



The Effect of *Vernonia amygdalina* on Chloroquine-induced Cardiotoxicity in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ROA designed the study, performed the analysis, interpreted the result wrote first draft of the discussion and the protocol. Author PMSA managed statistical analysis, interpreted the result and wrote first draft of the introduction, methods, worked on drafting sections of the discussion for the manuscript. Author GGA managed the literature searches and edited all portions of the manuscript. Author OOO managed discussion section and statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cardiotoxicity could result from chemotherapeutic drugs or other medications used in disease control such as antimalarial drugs.

Chloroquine (C.Q), an antimalarial agent has also been used in the treatment of rheumatoid arthritis, giardiasis, extra-intestinal amebiasis and lupus erythematosus. However, its cardiotoxic roles have been documented.

Vernonia amygdalina del. (V.A) has been reported to exhibit antioxidant and cytoprotective activities. These ameliorative and protect effects have been attributed to the presence of flavonoids. There is a paucity of data to support the cardioprotective potentials of this important nutraceutical. We aimed to evaluate the possible effects of *Vernonia amygdalina* on Chloroquine-induced cardiotoxicity in Wistar models.

Materials and Methods: Twenty-four male adult Wistar rats were randomized into four groups of six rats each: I, Control: given normal feed and water ad libitum for 28 days; II, administered 30

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mg/kg body weight chloroquine orally for 28 days; III, administered 30 mg/kg body weight of chloroquine orally for 28 days and with 400mg/kg bodyweight of *Vernonia amygdalina* for another 14 days; VI, administered *Vernonia amygdalina* 400mg/kg body weight for 28 days. Antioxidant parameters [malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD)], and histology of rat cardiac muscles were examined in the different groups.

Results: There was a significantly ($p < 0.05$) increase in MDA level, reduced GSH level, increased SOD activity, and altered microanatomy of the rat cardiac muscle in the positive control group when compared with those of the negative control group. The changes in MDA and GSH concentration and SOD activity parameters were significantly ($p < 0.05$) mitigated in rats co-treated with V.A when compared with the positive control rats. Similarly, co-administration of V.A with C.Q inhibited chloroquine induced-cardiotoxicity by reducing the altered microanatomy of the cardiac muscle of the rat.

Conclusion: It was concluded that V.A ameliorated chloroquine-induced cardiotoxicity in rats via its antioxidant property.

Keywords: Cardiotoxicity; chloroquine; *Vernonia amygdalina*; oxidative stress; cardiac muscle.

1. INTRODUCTION

Cardiotoxicity could result from chemotherapeutic drugs or other medications used in disease control such as antimalarial drugs.

Chloroquine, 7-chloro-4-(4-diethylamino-1-methylbutyl amino) quinoline, has been shown to have an antimalarial activity similar to and superior to that of quinacrine in avian malaria, and in trophozoite- and sporozoite-induced vivax and falciparum malaria. Approximately 50-70% of chloroquine in plasma is bound to plasma proteins [1]. Chloroquine, an antimalarial agent used in the treatment of rheumatoid arthritis, giardiasis, extra-intestinal amebiasis and lupus erythematosus [2,1].

Chronic administration of chloroquine may result in cardiac damage by the generation of excess free heme or reactive oxygen species to induce cardiomyocyte [3]. Yogasundaram, et al. [4] revealed that chloroquine caused defects in the structures of the heart. Chloroquine toxicity induced cardiomyopathy has been reported in the literature [5,6] (Blignaut, et al. 2019).

The quest for plants with medicinal properties has received attention as scientists are in need of plants for a wide range of biological activities such as antibiotics, antivirals, anticancers and antioxidants [7].

Vernonia amygdalina del. is commonly called bitter leaf because of its bitter taste, a member of the Asteraceae family and a small ever green shrub that grows in tropical Africa was reported to exhibit antioxidant and cytoprotective activities [8]. Ethnomedically, the leaves are consumed

either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various illnesses [9]. Phytochemical analysis of *V. amygdalina* reveals high levels of flavonoids, saponin, tannins, and alkaloids [10]. The antioxidant activity of *Vernonia amygdalina* has been attributed to the presence of flavonoids [8]. It also contains bitter sesquiterpene lactones, vernolepin, vernodalin, vernomygdin, and steroid glucosides from which its antitumor 17, 18 and antimicrobial 19 properties were derived. However, there is a paucity of data to support the cardioprotective potentials of *Vernonia amygdalina del.* This study is aimed to assess the effect of *Vernonia amygdalinadel* on the oxidative markers and histology of the cardiac muscle.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty- four adult male albino rats (100-120 g) were randomly divided into four groups of six rats. Members of each group were housed in transparent plastic cages and allowed to acclimatize to the animal house condition for at least one week prior to the experiment in 12h light-dark cycle. Rats were given free access to feed and water *ad libitum*.

