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Cardioprotective Potentials of Aqueous Extract of *Chrysophyllum Albidum* (G. Don-Holl.) Pulp on Isoproterenol-Induced Cardiotoxicity in Wistar Rats

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Abstract Cardiovascular diseases are the number one cause of death globally. A number of medicinal plants have shown their beneficial effect on cardiovascular system. The study investigated the cardioprotective potential of aqueous *C. albidium* pulp extract on isoproterenol-induced cardiotoxicity. The extract of *C. albidum* was screened for the presence of phytochemicals and the concentrations of phenolics and total flavonoids were also evaluated. Male wistar rats (30) were equally grouped into five. Group 1 rats received normal saline only, Group 2 rats received normal saline and induced with ISO, Group 3 rats were orally pretreated with propanolol and induced with ISO while Group 4 and 5 rats were orally pretreated with 100mg/kg bwt and 200mg/kg bwt *C. albidum* pulp respectively and induced with ISO. The activities of CK-MB, LDH, ALT, AST and the concentrations of lipid profile were estimated in the blood plasma of the experimental rats. Data was analyzed using appropriate descriptive and inferential statistics. The findings of the study revealed the presence of phytochemicals such as alkaloids, tannins, flavonoids, cardiac glycosides. There was a significant decrease in the activities of CK-MB, LDH, ALT and AST in the plasma of the pretreated *C. albidum* pulp extract groups. Pretreatment with the *C. albidum* pulp extract at 100mg/kg bwt and 200mg/kg bwt caused significant alterations in the lipid profile of the animals. The result of this study suggested that aqueous *C. albidum* pulp extract may serve as a potential cardioprotective agent against cardiovascular diseases.

Keywords: *cardiotoxicity, ISO (Isoproterenol), chrysophyllum albidum, antioxidants*

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1. Introduction

Cardiovascular diseases (CVD) comprise the most prevalent disorder globally whose prevalence rises progressively with age from 5% at age 20 to 75% at age above 75 years [1]. CVDs include high blood pressure, coronary heart disease, congestive heart failure, stroke which account for 17.5 million deaths per annum worldwide [2]. Growing evidence suggests that highly reactive oxygen species (ROS) and reactive nitrogen species (RNS) of endogenous or environmental origin play a significant role in the genesis and progression of various forms of CVD [3].

Agents from natural sources could serve as alternatives to currently available synthetic drugs in the management of cardiovascular-related disorders. This is important owing to the toxic side effects of most synthetic drugs and their high costs which make them not readily accessible to many patients in developing countries [4]. The implication of oxidative stress in the etiology of cardiovascular diseases suggests that antioxidant therapy represents a promising avenue for treatment [5].

Isoproterenol (ISO) is a sympathomimetic β-adrenergic receptor agonist that causes severe stress to the myocardium resulting to an infarct-like necrosis of heart muscle and produces stimulation that increases its rate and force of contraction [6]. Cardiac toxicity induced by ISO has been reported to exhibit many metabolic and morphologic aberrations in the heart tissues of experimental animals similar to those observed in human myocardial infarction [7]. Oxidative stress which has been observed in cardiovascular diseases has been reported as one of the main mechanisms through which ISO exerts its toxic effects. Auto-oxidation of ISO produces quinones, which interact with sulphydryl groups of various proteins leading to the production of superoxide anions and hydrogen peroxide [8].

C. albidum commonly known as African star apple is one of the indigenous wild fruit tree that belongs to the family Sapotaceae and has up to 800 species whose

natural occurrence is widely distributed throughout the tropical East, Central and West Africa regions particularly in Nigeria, Uganda, Niger Republic, Cameroon and cote d' ivoire [9]. In Folklore medicine, the roots of *C. albidum* are used to treat sprains, bruises and wounds in Southern Nigeria [10], inhibit microbial growth and arrest bleeding from fresh wounds [11] while in Western Nigeria, the bark is used for the treatment of malaria and yellow fever and the leaf is used as an emollient for the treatment of skin eruption, stomachache and diarrhea [10]. However, a lot of scientific findings on the cardioprotective activity of the aqueous extract from the pulp of the fruit has not been investigated, hence the present study.

