



The Antidiabetic Potential of Chloroquine in Streptozotocin-Induced Diabetes and its Complications in Rats

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ABSTRACT

This study assessed the therapeutic effects of chloroquine on diabetes and its renal, cardiac and hepatic complications. A single intraperitoneal streptozotocin (STZ) injection in an animal model was used for this study. Blood glucose level, body weight, markers of oxidative stress such as malondialdehyde (MDA), hydrogen peroxide (H₂O₂) generation, protein carbonyl, nitric oxide (NO), reduced glutathione and antioxidant enzymes such as superoxide dismutase, glutathione peroxidase were evaluated. Histopathology of various organs was done to evaluate structural changes. Immunohistochemical changes using CTnI, Kim-1, PPAR γ and Nrf2 were also performed. It was shown that chloroquine administration significantly improved blood glucose levels and body weight. Structural changes such as necrosis, inflammatory cell infiltration, and congestion induced by STZ injection were ameliorated with chloroquine treatment. Untreated diabetic animals showed marked increase in levels of oxidative stress and inflammatory markers such as MDA, H₂O₂, NO, MPO, and depletion in both non-enzymatic and enzymatic antioxidant defense system, upregulation of Kim1, CTnI, downregulation of PPAR γ and Nrf2. Treatment with chloroquine ameliorated renal and cardiac injury coupled with increased expressions of Kim-1 and CTnI. It can therefore be postulated that chloroquine exhibited antidiabetic property through upregulation of PPAR γ . Its anti-inflammatory and antioxidant properties were confirmed through the upregulation of Nrf2 and improved antioxidant status.

Key words: Drug repurposing; Chloroquine; Oxidative damage; Diabetes and complications.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by alterations in glucose and lipid metabolism and high morbidity, and hyperglycemia in diabetes is thought to be responsible for the various aberrations (Day *et al.*, 2017; Dowidar *et al.*, 2020), relating to heart, kidney and liver diseases. It has been projected that by 2030, 439 million people will be diabetic (Shaw *et al.*, 2010). WHO also projects that diabetic death will double between 2005 and 2030 (WHO, 2010). Oxidative damage has been incriminated in the

pathogenesis of diabetes, with a left shift that favors pro-oxidants rather than the antioxidant defense system. The oxidative damage leads to cell death, destruction of DNA and other macromolecules, thought to be responsible for the complications such as nephropathy, hepatopathy and cardiomyopathy seen in diabetes mellitus (Wu *et al.*, 2018).

Chloroquine is a popular 4-aminoquinolone antimalarial agent used in the treatment of malarial infections and later against autoimmune diseases. It has also been repurposed for the treatment of various cancers (Verbaandrd *et al.*, 2017), including myelomas (Montanari *et al.*, 2014), breast cancer (Maycotte *et al.*, 2015), hepatic cancer (Sun *et al.*, 2013)

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and colon cancer (Zhen *et al.*, 2009). Besides cancer, chloroquine has been repurposed for the treatment and prevention of Zika viral infection, transmitted to humans by mosquitoes (Shiryaev *et al.*, 2017).

Streptozotocin is a beta cell cytotoxic agent; it damages pancreatic islets and also triggers inflammation. This inflammation exacerbates the pancreatic islets damage, leading to aberrations in insulin production with consequent hyperglycemia (Furman 2016). Halaby *et al.* (2013) earlier reported that chloroquine stimulates glucose uptake and glycogen synthase in muscle cells through activation of Akt.

The study therefore is set to assess the antidiabetic potential of chloroquine in Wistar rats and to determine its mechanism of action using immunohistochemistry, histopathology, anti-inflammatory and anti-oxidative effects.

MATERIALS AND METHODS

Induction of diabetes

Male Wistar rats (30) weighing between 100 and 160 grams were obtained from the Experimental animal unit of the Faculty of Veterinary Medicine for the experiment. The animals were housed in the animal house unit of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan with a 12-hour light duration and $25\pm 2^{\circ}\text{C}$ temperatures. Pre-conditioning of the rats was done for two weeks before commencement of the experiment with access to standard rat pellets and fresh water *ad libitum*. All the animals received humane care according to the criteria outlined in the Public Health Service Policy on Humane Care and the Use of Laboratory Animals. The University of Ibadan Ethics Committee on animal use approved the study with approval number UI-ACUREC/App/03/2017/011.

The animals were fasted for 16 hours prior to the induction of diabetes; this was done by single intraperitoneal administration of 50 mg/kg Streptozotocin (STZ). Oral glucose was given to counteract the initial massive hypoglycemia that accompanies STZ administration. Blood glucose level was assessed 72 hours post STZ administration. Rats with blood glucose level above 250 mg/dl were considered diabetic and were used for the study.