2.2 Plant Extraction

Leaves of *Vernonia amygdalina del.* were obtained for further processing. The plant extraction was done according to the method of Akinola, et al. (2005). They were air-dried and grounded into powdered form. 820 g of the ground dried leaves were soaked in 70% ethanol

for 72hours filtered using Whatman filter paper lined in a funnel and concentrated using a rotary evaporator at 50°C for 3hours. The yield was allowed to dry at room temperature. The total yield from the concentrated extract was 62.99 g.

2.3 Animal Grouping

Group 1: Control rats were given normal feed and water ad libitum for 28 days.

Group 2: Rats were administered chloroquine 30mg/kg body weight orally for 28 days [3,11].

Group 3: Rats were administered chloroquine 30 mg/kg body weight orally for 28 days and with *Vernonia amygdalina* 400mg/kg body weight for [3,12,11].

Group 4: Rats were administered *Vernonia amygdalina* 400 mg/kg body weight for 28 days [12].

2.4 Experimental Animal Sacrifice

After four weeks of this experiment, the animals were fasted for 12 hours overnight and anaesthetized with the administration of 34mg/kg of ketamine and 5 mg/kg of xylazine. The animals were placed on the left recumbent position, and the limbs were held by an assistant, and anesthetic drugs were administered intra-peritoneal.

The animals were then secured in a supine position. A midline incision was made on the ventral surface of the rats extending from the neck to the groin. This exposed the thoracic and abdominal cavities. A needle was introduced into the left ventricle and with the aid of a 5mls syringe; 4mls of blood was collected into a plain sample bottle for serological analysis.

2.5 Tissue Histology

The hearts were dissected and their weights were taken. Each heart was divided into two

halves; one-half was fixed in 10% formalin and was used for histological examination and stained using hematoxylin and eosin staining technique.

2.6 Biochemical Analysis

The other half was fixed in ice-cold phosphate-buffered saline (PBS) pH 7.4 using a 1:4 tissue weight to buffer solution for biochemical analysis. Biochemical parameters analyzed were: lipid peroxidation, superoxide dismutase, and glutathione. The biochemical activities were determined according to the method of Varshney and Kale [13], Misra and Fridovich [14] and Beutler, et al. [15] respectively.

2.7 Statistical Analysis

The analysis was undertaken using Graph Pad Prism software version 5.04, 2010. The data were analysed using one way ANOVA (Analysis of Variance) followed by LSD (Least Square Deviation) post-test analysis. The confidence interval was calculated at 95% and the level of significance was set at $p < 0.05$.

3. RESULTS

3.1 Body Weight

The mean body weight for the control and the experimental treated groups on days 1, 14 and 28 are presented in Table 1.

The mean body weight when compared with the control group was significantly lower in the chloroquine only group, Chloroquine + *Vernonia amygdalina* del. group and *Vernonia amygdalina* only group on days 14 and 28 at $p < 0.05$. A significant increase was also noticed in the Chloroquine + *Vernonia amygdalina* del. group and *Vernonia amygdalina* del. group when compared with Chloroquine only group on days 14 and 28 at $p < 0.05$.

Table 1. The mean body weight for the control and the experimental treated groups on days 1, 14 and 28

Groups/Days	Day 1	Day 14	Day 28
Control	143.29±2.36	171.57±11.49	211.43±22.12
Chloroquine only	140.00±11.90	155.54±10.39 ^a	163.57±13.76 ^a
Chloroquine + <i>V. amygdalina</i>	139.29±5.35	158.13±10.61 ^{ab}	165.00±16.07 ^{ab}
<i>V. amygdalina</i> only	135.00±4.08	158.71±10.77 ^{ab}	184.29±18.35 ^{ab}

Values (n=6) are taken as mean±SD and are expressed in grams. *V. amygdalina*- *Vernonia amygdalina* del. ^acompared with control $p < 0.05$, ^b compared with chloroquine $p < 0.05$

3.2 Organ (Heart) Weight

The mean heart weight for the control and experimental treated group are presented in Table 2. The mean heart weight for the chloroquine only group, Chloroquine + *Vernonia amygdalina* del. group and *Vernonia amygdalina* del. only group showed a significant decrease when compared with control but only the *Vernonia amygdalina* del. only group is significantly higher when compared with the Chloroquine treated group.

