Chloroquine treatment attenuates alloxan-induced hyperglycemia and improves dyslipidemia via inhibition of PTEN receptor

ABSTRACT

Background: Diabetes mellitus is a life-threatening disease associated with worsening of glycemic control and progressive metabolic dysfunctions.
Purpose: To evaluate the effect of chloroquine on blood glucose level and lipid profile of alloxan-induced diabetic mice as well as observe its interaction with PTEN, a negative regulator of insulin signaling pathway.
Methodology: 30 mice were used for the experiment. A group (n=5) was kept as healthy control while the others were pre-treated with 140mg/kg alloxan monohydrate in distilled water. Alloxan-induced diabetic mice were randomly divided into four groups; diabetic model control group, 60 mg/kg chloroquine, 120 mg/kg chloroquine and 10mg/kg of glibenclamide treatment groups respectively. Treatment was done once daily for seven days while the blood glucose level was investigated acutely and then daily throughout the experimental period. A molecular docking study was conducted to evaluate the interaction of chloroquine with PTEN using PYRX software, and an automated COBAS C 311 machine was used to analyze the lipid profile after the treatment period.
Results: oral administration of chloroquine gradually and significantly lowered the raised blood glucose level in a time and dose-dependent manner. Repeated study with 120 mg/kg of chloroquine revealed a decline in blood sugar from 221.5 ± 3.6 on day 1 to 85.5 ± 2.4 on day 7 in comparison to glibenclamide whose sugar level reduced to 75.5 ± 3.7 at the end of day 7. The docking study revealed a non-competitive inhibition with an inhibition score of -6.1in comparison to Metformin which had a score of -4.4 and glibenclamide with a score of -9.0. This interaction resulted in a conformational change of the receptor, hence enhancing glucose uptake and reducing the raised hyperglycemia. Treatment with chloroquine was also observed to reduce the total cholesterol and triglyceride from 162 in the model group to 144 and 141 to 116 mMol/L respectively. The levels of LDL and VLDL following treatment with chloroquine were not statistically different from the non-diabetic healthy mice. These values were very similar to those obtained with glibenclamide-treated diabetic mice.
Conclusion: Chloroquine therefore possess potent antidiabetic properties and can improve dyslipidemia imposed by hyperglycemia probably due to its inhibition of PTEN, a negative regulator of insulin resistance.

Keywords: Diabetes; chloroquine; PTEN; blood glucose level; dyslipidemia

1. INTRODUCTION

Diabetes mellitus is a complex and chronic metabolic disease characterized by persistent hyperglycemia [1], polydipsia, and polyphagia [2][3]. The disease is accompanied with impaired carbohydrate, fat, and protein metabolism due to insulin secretion defects and/or peripheral tissue insulin action [4] Diabetes mellitus is an extensive public health emergency with increased incidence rate of 10.5% occurring globally in 2022. Amidst successful medical management of diabetes mellitus, about four million deaths were recorded globally [3][5]. Type 1 and Type 2 diabetes are the two major forms of diabetes resulting from autoimmune destruction of beta cells and insulin resistance respectively.There are several risk factors that contribute to the development of diabetes; ranging from genetic susceptibilities that predispose to insulin resistance. Lifestyle factors like sedentary and lack of physical activity, dietary factors, age and gender as well as certain medical conditions [6].

Insulin resistance, a key mediator of type 2 diabetes leads to increased production of very low-density lipoprotein (VLDL) particles, which in turn increases triglyceride levels and contributes to the development of dyslipidemia [7]. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles[8]. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance [9]. It is worthy of note that conventional treatments have really helped with the management of this disease, howbeit high cost. reduced efficacy and proliferation of adverse effects have limited available treatments and increase the search for more agents with potential of ameliorating the persistent side effects of diabetes mellitus.