2. Materials and Methods

2.1. Materials

2.1.1. Reagents and Chemicals

All chemicals used in the study were of analytical grade. Diagnostic kits for the assays of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were products of Randox Laboratory Limited, Crumlin, U.K. Lactate dehydrogenase (LDH) and Creatinine kinase- muscle/brain (CK-MB) kits were products of Agappe Diagnostics, Cham, Switzerland, Isopreterenol (ISO) was purchased from Sigma Aldrich. The drug (ISO) solution was freshly prepared in Normal saline (0.85% (w/v) NaOH) before each experiment.

2.1.2. Plant Material

Fresh *C. albidium* fruits were obtained from Owode - Ede market, Ede, Osun State, Nigeria.

2.1.3. Experimental Animals

Thirty adult male Wistar rats (*Rattus norvegicus*) with average weight 195 ± 2.60 g were obtained from Ladoke Akintola University of Technology (LAUTECH) Animal House in Ogbomoso, Oyo State, Nigeria. The animals were fed with standard rat pellet (ACE Feeds limited, Omo West Area, Osogbo, Osun State, Nigeria) and watered *ad libitum*. The animals were maintained under standard laboratory conditions of temperature (25 ± 1°C) in the Faculty of Science Animal House, Adeleke University, Ede, Osun State and were acclaimatized for 14 days. The principle of laboratory animal care (NIH publication No 85-23) guidelines were followed in this study.

2.2. Methods

2.2.1. Preparation of Aqueous *C. albidum* Pulp Extract

Fresh fruits were washed with distilled water to remove dirt thereafter the exocarp and seeds gently removed. The fleshy pulp was milled with a mechanical grinder and sieved with a double layered muslin cloth to get the juice. The juice was allowed to settle after which the solution was decanted and filtered with Whatman No. I filter paper. The filtrate was lyophilized to obtain the aqueous extract of *C. albidum* pulp which was stored at -20°C until further use.

2.2.1.1. Phytochemical Screening of Aqueous *C. albidum* Pulp Extract

The aqueous extract of *C. albidum* pulp was screened for the presence of secondary metabolites according to a standard procedure that were based on those of [12,13,14].

2.2.1.2. Thin Layer Chromatography Profile of Aqueous *C. albidum* Pulp Extract

The phytochemical analysis of the extract using thin layer chromatography was carried out using the method described by [15]. The Aluminium pre-coated plate was subjected to heat (70°C) for drying and activation. The plate was allowed to cool after which the extract was spotted on a TLC plate 2 cm above its bottom using a capillary tube. The aqueous extract of *C. albidum* pulp was subjected to thin layer chromatography using different solvent systems and observed for characteristic spots under Ultra–Violet (UV) light chamber. A solvent phase of Ethylacetate: Methanol: water (10:1.4:1) was used for the separation of the Alkaloid, Toluene: Acetone: formic acid (4.5:4.5:1.0) was used for the separation of flavonoids, Benzene: Ethylacetate (1:1) and Tannins was used for the separation of triterpenoids and Ethylacetate: formic acid: methanol (3.3:0.8:0.2) was used for the separation of Tannins respectively. The movement of the analyte was expressed by its retention factor (R*f*). Values were calculated for the sample.

$$Rf = \frac{Distance\ traveled\ by\ solute}{Distance\ travel\ by\ solvent}$$

Where- (R*f*-Retention factor).

2.2.1.3. Estimation of Phenolics Content of *C. albidum* pulp Extract

The total phenolics content in the extract was determined spectrophotometrically according to the method of [16] using tannic acid (100 µg/ml) as standard.

The standard curve was prepared by pipetting 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of tannic acid solution (100 µg/ml of tannic acid) in triplicate into clean and dried test tubes. The volume was made up to 1 ml with distilled water. Folin-Ciocalteu's phenol reagent (1:10) dilution (1.5 ml) was added to each and followed by the addition of 1.5 ml of Sodium carbonate (7.5%). The reaction mixtures were incubated for $1^1/_2$ hours at room temperature. The absorbance was read against the blank at 725 nm. The standard calibration was prepared by plotting the absorbance against the concentrations of tannic acid.