Table 2. The mean organ (heart) weight for the control and experimental treated groups

Group/Weight	Organ weight
Control	0.77±0.038
Chloroquine only	0.58±0.052 ^a
Chloroquine + <i>V. amygdalina</i>	0.63±0.054 ^a
<i>V. amygdalina</i> only	0.63±0.053 ^{ab}

Values (n=6) are taken as mean±SD and are expressed in grams. *V. amygdalina*- *Vernonia amygdalina* del. ^acompared with control p<0.05, ^b compared with chloroquine p<0.05

3.3 Biochemical Parameters

The activity of the following antioxidant enzymes; Malondialdehyde, Glutathione and Superoxide Dismutase for the control and experimental groups are shown in the Table 3.

3.3.1 Effect of treatment on Malondialdehyde (MDA)

The Chloroquine only group and the Chloroquine + *Vernonia amygdalina* treated group showed a significant high statistical difference when compared with the control group but no statistical difference was recorded with the *Vernonia amygdalina* only group. A low significant difference was also recorded in Chloroquine + *Vernonia amygdalina* group and the *Vernonia amygdalina* only group when compared with the Chloroquine only group.

3.3.2 Effect of treatment on glutathione

The mean of the control group has a low significant difference when compared with the Chloroquine only but no significant difference with the Chloroquine + *Vernonia amygdalina* group and *Vernonia amygdalina* group at p<0.05. The Chloroquine + *Vernonia amygdalina* group and *Vernonia amygdalina* group showed a statistical difference when compared with the Chloroquine only group.

3.3.3 Effect of treatment on superoxide dismutase

The mean of the control group has a significant difference when compared with the Chloroquine only group but no difference when compared with the Chloroquine + *Vernonia amygdalina* group and *Vernonia amygdalina* only group. The Chloroquine + *Vernonia amygdalina* group and the *Vernonia amygdalina* only group also showed a significant difference when compared with the Chloroquine only group at p<0.05.

3.4 Histology

Group A showed the normal histological features of the heart of rat. Group B showed interstitial fibrosis and moderate hypertrophy of the cardiomyocytes. Group C showed mild interstitial fibrosis while Group D showed the normal histology of the rat.

4. DISCUSSION

Chloroquine, an agent used in the treatment of prophylaxis of malaria, anti-inflammatory effects in dermatological, rheumatological, connective tissue disorders [16,17] sarcoidosis and treatment of cancer [18,19] has also been reported to be cardiotoxic [20,11].

The present study revealed there is a significant high reduction in the mean body weight and heart weight of the chloroquine treated group on days 14 and 28 when compared with control. This significant reduction in mean body weight was also reported by Anis, et al. [21]. Ajiboso, [22] also recorded loss of body weight in his study. This may be due to the anorexia (withdrawal from food), withdrawal from food was noted as the main factor that affected the body weights. During starvation, glucose reserve is empty mostly in a longer period of starvation; glucose must therefore be formed from non-carbohydrate sources such as glycerol and amino acid for survival [23]. These amino acids can be gotten from protein in the diet since the animals withdrew from food; the protein in the diet is simply unavailable. Therefore, the only source of these amino acids is the breakdown of proteins in the skeletal muscle; this therefore leads to a decrease in total body mass [22].

In this study, chloroquine administration increased the malondialdehyde, superoxide dismutase and glutathione level in the cardiac muscle of the rats treated with the drug.

Malondialdehyde is a naturally occurring marker for oxidative stress and endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation whose adducts is known to exist in DNA isolated from healthy human beings. It results from lipid peroxidation of polyunsaturated fatty acids [24]. The increase in the malondialdehyde level in this study suggests that the organ has been subjected to oxidative stress. This is similar to the findings of Mohammed, et al. [11] that MDA level was significantly elevated in the group treated with chloroquine. Elevation in cardiac MDA levels

observed with respect to treatment with single agents could be attributed to cardiac injury induced by oxidative stress [25,26]. This may be due to the induction of lipid peroxidation by this drug. Chloroquine enhances the ability of heme to catalyse lipid peroxidation [24]. A slight significant difference was also noticed when the Chloroquine treated with *Vernonia amygdalina* group was compared with the Chloroquine only group. This indicates that *Vernonia amygdalina* has an ameliorative effect on chloroquine thus reducing the oxidative stress caused by the chloroquine on the heart tissue.