Chloroquine, a 4-aminoquinoline compound with well-established anti-malarial properties has been shown to possess immunomodulatory and anti-inflammatory properties, making it a promising candidate for repurposing in the treatment of various autoimmune and inflammatory conditions [10][11]. Chloroquine's ability to modulate immune responses has led to investigations into its potential use in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus as well as other diseases owing to its diverse mechanism of action. One of the key mechanisms is its ability to inhibit autophagy, a cellular process involved in the degradation and recycling of cellular components which has been implicated in the pathogenesis of diabetes. Chloroquine has also been shown to modulate insulin signaling pathways, leading to improved glucose homeostasis. Thus, this study aimed to explore the antidiabetic and hypolipidemic potential of chloroquine in alloxan-induced diabetic mice while evaluating its interaction with PTEN, a negative regulator of insulin signaling.

2. MATERIAL AND METHODS

The following drugs were used for the study: Alloxan monohydrate (Sigma-Aldrich, Switzerland) Maxiquine® (Chloroquine phosphate tablet B.P 250mg) manufactured by Vitabiotics (NIG) LTD. **Glanil®** (Glibenclamide 5mg B.P) and ketamine hydrochloride manufactured by Sygen Pharmaceuticals Limited.

2.1*Animal experimentation*

Swiss mice of both sexes weighing between 15 to 26 grams were used for the study. The mice were obtained from the animal house of the Department of Pharmacology and Toxicology at the University of Uyo, Nigeria. Throughout the experimental period, the mice were kept in standard cages at room temperature with a 12-hour cycle of light and darkness. They were provided with rodent pellet diet and water as needed. Experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and the ARRIVE guidelines, and were approved by the college of Health Science Animal Ethics Committee, University of Uyo.

2.2*Induction of Diabetes and Treatment*

The Swiss mice were fasted for 24 hours prior to the experiment but were allowed access to water. They were rendered diabetic by injecting freshly prepared alloxan monohydrate in distilled water (140mg/kg) intraperitoneally. One hour after alloxan administration, the animals were allowed access to food and administered 5% dextrose saline solution to prevent hypoglycemic shock. After 72 hours, mice with blood glucose level above 150mg/dL were selected for the study. The selected animals were randomly divided into 5 groups: negative control (healthy non-diabetic animals), diabetic positive control, 60 mg/kg chloroquine low dose group, 120 mg/kg chloroquine high dose group and 10 mg/kg glibenclamide. Each group received a once daily oral treatment for seven days. The changes in body weight and fasting blood glucose level (BGL) of all the mice were recorded at regular intervals throughout the experimental period. For acute study, the blood glucose level (BGL) was monitored after 1-, 2-, 4- and 6-hours following administration of a single dose of the drugs and at the end of 1, 3 ,5 and 7 days for short term treatment. The blood glucose level (BGL) was monitored by tail tipping method. The blood was dropped on the blood glucose test strip, and inserted into the microprocessor digital blood glucometer and the readings were recorded.

2.3*Interaction of chloroquine with Phosphatase and TENsin homolog (PTEN)*

Chloroquine was retrieved in SDF format from PubChem database while PTEN was retrieved from NCBI with PDB ID 5bzx. Both structures were prepared and saved in their appropriate format before molecular docking studies using PYRX software. The least energy conformation was chosen and the pose analyzed accordingly.

2.4*Evaluation of hypolipidemic activity of the drugs*

Twenty-four hours after the last dose, the animals were anesthetized with ketamine and blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuge at 1000rpm for 10min to obtain the serum. Total cholesterol, triglycerides and high-density lipoprotein (HDL) levels were analyzed using automated COBAS C 311 machine.

2.5 Statistical Analysis

Statistical analysis of the data was done using Graph Pad Prism Software version 5.0 for windows (Graph Pad software, San Diego California USA). Results were expressed as mean ± standard error of mean. The difference between treatment groups and controls was evaluated by performing a one-way ANOVA followed by Tukey's multiple comparison post-hoc test to ascertain the level of significance. $P \leq 0.05$ were considered statistically significant.