Experimental design

Rats with blood glucose higher than 250 mg/dL were used for the study. Thirty male Wistar albino rats were randomly divided into four groups A-D, with Group A as the control group and non-diabetic. This group received distilled water only. Group B was the diabetic non-treated group and was given single intraperitoneal dose of STZ, Group C animals were induced with STZ but treated with 5 mg/kg of Chloroquine daily throughout the study while Group D was the diabetic group treated with 25 mg/kg of Pioglitazone daily. Blood glucose levels were determined using Accucheck glucometer according to manufacturers' instructions on days 0, 1, 7, 14 and 21. The blood glucose levels were reported as mg/dl. On day 22, serum was obtained by centrifuging blood samples while renal, hepatic and cardiac homogenates were obtained for biochemical evaluation. Some sections of the renal, cardiac, hepatic, pancreatic tissues were used for

histopathology and immunohistochemistry. The rats were sacrificed by cervical dislocation.

Biochemical assays

The hydrogen peroxide level, malondialdehyde (MDA) content as an index of lipid peroxidation quantification in the PMFs of the tissues, advanced oxidation protein product (AOPP) contents determination, measurement of protein carbonyl (PCO) contents in the tissues, superoxide dismutase (SOD) assay, reduced glutathione (GSH) estimation, glutathione peroxidase (GPx) activity, glutathione-S-transferase (GST) activity and protein concentration determination were performed as described by Oyagbemi *et al.* (2016). The serum nitric oxide levels were determined using Griess reagent according to the method earlier described by Olaleye *et al.* (2007). The myeloperoxidase (MPO) and xanthine oxidase activities were also as determined by Oyagbemi *et al.* (2017). On the other hand, serum urea and creatinine were determined by using Randox Kits according to manufacturers' instructions.

Histopathology

The organs were isolated and fixed in ten percent buffered formalin, embedded in paraffin and sectioned (5 microns) using a microtome; the sections were stained with hematoxylin and eosin and observed under a light microscope. They were evaluated for structural changes (Drury, 1976).

Immunohistochemistry

The tissue sections were de-paraffinized and subsequent antigen retrieval performed by microwave heating in 0.01 M citrate buffer (pH 6.0) to boil. After blocking in normal goat serum (10%, HistoMark[®], KPL, Gaithersburg MD, USA), the tissue sections were incubated with anti-peroxisome proliferator-activated receptor gamma (PPAR γ) (bs-4590R), cardiac troponin I (bs-0799R), nuclear factor erythroid-2 (NFE-2)-related factor-2 (Nrf2; bs-1074R), NF-kB (bs-10037R) by Bioss and Kim-1 (P1354) by BioVisions. Bound antibody detection was carried out using biotinylated (goat anti-rabbit, 2.0 $\mu\text{g/ml}$) secondary antibody and thereafter, streptavidin peroxidase (Horse Radish Peroxidase-streptavidin) in line with manufacturer's protocol (HistoMark[®], KPL, Gaithersburg MD, USA). Immuno-reactive positive expression regions were viewed from low magnification on each slice and later with 400 \times magnifications using a photo microscope (Olympus) and a digital camera (Toupcam[®], Touptek Photonics, Zhejiang, China).

Statistical analysis

Data were expressed as mean \pm standard deviation (SD). Results were analysed using student's t-test and one-way ANOVA followed by Tukey's post-hoc test using Graph prism 5 and $P < 0.05$ considered to be statistically significant.

RESULTS

Weekly weight and blood glucose levels

There was significant weight loss in the diabetic non-treated groups throughout the study, whereas both groups treated with chloroquine (5 mg/kg) and pioglitazone

Table 1: Effects of chloroquine and pioglitazone on weekly body weight (g) and blood glucose (mg/dl) in stz-induced diabetes

Group	Initial	Day 1	Day 7	Day 14	Day 21
A weight	148±8.37	154±11.4	168±17.89	182±16.43	190±20.00
(BG)	66.8±2.49	66.8±2.49	101.4±9.24	87.8±3.70	107.2±3.19
B weight	164±5.48 ^a	152±8.37	152±16.43 ^a	154±15.17 ^a	126±23.02 ^a
(BG)	90±18.67 ^a	312.8±59.20 ^a	281.8±48.13 ^a	275.8±25.41 ^a	344.6±68.56 ^a
C weight	145±5.77 ^b	140±8.16 ^{a,b}	155±12.91	165±17.32	165±28.87 ^b
(BG)	61.75±4.79 ^b	326.5±74.84 ^a	186±78.52 ^{a,b}	195.5±109.79 ^a	230±127.14 ^{a,b}
D weight	155±5.77 ^b	137.5±9.57 ^{a,b}	142.5±26.30 ^a	150±21.60 ^a	127.5±20.61 ^a
(BG)	52.5±2.65 ^{a,b}	411.25±38.07 ^{a,b}	249.25±55.01 ^a	241±108.59 ^a	236.5±100.91 ^{a,b}

Values expressed as Mean±SD. Alphabets indicates significant difference across groups at P<0.05. A (Control), B (Diabetic non-treated 50mg/kg STZ), C (Diabetic + 5mg/kg Chloroquine), D (Diabetic + 25mg/kg Pioglitazone). BG: blood glucose

Table 2: Effects of chloroquine and pioglitazone on cardiac markers of oxidative stress