A diluted solution of aqueous *C. albidum* pulp extract (0.2 ml and 0.5 ml of 1mg/ml) in triplicate was made up to 1 ml with distilled water. The reaction mixture was treated as described for standard, tannic acid and the total phenolics concentrations were extrapolated from the standard calibration curve and expressed as mg tannic acid equivalent per gram of the extract (mg TAE/g of extract).

2.2.1.4. Estimation of Total Flavonoid Concentration

The total flavonoid content in the aqueous *C. albidum* pulp extract was determined according to the spectrophotometric method described by [17] with rutin (0.1 mg/ml) as standard.

The standard curve was prepared by pipetting 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of rutin solution (0.1 mg/ml) in triplicate into clean and dried test tubes. The volume was made up to 2 ml with distilled water. This was followed by the addition of 0.3 ml of freshly prepared 5 % (w/v) $NaNO_2$, 0.3 ml of 10 % (w/v) $AlCl_3$ and 2 ml of 4 % (w/v) NaOH. The reaction mixtures were incubated for 10 min at room temperature and absorbance was taken against the blank at 500 nm. The standard calibration curve was prepared by plotting the absorbance against rutin concentrations.

The extract (0.2 ml and 0.5ml of 1 mg/ml) in triplicate was pipetted and made up to 2 ml with distilled water in clean test tubes followed by the addition of 0.3 ml (5%) $NaNO_2$, 0.3 ml (10%) $AlCl_3$ and 2 ml (4%) NaOH. The reaction mixture was treated as described for standard, rutin and the total flavonoid concentrations were extrapolated from the standard calibration curve and expressed as mg rutin equivalent per gram of the extract (mg RE/g of extract).

2.2.2. Animal Grouping and Treatment

The experimental rats were divided into five groups of six animals each and treated as follows:

Group 1 (Normal control): Rats were fed with standard diet + normal saline

Group 2 (Cardiac control): Rats were fed with standard diet + normal saline + Isoproterenol hydrochloride (85 mg/kg).

Group 3 (Drug control): Rats were fed with standard diet + propanolol (standard drug) (1.8mg/kg bwt) + Isoproterenol hydrochloride (85 mg/kg)

Group 4: Rats were fed with Standard diet + 100 mg/kg bwt of aqueous *C. albidum* pulp extract + Isoproterenol hydrochloride (85 mg/kg)

Group 5: Rats were fed with Standard diet + 100 mg/kg bwt of aqueous *C. albidum* pulp extract + Isoproterenol hydrochloride (85 mg/kg).

Normal saline, propranolol or extract were administered orally to experimental rats, once daily for 14 consecutive days. The rats (other than Group 1) were challenged with subcutaneous dose of isoproterenol at an interval of 24 hours for 2 days (14^{th} and 15^{th}) to induce experimental cardiotoxicity. Animals were sacrificed 24 hours after the last dose of isoproterenol.

2.2.3. Sacrifice and Collection of Tissues (Blood and Heart) of Experimental Animals

The blood was collected by cardiac puncture into tubes containing EDTA. The hearts were surgically removed and immediately rinsed in normal saline (0.85% w/v NaCl) to remove blood cells.

2.2.4. Preparation of Plasma and Heart Homogenate

The blood collected was centrifuged on Bench Centrifuge Model 800D (Pathway Medicals England, U.K) at 3000 rpm for 10 min. The blood plasma (supernatant) was collected into labeled sterile bottles and stored in freezer at -20^{o}C for further biochemical analyses. The heart homogenates (10% w/v) were prepared according to the method reported by [18]. The heart (1 g) was cut into thin slices and homogenized in 10 ml of freshly prepared 100 mM phosphate buffer, pH 6.8. The homogenates were centrifuged at 3000 rpm for 10 min. The supernatants were carefully transferred into clean vials and stored frozen for further biochemical assays.