3. RESULTS

3.1 Chloroquine reduces blood glucose levels of alloxan induced diabetic mice after a single dose

Table 1. Effect of single dose administration of chloroquine on blood glucose level

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL (mg/dL) IN HOURS (Mean ± SEM				
	(mg/ml)	0hr	1hr	2hr	4hr	6hr
Negative Control	10	68.75±3.68	65.50±2.17	60.25±0.47	58.75±0.62	57.50±1.04
Diabetic model Control	10	215.0±3.67[c]	215.25±3.47[c]	215.75±4.57[c]	216.75±4.28[c]	219.75±4.00[c]
CQ Low Dose	60	230.25±19.3[c]	224.0±15.12[c,d]	212.50±11.53[c]	209.75±11.60[c,d]	206.0±11.83[c,d]
CQ High Dose	120	226.0±7.15[c]	216.25±8.45[c]	211.0±4.50[c]	198.25±0.85[c,d]	187.25±1.79[c,d]
Glibenclamide	10	221.0±3.48[c]	200.25±3.17[c]	177.0±5.30[c,d]	168.0±5.24c,f	158.25±4.51[c,f]

Significant at [a]p<0.05, [b]p < 0.01, [c]p < 0.001, when compared to normal control group;Significant at [d]p<0.05, [e]p<0.01, [f]p<0.001 compared to diabetic control group.

A single oral administration of chloroquine was observed to significantly reduce the fasting blood glucose of alloxan-induced diabetic mice across the various time point examined (Table 1). A significant decrease was observed an hour following treatment with 60 mg of chloroquine. This effect was observed to be dose dependent as the time progresses. Within a short period of seven days, daily chloroquine administration (Table 2) also revealed a significant reduction in blood glucose level.

Table 2. Effect of daily administration of chloroquine on blood glucose level of alloxa-induced diabetic mice

TREATMENT	DOSE (mg/ml)	BLOOD GLUCOSE LEVEL (mg/dL) IN HOURS (Mean ± SEM) 0hr	1st Day	3rd Day	5th Day	7th Day
Negative Control	10	68.75±3.68	58.25±1.93	60.0±3.71	60.25±2.25	63.0±2.04
Diabetic model Control	10	215.0±3.67 [c]	221.25±3.63[c]	221.50±3.09	192.0±2.16[c,f]	181.0±1.95[c,f]
CQ Low Dose	60	230.25±19.37[c]	210.50±8.31[f]	109.50±3.37[c,f]	129.75±5.36[c,f]	84.25±2.39[c,f]
CQ High Dose	120	226.0±7.15 [c]	190.5±0.86[c,e]	178.50±1.32[c,f]	129.75±5.07[c,f]	85.50±2.39[c,f]
Glibenclamide	10	221.0±3.48 [c]	145.75±3.96[c,f]	133.0±4.76[c,f]	108.75±4.47[c,f]	75.50±3.70[a,f]

Significant at [a]p<0.05, [b]p < 0.01, [c]p < 0.001, when compared to normal control group; Significant at [d]p<0.05, [e]p<0.01, [f]p<0.001 compared to diabetic control group.

3.2*Chloroquine inhibits PTEN receptor*

Chloroquine showed a favourable interaction with PTEN. A classical hydrogen bond interaction was observed with phenylalanine 279 at a close distance of 1.83 and Pi=Alkyl bonds with proline 281 and 283. A Pi-Sigma bond was also observed with Tyrosine 180 (Figure 1 and 2). The inhibition score was -6.1 with chloroquine, 4.4 with mefloquine and 9.0 with glibenclamide.