Group	MDA	H ₂ O ₂	PCO	GPx	GST	SOD	GSH
A	0.60±0.07	37.45±1.67	65.81±3.05	230.5±27.5	2.4±0.8	11.1±1.1	109.2±9.2
B	1.18±0.15 ^a	43.16±2.06 ^a	104.69±2.26 ^a	185.7±25.7 ^a	1.6±0.3	8.6±1.4 ^a	92.4±0.4 ^a
C	0.77±0.10 ^{a,b}	36.80±2.81 ^b	65.66±5.8 ^b	267.5±6.1 ^{a,b}	2.4±0.9	11.5±1.1 ^b	104.7±0.9 ^b
D	0.67±0.09 ^b	37.52±2.32 ^b	65.96±13.93 ^b	247.2±12.6 ^b	2.0±0.8	11.2±0.4 ^b	103.5±2.3 ^b

Values expressed as Mean ±SD. Alphabets indicates significant difference across groups at P<0.05. A (Control), B (Diabetic non-treated 50 mg/kg STZ), C (Diabetic + 5 mg/kg Chloroquine), D (Diabetic + 25 mg/kg Pioglitazone). H₂O₂ (μmol/mg protein); MDA (μmol of MDA formed/mg protein); PCO- (nmol /mg protein); GPx (units/mg protein); SOD (units/mg protein); GST (mmole1- Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); GSH (μmo /mg protein).

Table 3: Effect of chloroquine and pioglitazone on renal markers of oxidative stress

Group	MDA	H ₂ O ₂	PCO	GPx	GST	SOD	GSH
A	0.42±0.03	45.20±3.84	47.23±3.88	154.22±17.42	3.44±1.53	7.56±0.56	105.8±0.9
B	0.70±0.22 ^a	136.10±9.37 ^a	85.03±6.8 ^a	122.10±3.49 ^a	1.73±0.97 ^a	5.78±0.30 ^a	98.8±3.2 ^a
C	0.36±0.07 ^b	43.76±3.33 ^b	37.46±6.52 ^{a,b}	134.09±17.66 ^a	2.38±1.17	6.36±1.50 ^a	104.8±1.6 ^b
D	0.32±0.07 ^b	44.66±3.40 ^b	45.68±7.49 ^b	144.98±13.64 ^b	3.28±1.73	6.46±0.73 ^a	104.9±2.9 ^b

Values expressed as Mean ±SD. Alphabets indicates significant difference across groups at P<0.05. A (Control), B (Diabetic non-treated 50 mg/kg STZ), C (Diabetic + 5 mg/kg Chloroquine), D (Diabetic + 25 mg/kg Pioglitazone). H₂O₂ (μmol/mg protein); MDA (μmol of MDA formed/mg protein); PCO- (nmol /mg protein); GPx (units/mg protein); SOD (units/mg protein); GST (mmole1- Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); GSH (μmol/mg protein).

Table 4: Effect of chloroquine and pioglitazone on hepatic markers of oxidative stress

Group	MDA	H ₂ O ₂	PCO	GPx	GST	SOD	GSH
A	0.10±0.01	119.73±6.72	14.01±0.83	30.0±4.6	6.0±1.6	1.6±0.2	106.7±3.5
B	0.15±0.01 ^a	194.39±2.75 ^a	34.04±1.27 ^a	22.3±1.5 ^a	3.4±1.4 ^a	1.0±0.1 ^a	88.3±10.9 ^a
C	0.11±0.01 ^b	144.14±13.38 ^{a,b}	22.30±2.29 ^{a,b}	26.8±1.5 ^b	7.5±1.1 ^b	1.4±0.2 ^{a,b}	105.7±1.6 ^b
D	0.11±0.01 ^b	139.31±11.22 ^{a,b}	13.73±4.80 ^b	28.3±4.5 ^b	6.3±1.5 ^b	1.3±0.3 ^{a,b}	105.7±0.8 ^b

Values expressed as Mean ±SD. Alphabets indicates significant difference across groups at P<0.05. A (Control), B (Diabetic non-treated 50 mg/kg STZ), C (Diabetic + 5 mg/kg Chloroquine), D (Diabetic + 25 mg/kg Pioglitazone). H₂O₂ (μmol/mg protein); MDA (μmol of MDA formed/mg protein); PCO - (nmol /mg protein); GPx (units/mg protein); SOD (units/mg protein); GST (mmole1- Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); GSH (μmol/mg protein).

Table 5: Effect of chloroquine and pioglitazone on serum markers of oxidative stress and inflammation

Treatments	MPO	NO	AOPP	XO	TP
A	37.08±3.45	0.95±0.19	19.38±1.98	1.37±0.4	4.39±0.08
B	118.55±9.67 ^a	1.71±0.17 ^a	34.29±4.32 ^a	1.72±0.3 ^a	6.79±0.61 ^a
C	35.75±2.11 ^b	0.96±0.43 ^b	16.92±2.85 ^b	1.32±0.3 ^b	5.19±0.61 ^b
D	82.96±9.82 ^{a,b}	1.33±0.03 ^{a,b}	17.41±3.50 ^b	1.38±0.04 ^b	5.01±1.23 ^b

Values expressed as Mean ±SD. Alphabets indicates significant difference across groups at P<0.05. A (Control), B (Diabetic non-treated 50 mg/kg STZ), C (Diabetic + 5 mg/kg Chloroquine), D (Diabetic + 25 mg/kg Pioglitazone). MPO (μmol/minute); NO (μmol/mg protein); AOPP (μmol/mg protein); XO (units/mg protein)

(25 mg/kg) showed improvements in weight from day 7 of the study (Table 1). The blood glucose levels of the diabetic non-treated group recorded significant increase. However, treatment with chloroquine and pioglitazone significantly reduced the blood glucose levels although not to the levels seen in the control group (Table 1).