Table 3. The mean MDA, glutathione and superoxide dismutase in the control and experimental treated groups

Parameters/ Groups	MDA ($\mu\text{moles/mg protein}$)	Glutathione ($\mu\text{g/ml/mg protein}$)	Superoxide dismutase (unit/mg protein)
Control	0.16 \pm 0.021	0.33 \pm 0.043	38.99 \pm 3.47
CQ only	0.63 \pm 0.088 ^a	0.55 \pm 0.040 ^a	46.32 \pm 6.91 ^a
CQ + V.A	0.30 \pm 0.0026 ^{ab}	0.38 \pm 0.049 ^b	38.45 \pm 1.06 ^b
V.A only	0.13 \pm 0.028 ^b	0.29 \pm 0.039 ^b	33.97 \pm 2.73 ^b

Values (n=6) are taken as mean \pm SD and are expressed in grams. CQ- Chloroquine, V. A - *Vernonia amygdalina* del. ^acompared with control $p < 0.05$, ^b compared with chloroquine $p < 0.05$

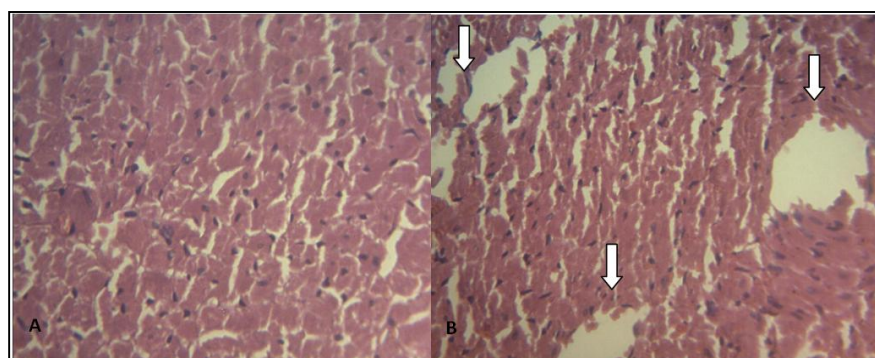


Fig. 1. Photomicrograph showing the section of the normal histological features of the heart of rat in the control group (A) and the section of the heart of a rat treated with chloroquine showing interstitial fibrosis and moderate hypertrophy of the cardiomyocytes (B). H & E X400

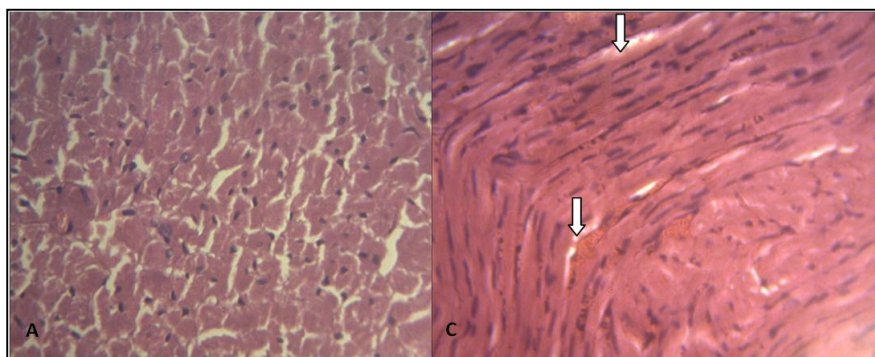


Fig. 2. Photomicrograph showing the section of the normal histological features of the heart of rat in the control group (A) and the section of the heart of rat treated with chloroquine and *Vernonia amygdalina* del. showing mild interstitial fibrosis (C). H & E X 400