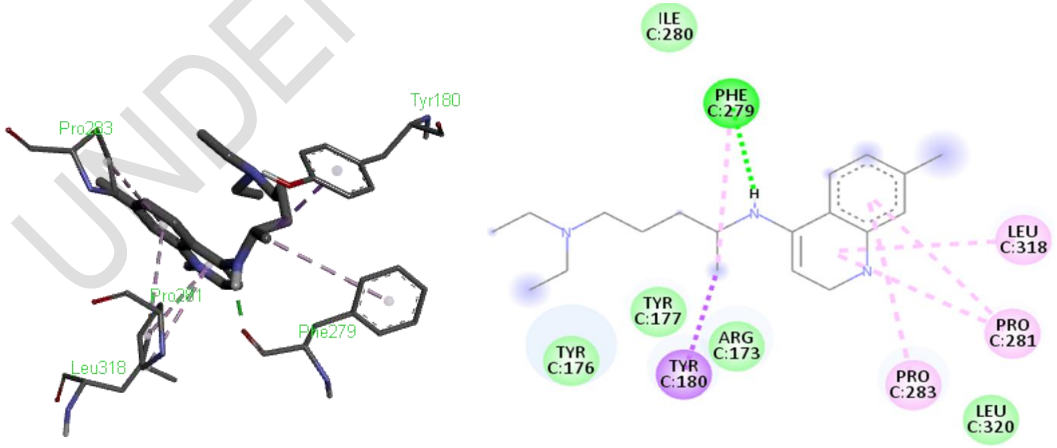

Fig.1. 3D and 2D interaction of chloroquine with PTEN, a negative regulator of insulin sensitivity

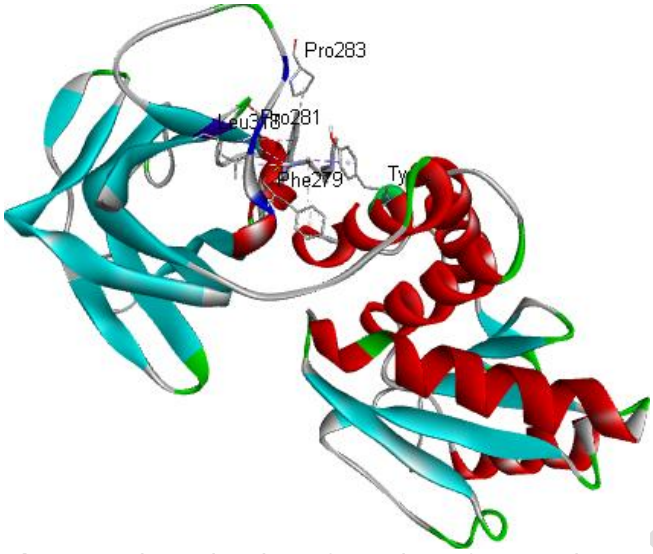

Fig 2. 3-Dimensional conformation of PTEN in complex with chloroquine at the ligand binding site**.**

3.3.*Effect of Chloroquine on Lipid Profile of Alloxan-Induced Diabetic Mice*

The administration of Chloroquine (60mg/kg and 120mg/kg) attenuates lipid disorder found in alloxan-induced diabetic mice. Total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein(VLDL) were significantly reduced upon treatment with chloroquine when compared to diabetic control group (Table 3). There was also an improvement of the levels of HDL following treatment with chloroquine.

Table 3. Effect of chloroquine on lipid profile of alloxan-induced diabetic mice

TREATMENT	**DOSE mg/kg**	**TOTAL CHOLESTEROL (mMol/L)**	**TRIGLYCERIDE (mMol/L)**	**HDL-C (mMol/L)**	**LDL-C (mMol/L)**	**VLDL (mMol/L)**
Control (normal mice)	10 mg/ml	128.15±26.96	117.05± 26.96	54.0± 16.40	52.82± 10.97	19.52± 4.50
Diabetic control (distilled water)	10	162.62± 7.62^{c}	141.85± 3.96^{c}	52.50± 7.37^{c}	56.47± 9.47	23.65± 0.65
Chloroquine	60	144.52± 12.53^{c,e}	116.1± 10.70^{f}	72.75± 6.11^{c,d}	52.40± 5.43	19.37± 1.77^{d}
Chloroquine	120	152.82± 9.63^{c,d}	112.17± 5.64^{d}	66.30± 8.98^{c,e}	67.40± 17.57^{c,d}	18.70± 0.95