Cardiac, renal and hepatic markers of oxidative stress

STZ induced significant elevations in levels of cardiac, renal and hepatic lipid peroxidation product (malondialdehyde), hydrogen peroxide and protein carbonyl with improvements in the groups treated with chloroquine (5 mg/kg) and pioglitazone (25 mg/kg) (Table 2-4).

Serum markers of inflammation and oxidative stress

We reported significant increase in levels of advanced oxidative protein products, xanthine oxidase, nitric oxide and myeloperoxidase in the diabetic non-treated group whereas chloroquine (5mg/kg) and pioglitazone (25mg/kg) ameliorated this (Table 5).

Cardiac, renal and hepatic antioxidant activities

We reported significant decrease in cardiac SOD, GPx, GSH and GST in the diabetic non-treated groups whereas the activities of these enzymes in the groups treated with chloroquine (5 mg/kg) and pioglitazone (25 mg/kg) significantly increased (Table 2-4).

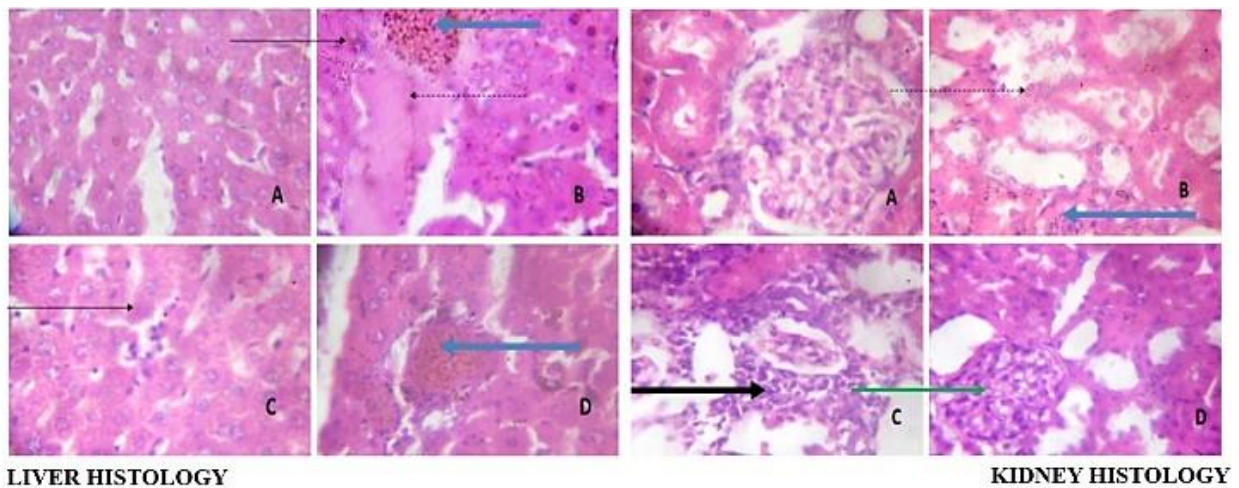


Fig. 1: The histology of the liver (right) and kidney (left): A shows No visible lesion. B: shows congestion (blue arrows), focal thrombosis (dotted arrow), and marked disseminated infiltration of zone 2 by inflammatory cells (slender arrows). C: shows mild infiltration of zone 2 by inflammatory cells (slender arrow). D shows disseminated congestion (blue arrows: Kidney: (A) shows normal morphology of the kidney. (B) shows inflammatory cell infiltration, congestion of vessels (blue arrows), areas of red cell cast (slender arrows), some tubules show desquamation (dotted arrows). (C) shows focal area of extensive interstitial infiltration by inflammatory cells (black arrows). D: glomeruli showing hypercellularity of the mesangial cells (green arrows). A- Normal control, B- Diabetic control, C- Chloroquine treated group, D- Pioglitazone treated group. Photomicrograph of the Liver and Kidney (x 100).