2.3. Biochemical Analyses

2.3.1. Enzyme Assays

The activities of Creatine kinase, Lactate dehydrogenase, Aspartate aminotransferase and Alanine aminotransferase were determined according to standard methods using diagnostic Kits from Agappe diagnostics, Cham, Switzrland and Randox Laboratory Limited, Crumlin, U.K respectively. The lipid profile assays: Triglycerol (TG), Total Cholesterol (TC), High Density Cholesterol (HDL-c) were also determined using diagnostic kits. The concentration of Low Density Cholesterol (LDL-c and Very Low Density Cholesterol VLDL-c in the plasma was calculated using Friedewald's equation [19] as reported by [20].

2.4. Histopathological Studies

The left ventricle of the heart was fixed in 10% (v/v) formal-saline for histological study. The fixed tissues were washed, dehydrated with alcohol and embedded in paraffin. Serial sections cut using a rotary microtome were stained with haematoxylin and eosin (H & E) [21]. The slides were examined and reviewed at the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria under light microscope (Future Winjoe Photomicroscope Camera Coupled with Olympus Binocular Microscope) and photomicrograph captured at x400.

2.5. Statistical Analysis

Data were expressed as mean ± Standard Error of Mean (SEM) and the differences between the mean values of the control and treated groups was determined by One-way Analysis of Variance (ANOVA) with a Duncan Post Hoc Test. SPSS 16 package was used for the analysis of the Data. The graphs were plotted using the Graph Pad Prism 5. Significant difference was considered when $p < 0.05$.

3. Results

3.1. Phytochemical Constituents of the Extract

Phytochemical screening of the aqueous extract revealed the presence of saponins, flavonoids, cardiac glycosides, steroids, triterpenoids and alkaloids in the *C. albidum* pulp extract.

Table 1. Phytochemical Constituents of *C. albidum* pulp extract

Phytochemical	
Saponin	+
Flavonoids	+
Tannins	-
Cardiac glycosides	+
Steroids	+
Triterpenes	+
Phylobtanins	-
Alkaloids	+
Xanthoproteins	-
Anthraquinones	-

+ means present, - means absent.

3.2. Phytochemical Analysis of *C. albidum* Pulp Extract

The thin layer chromatography (TLC) for the presence of alkaloids, flavonoids, triterpenes and tannins was carried out. The alkaloid of aqueous *C. albidum* pulp extract revealed the presence of 2 compounds having R*f* values of 0.70, 0.92 when a solvent phase of Ethylacetate: Methanol: water (10:1.4:1) was used. Flavonoid revealed the presence of 1 compound having R*f* value of 0.50 with a solvent phase of Toluene: Acetone: formic acid (4.5:4.5:1.0). Triterpenoids revealed the presence of 2 compounds having R*f* values of 0.75, 0.90 with a solvent phase of Benzene: Ethylacetate (1:1) and Tannins revealed the presence of 2 compound having R*f* values of 0.80, 0.96 when a solvent phase of Ethylacetate: formic acid: methanol (3.3:0.8:0.2) was used (Plate 1).