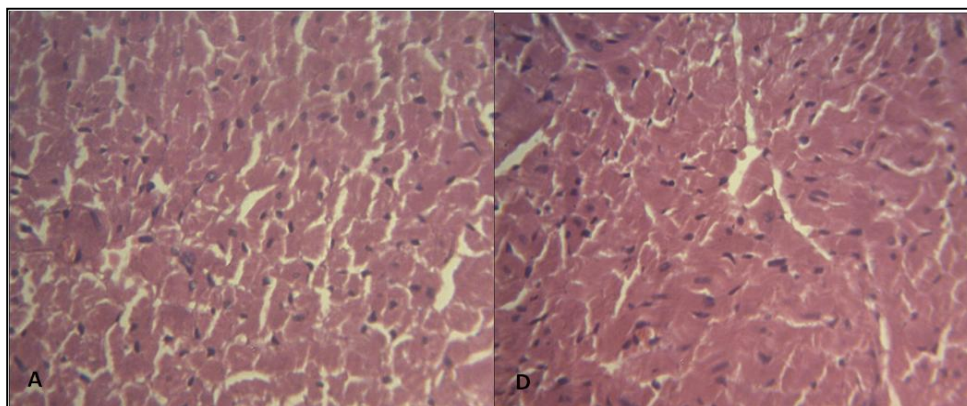


Fig. 3. Photomicrograph showing the section of the normal histological features of the heart of rat in the control group (A) and the section of the heart of rat treated with *Vernonia amygdalina* del showing the normal histology of the rat (D). H & E X400

Superoxide dismutase (SOD) accelerates the conversion of superoxide radicals to H_2O_2 . The rise in the Superoxide dismutase activity as also reported in this work suggests that a state of oxidative stress is generated in the cardiac muscle of adult Wistar rats induced with chloroquine. This also agrees with the reports of Ogunbayo, et al. [27] that increase in SOD in the blood of rabbit induced with oxidative stress using Chloroquine.

In the present study, the level of Glutathione was significantly increased as compared with the control group but there was no difference when compared with the other treatment groups. This result is contrary to findings by Mishra, et al. [28] which revealed that Glutathione level is significantly reduced in the chloroquine induced group as compared with the control. Glutathione is synthesized in the liver cells and then distributed through the circulatory system into different organs and subcellular compartments (Meister, 1983). It plays a crucial role in both scavenging ROS (Reactive Oxidative Specie) and detoxification of drugs and chemicals. GSH reacts directly with ROS and electrophilic metabolites, protecting essential thiolgroups from oxidation. Therefore, the perturbation in the redox status of GSH cannot only impair cell defence against toxic compounds but also result in enhanced oxidative stress and tissue injury.

The result of the morphological observation of the present study revealed that the administration of chloroquine caused necrosis and interstitial tissue fibrosis of cells of the heart of rats as while the control group presents the normal histology of the cardiac muscle. These

morphological findings were also similar to Izunya, et al. [3] observations. They found that chronic oral administration of chloroquine may cause cytoplasmic vacuolation, nuclear enlargement and apoptosis of cells. Mohammed, et al. [11] also confirmed that chloroquine induced marked lesions in the heart of adult Wistar rats. The lesions appeared in the form of hydropic degeneration, congestion, inflammation and necrosis. These structural changes may be caused by chloroquine induced oxidative stress, the heightened state of oxidative stress observed in the chloroquine group results in the peroxidation of the lysosome membrane leading to the instability of the lysosomes and to the accumulation of lipofuscin material and therefore further impairs lysosome and cellular function and promotes apoptosis [29,30]. A high dose of chloroquine is metabolically toxic by inducing lysosome dysfunction and by attenuating the mitochondrial buffering capacity leading to a state of heightened oxidative stress which further perpetuates lysosome and mitochondrial function and promotes apoptosis [31]. Evidences from other studies suggest the role of oxidative stress in the pathogenesis of heart dysfunction [32-35]. Furthermore, elevated ROS are implicated in the development of cardiac hypertrophy, reperfusion injury, remodelling and heart failure [36]. The mechanisms by which ROS can damage cardiac muscle are multiple and certainly involve direct toxicity by inducing both necrosis and apoptosis [37], impairing myocardial function [38] and inducing cardiac arrhythmias [39] changes were also noted but to a lesser extent with the group chloroquine group treated with *Vernonia amygdalina* del.

5. CONCLUSION

The present investigation has shown that though chloroquine may be a widely used antimalarial, antirheumatic drug and undergoing clinical trial for the treatment of solid tumours and cancer, its chronic administration may result in cardiac damage. Based on this research, the combined administration of Chloroquine and *Vernonia amygdalina* del. showed that *Vernonia amygdalina* del. has an ameliorative effect on the chronic effect of Chloroquine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research protocol was approved by the ethical committee of the University of Ibadan, Ibadan Oyo State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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