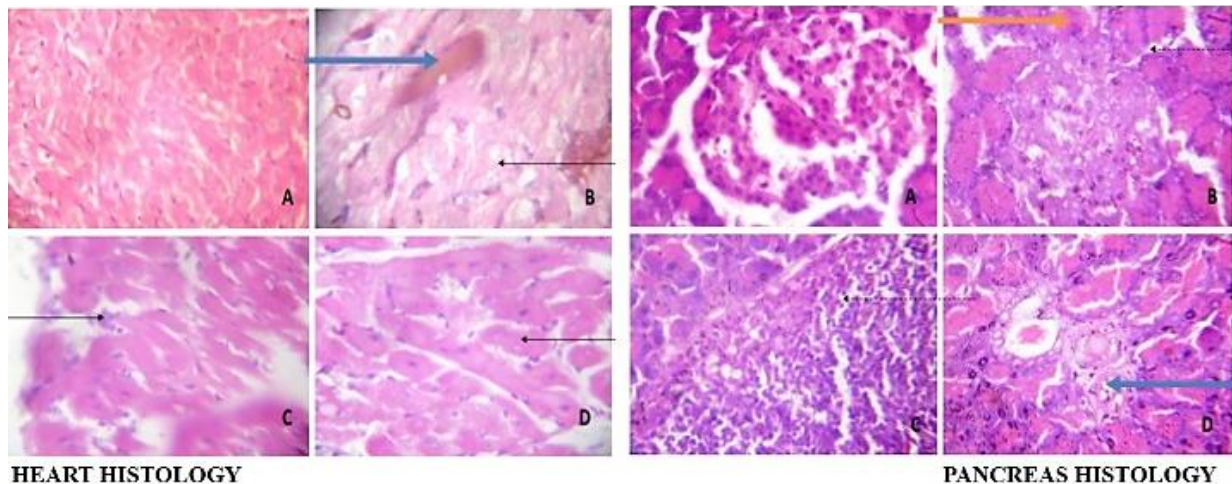


Fig. 2: The histology of the heart (right) and pancreas (left). A: No visible lesion. B: shows focal area of hemorrhagic lesion (blue arrow), focal area of mild necrosis (slender arrow). C: shows focal area of very mild myocardial infiltration by inflammatory cells (slender arrows). D: focal area of myocardial infarction (slender arrow): Pancreas: A: is normal. B: Islets are few and appear necrotic (green arrow), acinar cells show hyperplasia (dotted arrow). C: shows area of infiltration by inflammatory cells and attached lymph node (black arrows). D: shows focal area of congestion (blue arrow), the intralobular ducts appear normal with eosinophilic pancreatic secretion (slender arrow). A- Normal control, B- Diabetic control, C- Chloroquine treated group, D- Pioglitazone treated group. Photomicrograph of the heart and pancreas X100.

There were significant depletions in renal and hepatic GSH, GPx, and GST activities in the diabetic non-treated group, but treatment with chloroquine (5 mg/kg) and pioglitazone (25mg/kg) induced a significant increase in their levels although not to control group level (Tables 2-4).

Histopathology

The diabetic non-treated group showed congestion, focal thrombosis, marked disseminated infiltration of inflammatory cells while the groups treated with chloroquine (5mg/kg) and pioglitazone (25mg/kg) showed milder lesions in the hepatic tissue (Fig. 1). The renal tissue of the diabetic non-treated group exhibited inflammatory cell infiltration, congestion of vessels, areas of red cell cast, some tubules showed desquamation whereas the group

treated with chloroquine (5mg/kg) showed focal area of infiltration by inflammatory cells and the group treated with pioglitazone (25mg/kg) showed hypercellularity of the glomerular mesangial cells (Fig. 1). The diabetic non-treated group showed focal area of hemorrhagic and mild necrosis, while group treated with chloroquine (5mg/kg) showed focal area of mild myocardial infiltration by inflammatory cells and the group treated with pioglitazone (25mg/kg) showed focal area of myocardial infarction in the cardiac tissues (Fig. 2). In the pancreatic tissue, the diabetic non-treated group exhibited few and necrotic pancreatic islets, hyperplasia of acinar cells, while the groups treated with chloroquine (5 mg/kg) and pioglitazone (25mg/kg) showed milder lesions (Fig. 2). The control group showed normal morphology of the heart, kidney, liver and pancreas.

