

## Effect of Chloroquine on Blood Glucose and Cholesterol Levels in Alloxan-induced Diabetic Rabbits

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**Abstract:** The effect of treatment with chloroquine and normal saline on blood glucose and serum cholesterol levels was investigated in alloxan-induced diabetic and non-diabetic rabbits. It was observed that high dose of chloroquine (25 mg kg<sup>-1</sup> body wt) administration twice daily for three consecutive days led to an increase in the blood glucose and decrease in the serum cholesterol levels. The possible mechanism for the hyperglycaemic and hypocholesterolemic effects being observed was x-rayed in the light of other reported observations of chloroquine administration.

**Key words:** Chloroquine, alloxan, blood glucose, serum cholesterol, hyperglycemia, hypocholesterolemia, rabbits

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### Introduction

Chloroquine - induced changes in blood glucose and cholesterol in relation to tissue changes during alloxan-induced diabetes.

Chloroquine is a 4-aminoquinoline that has been widely used in African continent, particularly in Nigeria in the treatment of malaria and a vast number of diseases such as rheumatoid arthritis, lupus erythematosus and light eruptions (Ngaha and Akanji, 1982). Chloroquine and quinacridine acridines are active against the asexual and erythrocytic forms of the parasite. They ameliorate autoimmune diseases possibly by suppression of antigen formation. The parasites are more susceptible than the host cells, probably by inhibition of parasite DNA replication although there is also damage to the digestive and pigment vesicles.

Chloroquine is the most useful drug for malaria suppression and termination of acute treatment. It has no action against exoerythrocytic forms in the liver or other tissues: resistant strains of plasmodium, the causative pathogens are appearing. The drug is also active against other forms of protozoa especially giardia and amoeba. Typical derivatives of chloroquine include amodiaquinhydrochloride (camoquine), chloroquine phosphate or diphosphate (Aralen), hydrochloroquine sulphate and quinacrine hydrochloride (atabrine, mepacrine).

Chloroquine in a single large dose administration causes severe itching barley controllable

by antihistamines and lasting for several days. When administered in large doses or for longer periods, the drug can cause nausea, vomiting, dizziness; insomnia and blurred vision while toxic doses can cause circulatory collapse and death (Drill, 1971). It also inhibits histamine-N-methyl transferase (Pacific *et al.*, 1990); the enzyme involved in histamine activation as well as muramidase activity probably at the cellular / molecular level (Ngaha, 1984).

Reports that chloroquine was prescribed to diabetic patients and also used as a possible hypoglycemic agent (Quatraro *et al.*, 1988) and the variant observation (Adelusi and Ogonor, 1987) which reported that chronic administration of 30 - 35 mg chloroquine per kg body weight 3 times a week over a period of 2 weeks induced hyperglycemia in experimental animals stimulated this work. Other reports also implicated chloroquine in side effects on lipid metabolism such as phospholipidosis (Hosteller and Richman, 1982) and formation of inclusion of intralysosomal lipid in cornea and conjunctiva tissues (D' Amico and Kenyon, 1981).

Alloxan ( $C_4H_2N_2O_4$ ) is obtained from the oxidation of uric acid with nitric acid and produces a similar effect to diabetes mellitus when injected into experimental animals (Lange and Foye, 1956). Alloxan acts directly,

promptly and specifically on the B-cells of the pancreatic islets (Lanter, 1975). In alloxan-induced diabetes, hyperglycemia and glycosuria are more severe and the insulin requirement is high but show little ketonuria: the animals do not go into coma than depancreatised animals. The rise in blood glucose may persist for three or four hours causing many unfavourable secondary effects.

The present investigation was therefore, conducted to examine whether chloroquine administration during alloxan-induced diabetes caused changes in blood glucose and total cholesterol levels and relate any such phenomenon to changes that might occur in the level of these parameters in the tissues of chloroquine treated rats as described by Ngaha (1984) and Ngaha and Akanji (1982).

## **Materials and Methods**

### **Animal treatment and drug administration**

Twelve rabbits of the same age (10 months) obtained from the primate colony, Department of Biochemistry, University of Maiduguri, Nigeria were used for the determination of blood glucose and cholesterol levels in both diabetic and non-diabetic rabbits. The rabbits were evenly distributed into three groups of 4 male rabbits each. One group (non-diabetic) was used, as the controls while two groups were diabetic test groups.

After stabilization on Pfizer feeds the 2nd group of diabetic rabbits were given 25mg chloroquine per kg body weight intramuscularly. The injections were given twice daily for 3 consecutive days. The other diabetic group (3rd group) and controls were given pure physiological saline (0.5 ml) which was the solvent for the chloroquine twice daily for 3 consecutive days. On the 4th day after the last injection, analyses were carried out to determine blood glucose and cholesterol levels. The stock chloroquine solution contained 200 mg base  $5\text{ ml}^{-1}$  chloroquine diluted with physiological saline to give the concentration of 25 mg base.