Glibenclamide	10	146.35± 4.43[c,e]	112.42± 5.15[d]	58.62± 4.32[e]	67.67± 3.27[c,d]	19.90± 1.09

Significant at [a]p<0.05, [b]p < 0.01, [c]p < 0.001, when compared to normal control group;
Significant at [d]p<0.05, [e]p<0.01, [f]p<0.001 compared to diabetic control group.

4. DISCUSSION

The rise in the incidence rate of diabetes mellitus necessitates research into more drugs or other therapeutic options to manage this disease. Chloroquine has been reported to possess anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [12]. Chronic low-grade inflammation is implicated in insulin resistance and the progression of type 2 diabetes [13]. By attenuating inflammation, chloroquine may improve insulin sensitivity and glucose homeostasis. This study evaluated the effect of oral administration of chloroquine in alloxan-induced diabetic mice as well as examining the effect of chloroquine in dyslipidemia which is evident in diabetes.

Upon induction of diabetes with the alloxan, the blood sugar level rose drastically. This rise as earlier reported may be attributed to destruction of β-cells by the β-cytotoxin, alloxan [14]. This destruction resulted in the observed hyperglycemia. However, the raised blood glucose was reduced upon treatment with chloroquine, an agent with wide variety of action that has found application in diverse medical condition. This reduction in the induced hyperglycemia may be attributed to glucose uptake or modulation of the insulin signaling pathways by chloroquine [15]. It is also possible that chloroquine might have enhanced insulin secretion. Studies have demonstrated that chloroquine increases the release of insulin by stimulating the production and release of insulin granules from beta cells [16]. This effect is mediated by the inhibition of lysosomal function within the beta cells, leading to increased availability of insulin granules for exocytosis.

Chloroquine has been shown to modulate insulin signaling pathways, leading to improved glucose homeostasis [17]. Result from this study showed the interaction of chloroquine with tensin homolog (PTEN), a negative regulator of the insulin signaling pathway. The observed hydrogen and mixed hydrophobic interactions observed with chloroquine and PTEN, significantly altered the proteins conformation. By inhibiting PTEN, chloroquine possibly increased the activation of downstream components of the pathway, such as protein kinase B (Akt), which enhances glucose uptake and metabolism, hence reduction in the induced hyperglycemia. Similar to a study reported by Halaby *et al*. [17].

Furthermore, Chronic low-grade inflammation and increased oxidative stress has been reported to impair insulin signaling, triggering insulin resistance. Chloroquine has been widely reported with anti-inflammatory potential. This anti-inflammatory potential coupled with chloroquine inhibition of PTEN may have improved in vivo insulin sensitivity and reduce hyperglycemia in the experimental animals.

Dyslipidemia is a common metabolic abnormality observed in diabetes [18][19] which is characterized by altered lipid profiles, including elevated levels of triglycerides, reduced levels of high-density lipoprotein (HDL) cholesterol, and increased levels of low-density lipoprotein (LDL) cholesterol. Upon treatment with chloroquine, the lipid profile of the studied animals improved as observed by Vijayaraghavan 2010 [20]. In this study, treatment with Chloroquine, reduced the elevated triglycerides improved the levels of HDL, and reduced the levels of LDL and cholesterol. This observation could as well be a concerted effort of chloroquine's improvement of glucose sensitivity, its anti-inflammatory potential as well as a possible direct effect on lipid metabolism.

5. CONCLUSION

Chloroquine shows promise in reducing hyperglycemia in diabetes, further research is therefore needed to fully understand its mechanisms especially its possible interaction with downstream signaling molecules and its potential as a therapeutic agent for the treatment of diabetes.

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