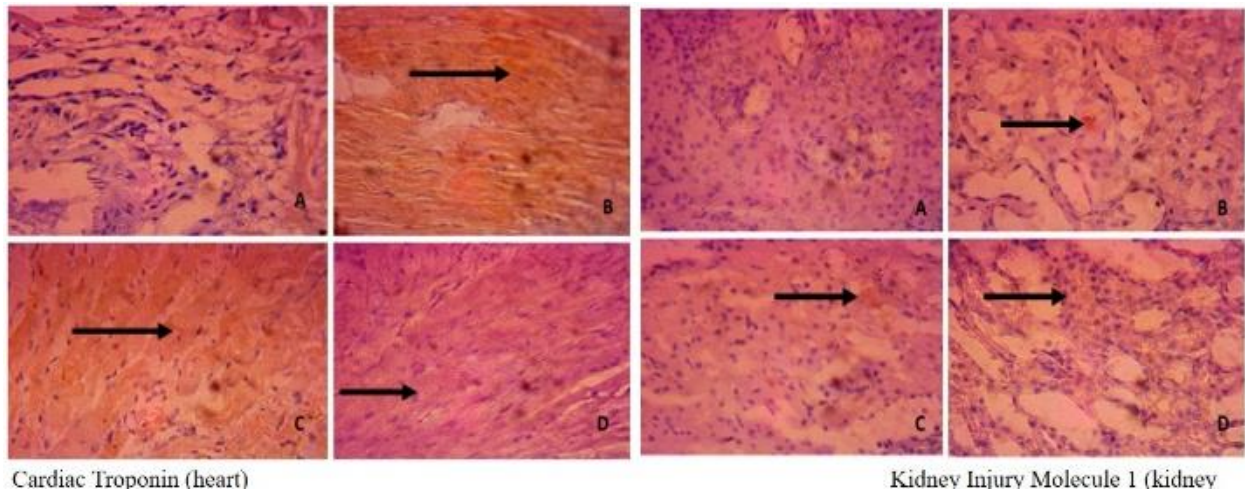


Fig. 3: Immunohistochemistry of Cardiac Troponin I (right) and Kidney Injury Marker 1 (left). A shows lower expression of CTnI than B; (B) shows higher expressions of CTnI than in C and D: C shows lower expression of B; D shows lower expressions of CTnI than Groups B and C. The result is indicative that CTnI is a biomarker of cardiomyopathy: Kidney: A shows lower expressions of Kim-1 than B. B shows higher expressions of Kim-1 than C and D: C shows lower expression of Kim-1 than in B; D shows lower expressions of Kim-1 than in Group B: A- Normal control, B- Diabetic control, C- Chloroquine treated group, D- Pioglitazone treated group. Slides were counterstained with high definition hematoxylin and viewed $\times 100$ objectives.

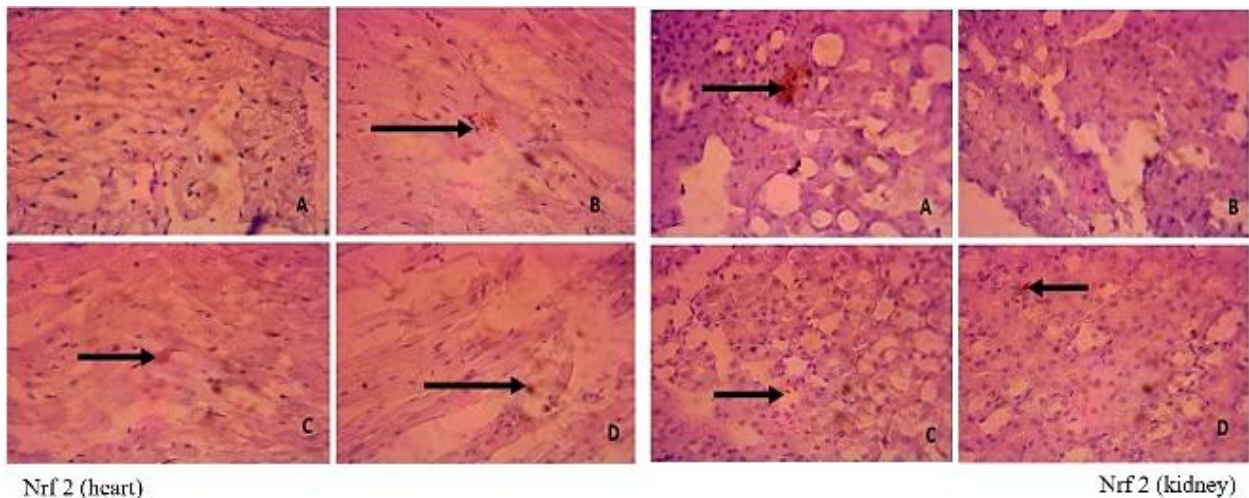


Fig. 4: Immunohistochemistry of cardiac (right) and renal (left) Nuclear factor like 2 (Nrf2). A shows lower expressions of Nrf2 than B; B shows higher expressions of Nrf2 than control, C and D: C shows lower expressions of Nrf2 than in B; D shows lower expressions of Nrf2 than in B: Kidney: (A): shows high expression of Nrf2; (B) shows lower expression of Nrf2 than control, C and D: (C) shows higher expression of Nrf2 than in B; (D) shows higher expression of Nrf2 than in B. Result is indicative that Chloroquine and Pioglitazone are nephroprotective: A- Normal control, B- Diabetic control, C- Chloroquine treated group, D- Pioglitazone treated group. Slides were counterstained with high definition hematoxylin and viewed $\times 100$ objectives.

Immunohistochemistry of CTnI, Kim-1, Nrf2 and PPAR γ

The diabetic non-treated groups showed increased expression of cardiac troponin in cardiac tissue, increased expression of cardiac Nrf2, decreased expression of cardiac PPAR γ , higher expression of renal KIM, higher expression of renal Nrf2, in kidney tissue, and lower expression of renal PPAR γ when compared with the control and groups treated with chloroquine (5mg/kg) and pioglitazone (25mg/kg) (Fig. 3-5).