The two diabetic groups (groups 2 and 3) were injected with 50 mg alloxan  $\text{kg}^{-1}$  body weight dissolved in pure physiological saline intraperitoneally once daily for 3 consecutive days. There after, chloroquine administration was commenced.

#### **Collection of blood samples**

Blood samples were collected separately from each rabbit by cutting the tip of the ear vein with clean surgical blade into clean centrifuge tube. The blood from each rabbit was collected into different centrifuge tubes. Blood glucose was assayed in each of the blood samples while serum was prepared from the remaining samples and used in determining cholesterol levels.

#### **Biochemical analyses**

##### **Determination of blood glucose**

Blood glucose level was determined in each of the blood samples by the glucose oxidase method of Mark (1956) as described by Verley (1967). The assay was based on the oxidation of glucose to glucuronic acid with gluconolactone as an intermediate. Hydrogen peroxide formed is broken down to water and oxygen by peroxidase in the presence of an oxygen acceptor, which is converted to a coloured compound, which can be determined calorimetrically at 626 nm.

The procedure involved standing the blood samples on ice for 1 min to ensure complete precipitation and centrifuging at 3000 ypm for 5 mins after which the supernatant was recovered and analysed subsequently for glucose.

Three ml of glucose oxidase reagent was added to 1.0 ml of the supernatant, mixed gently for not more than 10 seconds and left standing at room temperature for 10 minutes. Test tubes containing 1.0 ml of 2.5, 5.0, 10.0 mg per 100 ml glucose were set up as standards respectively. Each was treated with glucose oxidase reagent as described for the test above. A mixture of 1 ml of distilled water and 3ml of enzyme dye reagent was used as blank. This was allowed to stand for ten minutes after which the absorbances of the colour that developed in the test samples were read at 625 nm. The amount of glucose present in 100 ml of blood was calculated from the absorbances. The assays were done in quadruplets and results presented as mean $\pm$ S.D.

##### **Determination of total cholesterol**

Total cholesterol was determined in the sera samples of the control and diabetic rabbits following the method described by Verley (1967). This method based on the Lieberman Burchard reaction involved colorimetric determination of cholesterol in chloroform after precipitation of proteins and extraction of cholesterol from ethanol-ether mixture. 10 ml of ethanol-ether mixture were measured into a suitable centrifuge tube and 0.2 of serum prepared for analysis was added and the tube corked tightly and shaken vigorously for 1 minute.

The tube was allowed to lie almost horizontally so that the precipitate was fairly evenly distributed along the tube. After standing for an hour, it was centrifuged for few minutes to get a firm deposit. The residue after evaporation was dissolved in 5 ml chloroform.

The supernatant fluid were decanted completely into a boiling test tube and evaporated to dryness in a water bath. The residue after evaporation was dissolved in 5 ml of chloroform. Into another test tube was measured 5 ml of standard cholesterol solution containing 0.4 mg of cholesterol. To each test tube was added 2ml of acetic anhydride-sulphuric acid mixture, mixed and left to stand for 15 minutes in the dark at 25°C.

The absorbance developed was read at 680 nm. From the absorbance, the amount of cholesterol present in 100 ml blood was calculated and presented as mean±S.D. of quadruplet determinations.

## **Results**

The effects of treatment with chloroquine, alloxan and normal saline on the blood glucose and serum cholesterol levels on diabetic and non-diabetic rabbits are as presented in Table 1. As can be seen from the table, alloxan administration caused significant ( $P < 0.05$ ) increase in the blood glucose as well as in the concentration of cholesterol levels in the sera suggesting that the islets of the langerhans were destroyed. Similarly, intramuscular administration of chloroquine to the diabetic rabbits caused a significant increase in the blood glucose level of the group. However, the same treatment caused a significant ( $P < 0.05$ ) decrease in the level of serum cholesterol from the diabetic level of 215 mg 100 ml<sup>-1</sup> to 183 mg 100 ml<sup>-1</sup>. Chloroquine was thus, not able to bring down the cholesterol level to the normal level observed before the inducement of diabetes.

Treatment of diabetic and non-diabetic rabbits with intramuscularly administered normal saline did not cause any significant ( $P < 0.05$ ) changes in the levels of blood glucose or serum cholesterol in either the diabetic or non-diabetic rabbits.

## **Discussion**

This work has revealed that chloroquine can also increase blood glucose in diabetes (hyperglycemia). Hyperglycemia arises from two main sources, namely reduced rate of removal of glucose from the blood by peripheral tissues and increased release of glucose from liver and muscles into the blood stream.