DISCUSSION

The oxidative damage caused by excessive reactive oxygen and nitrogen species is a principal contributory influence to complications of diabetes mellitus. The free radicals generated during oxidative reactions of sugars to

proteins also contribute to oxidative stress. In this study, the resultant increase in blood glucose levels is thought to be due to destruction of beta cells in the islet of Langerhans (Akbarzadeh *et al.*, 2007), thus impairing the production of insulin leading to hyperglycemia. Elevated blood glucose levels upon STZ induction have been earlier reported (Nagarchi *et al.*, 2015). Hyperglycemia was significantly lower in the chloroquine and pioglitazone treated groups, although still higher than the levels recorded in the control group. Pioglitazone as a thiazolidinedione acts by improving tissue sensitivity to insulin, thereby enhancing glucose uptake (Papoushek, 2003; Mohamed and Fayed, 2020). This explains the lower glucose levels recorded upon treatment with pioglitazone as compared with the untreated diabetic group. Alteration in insulin metabolism is a major pathway in reducing hyperglycemia. Chloroquine possibly reduced hyperglycemia by inducing such alterations.

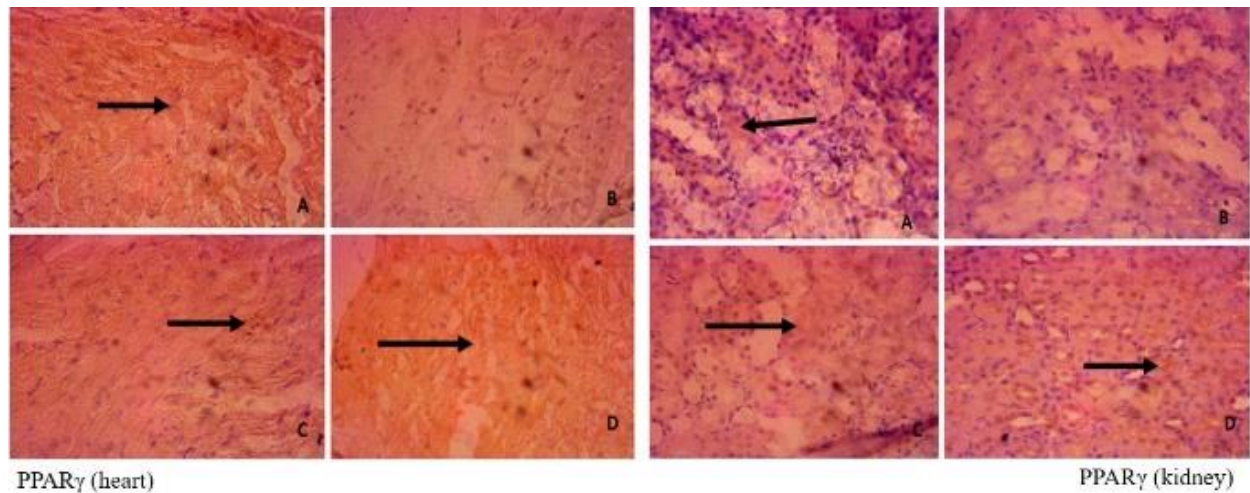


Fig. 5: Immunohistochemistry of cardiac (right) and renal (left) PPAR γ . A shows positive and high expression of PPAR γ than in B, C and D; B shows lower expression of PPAR γ than control A, C and D; C and D: C show lower expressions of PPAR γ than control and higher expression than B; D show higher expression of PPAR γ than in B and C. The result indicates that CQ and Pioglitazone have cardioprotective effect: Kidney: (A) shows positive and high expression of PPAR γ ; (B) shows lower expression of PPAR γ than control, C and D. (C) shows lower expression of PPAR γ than control and higher expression than in B. (D) shows higher expression of PPAR γ than in B and C. Result indicates that CQ and Pioglitazone are nephroprotective: A- Normal control, B- Diabetic control, C- Chloroquine treated group, D- Pioglitazone treated group. Slides were counterstained with high definition hematoxylin and viewed $\times 100$ objectives.

Diabetes promotes muscle atrophy by decreasing muscle protein synthesis thus leading to diabetic weight loss (Garnham *et al.*, 2017), the weight loss seen with STZ induction in this study was improved significantly upon treatment with chloroquine and pioglitazone.

In this study, we reported significant changes consistent with diabetic nephropathy, hepatopathy and cardiomyopathy seen with increased levels of markers of oxidative stress, organ damage, inflammation and decrease in antioxidant status. A common etiology of chronic kidney disease is diabetic nephropathy, and a mechanism is thought to be increased glomerular hyperfiltration, leading to increased glomerular pressure thus causing damage to the glomerular cells (Rahman *et al.*, 2012). This plays a role in the morphological changes seen in the kidneys in diabetes and explains the various pathological lesions such as congestion, infiltration of inflammatory cells seen in the diabetic non-treated group. Other responsible factors are oxidative stress and inflammation (Benneth and Aditya 2015). Diabetic nephropathy was characterized by increase in renal markers of oxidative stress such as advanced oxidative protein product (AOPP), malondialdehyde (MDA), protein carbonyl (PCO), hydrogen peroxide (H_2O_2) and also by depletion in the antioxidant status, confirming the role of oxidative stress and inflammation in the progression of diabetic nephropathy. Previous studies have found renal antioxidant enzymes such as catalase, GPx to be reduced in diabetes (Sadi *et al.*, 2012). Chloroquine, possibly through its anti-inflammatory pathway was able to protect against diabetic nephropathy. The cytotoxic products of lipid peroxidation such as malondialdehyde is involved in the pathogenesis of DM (Ayala *et al.*, 2014). Increased levels of hydrogen peroxide are associated with inflammation and oxidative stress. H_2O_2 upregulates endothelial NOs resulting in increased production of NO thus exacerbating inflammation. Superoxide dismutase (SOD) converts O_2^- to hydrogen peroxide, which is reduced to water by glutathione

peroxidase (GPx), therefore depletion in activities of GPx and SODs contribute to oxidative stress.