The blood glucose level of the diabetic rabbits was increased after chloroquine administration. This finding is not in complete agreement with the result of other workers, which showed that chloroquine lowered blood sugar in a diabetic patient with severe insulin resistance and in those suffering from type II (non-insulin dependent) diabetes mellitus (Quatraro *et al.*, 1988). The increase in the blood glucose level observed in this work might be due to dose of chloroquine administered (25mg per kg body weight twice daily for three (3) consecutive days).

Also from literature the chronic administration of 30-35 mg chloroquine per kg body weight, three (3) times a week over a period of two (2) weeks induced hyperglycemia in experimental rats (Adelusi and Ogonor, 1987). As a result of this observation the authors were of the opinion that chronic administration of chloroquine: as is the case in the treatment of rheumatoid arthritis, where doses higher than normal are used; may complicate the matter if rheumatoid patient happens to be diabetic as well. Comparing the dose of chloroquine administered to the

rabbits in this work (25mg per kg weight twice daily for three (3) consecutive days) with the one used in previous investigation by Adelusi and Ogonor 1987 (30-35 mg) per kg body weight three (3) times a week over a period of two (2) weeks, the dose administered could also be chronic, since the blood glucose level has increased significantly ( $P < 0.5$ ).

On the whole, the results presented indicated that chloroquine significantly ( $P < 0.05$ ) increased blood glucose when administered at high dose to rabbits. The mechanism underlying this effect of chloroquine was not worked out in the present work. But based on the available reports in the literature, this effect could be due to the inability of the rabbits to synthesis insulin, a polypeptide hormone in the presence of this drug. And thus, a decrease in insulin is as a result of decrease in protein synthesis (Grodsky, 1983) that might be due to the high dose of chloroquine administered. This drug might have blocked the enzymatic synthesis of DNA and RNA by forming complexes with DNA, thereby preventing it from acting as template for its own replication or transcription of RNA (Meyer *et al.*, 1976).

This action of chloroquine in diabetic rabbits could be analyzed from two points. First is the action of chloroquine mediated via insulin. If this is the case, the hyperglycaemic effects of the drug could be interpreted on the basis of the ability of the chloroquine to stimulate the degradation of available insulin. Insulin is normally degraded by proteases in the liver (Stryer, 1988); fibroblast, adipose and blood (Blazer *et al.*, 1984). Therefore, a high dose of chloroquine administration will further stimulate the degradation of insulin leading to hyperglycemia.

The second point on which the interpretation of the hyperglycaemic action of chloroquine in diabetic rabbits could be based, is action on the pancreas itself. If the drug inhibited insulin production and then prevented the regeneration of the damaged pancreatic cells, then hyperglycaemic effect of chloroquine could be attributed to its later effect.

This work has also shown that the level of serum cholesterol was decreased in the same diabetic rabbits when the blood was analyzed. Also, the mechanism underlying the decrease in serum cholesterol level was not investigated. But it might be due to the blockage in the reabsorption of bile acids. This is by interrupting the enterohepatic circulation of the bile acids. Thus, chloroquine might act as the blocking agent, which blocks the enterohepatic cycle.

Table 1: The effect of treatment with chloroquine and alloxan on blood glucose and serum cholesterol levels of diabetic and non-diabetic rabbits

Treatment	Biochemical parameters			
	Blood glucose mg 100 ml <sup>-1</sup>		Serum Cholesterol mg 100 ml <sup>-1</sup>	
	Before treatment	After treatment	Before treatment	After treatment
Group 1 - Diabetic (Chloroquine treated, (25 mg)	131±0.50b	154±1.20c	215±1.50b	183±1.30c
Group 2 - Diabetic rabbits (non-Chloroquine treated)	120±2.00d	119±1.40d	200±2.30d	198±2.20d
Group 3- Controls- (Non-diabetic Saline treated)	65±0.75a	64±1.10a	179±2.80c	177±3.10a

Tabulated values are mean±S.D. of four determinations

Statistical comparison was done in a row and values with different superscripts are significantly different ( $P < 0.05$ )

Hence, the liver will draw more cholesterol from the blood for conversion into bile acids. That is, the conversion of cholesterol to bile is greatly enhanced in an effort to maintain the pool of bile acids. Low-density lipoproteins (LDL) receptors in the liver are up regulated, causing increased uptake of LDL with consequent lowering of blood cholesterol (Stryer, 1988). Therefore, at high chloroquine dose, (25mg kg<sup>-1</sup> body weight twice daily for three (3) consecutive days) exhibit a hypocholesterolemic effect. The increase may also be attributed to the chloroquine interfering with the synthesis of cholesterol.

However, further studies need to be done to find out which factor is exactly responsible for the decrease. It is, therefore, good as a subject of further investigation to study the mechanism involved in the higher blood glucose and the decrease in the cholesterol levels observed.

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