Occurrence of acute liver injury as a complication of diabetes is on the rise, and there is evidence linking liver disease and diabetes (El-Serag and Everhart, 2002). The liver is a target because of the important role it plays in glucose metabolism. Inflammation and oxidative stress are fundamental mechanisms of liver damage as there are significant involvement of pro-inflammatory cytokines such as IL-6 and Tumor necrosis factor (TNF- α) in diabetes, the imbalance in lipid and protein metabolism also promotes oxidative stress, thus exacerbating the damage to the liver (Jamaludia *et al.*, 2016). In this study, lipid peroxidation product was significantly elevated in the liver of the diabetic untreated group. There were also significant depletions in antioxidants such as GSH, SOD, and GPx. A combination of all these is probably responsible for the STZ induced liver damage.

Also, diabetes cardiomyopathy was characterized by increase in levels of cardiac markers of oxidative stress such as MDA, hydrogen peroxide with depletion in cardiac SOD, GST, GPx, and GSH. The depletion in the amount of these antioxidant enzymes makes the tissues more vulnerable to the complications associated with diabetes (Lipinski, 2001).

This study shows that serum levels of nitric oxide are increased in diabetes, presumably due to increased production of NO via the NOs pathway. Previous studies have shown that NO production is increased in diabetes (Choi *et al.*, 1999). Streptozotocin, is a nitrosourea, and is therefore a potential NO free radical donor (Goud and Dwarakanath, 2015). The nitric oxide generated is also thought to contribute to the destructive effects of STZ on beta pancreatic cells (Wada and Yagihashi, 2004). Our finding supports this report.

Xanthine oxidase forms superoxide anion and hydrogen peroxide from hypoxanthine and xanthine (Wolin 2000) suggesting that xanthine oxidase is another

source of oxidative stress in diabetes, and this explains the significant increase in XO levels recorded in this study.

Cardiac troponins (CTnI) are released when damage occur to the myocytes and are therefore considered cardiac leakage of enzymes, myocardial infarction, direct trauma to the heart and infections are cases that result in elevated levels of CTnI, and though increased levels of CTnI indicate cardiac injury, the mechanism of this injury is unknown (Wela and Sleeper, 2008). Diabetic cardiomyopathy is one of the most important complications of diabetes, the injury is detectable with increased expression of CTnI in the diabetic non-treated group while chloroquine and pioglitazone resulted in reduced expression thus suggesting that they protected against diabetic cardiomyopathy by inhibiting release of troponin by the cardiac myocytes.

Kidney injury molecule-1 (Kim-1) is a transmembrane glycoprotein that is produced in diseases associated with tubulo-interstitial injury such as diabetes; it is also upregulated following renal ischemia (El-Attar *et al.*, 2017). We thus assume that the increase in Kim-1 expression in the diabetic non-treated is due to the damage caused by STZ and that Chloroquine and pioglitazone protects against diabetic nephropathy by inhibiting expression of Kidney injury molecule.

Nrf2 plays a role in the metabolism of glucose by upregulating glycolytic enzymes and downregulating glycolytic enzymes in the liver, adipose tissue and kidney (Rajappar *et al.*, 2017), thus activation of Nrf2 therefore protects against diabetic associated complications. Chloroquine in this study activates Nrf2 and thus protects against diabetic nephropathy and cardiomyopathy. Nrf2 also regulates the expression of antioxidant enzymes; its downregulation in the diabetic untreated group could also be a contributory factor to the low levels of antioxidant defense status exhibited. Nrf2 signaling improves insulin resistance by suppressing oxidative stress.

Expression of PPAR γ is high in tissues like kidney, heart, liver and adipose tissues. Its activation improves the tissue sensitivity to insulin and glucose uptake (Pablo *et al.*, 2014). It also acts by lowering blood triglycerides. The activation of PPAR γ has previously been reported to decrease the incidence of diabetic nephropathy (Baylis *et al.*, 2003). Pioglitazone, a PPAR γ agonist was used as the reference drug in this study and this explains the increased expression in PPAR γ reported in the group treated with pioglitazone. Chloroquine also improved glucose uptake by activating PPAR γ pathway.

Conclusion

We can therefore postulate that chloroquine exhibited antidiabetic property through upregulation of PPAR γ . Its anti-inflammatory and antioxidant properties were confirmed through the upregulation of Nrf2.

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