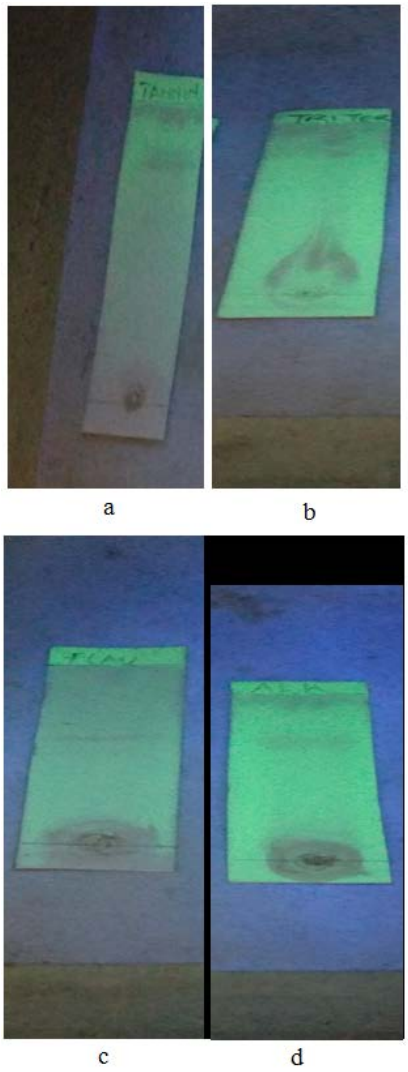

Plate 1. Separation of compound by using different solvent system for thin layer chromatography of *C. albidum* (Plate a: TLC of Tannins, Plate b: TLC of Triterpenes, Plate c: TLC of Flavonoids**,** Plate d: TLC of Alkaloids)

3.3. Total Flavonoids and Phenolics Content of *C. albidum* Pulp Extract

The concentration of total flavonoids and phenolics content of aqueous *C. albidum* pulp extract as shown in Table 2 is expressed in mg/g (RE) of Rutin standard and mg/g (TAE) of Tannic acid standard respectively. The aqueous *C. albidum* pulp extract contained 128.30 ± 3.48 RE concentration of total flavonoids and 115.03 ±2.91TAE of phenolics.

Table 2. Total Flavonoid and Phenolics Contents of Aqueous *C. albidum* Pulp Extract

Phytochemical Constituent	Concentration (mg/g)
Total phenolics	115.03 ±2.91 (TAE)
Total flavonoid	128.30 ± 3.48 (RE)

Note: Each value is expressed as mean ± SEM, n=3. TAE; Tannic acid Equivalent, RE; Rutin Equivalent.

3.4. Effect of Aqueous *C. albidum* Pulp Extract on LDH, CK-MB, ALT and AST Activities in Isoproterenol induced Cardiotoxicity

In Table 3 is the summary of the effect of aqueous *C. albidum* pulp extract on the activities of Lactate dehydrogenase (LDH) and Creatinine Kinase-myocardial band (CK-MB) on the plasma of Isoproterenol induced rats. Compared to the negative control (Group 2), the extract caused a significant reduction in LDH and CK-MB activity of the plasma in the Group 4 and Group 5 rats. The extract at 200 mg/kg bwt lowered the enzyme activity more when compared to the 100 mg/kg bwt. Furthermore, propranolol was observed to significantly lower the enzymatic activities of LDH and CK-MB when compared to the aqueous *C. albidum* pulp extract (Group 4 and Group 5) and the negative control group (Table 3).

Table 3. Effect of Aqueous *C. albidum* Pulp Extract on LDH and CK-MB Activities in Isoproterenol induced Cardiotoxicity

Group	LDH (U/L)	CKMB (U/L)
1	34.73 ± 4.92^{c}	191.22 ± 34.52^{c}
2	133.58 ± 19.23^{a}	1088.70 ± 47.40^{a}
3	61.45 ± 6.43^{bc}	491.11 ± 30.50^{b}
4	85.49 ± 3.38^{b}	582.65 ± 68.32^{b}
5	69.46 ± 3.38^{b}	548.09 ± 75.60^{b}

Each value represented Mean ± SEM (n=5). Each mean value in a row was compared with the Cardiac control with superscript 'a' and different alphabet in superscript indicates a significant difference ($p < 0.05$) between the cardiac control and a considered value.

3.5. Effect of Aqueous *C. albidum* Pulp Extract on ALT and AST Activities in Isoproterenol induced Cardiotoxicity

The aqueous *C. albidum* pulp extract significantly reduced the activity of the ALT and AST in the plasma of the rats. Also, the aqueous *C. albidum* pulp extract at 200 mg/kg bwt lowered the enzyme activity compared to 100 mg/kg bwt. Furthermore, the *C. albidum* pulp extract was observed to compare favorably with the standard drug (Propanolol) at the concentration used (Table 4).

Table 4. Effect of Aqueous *C. albidum* Pulp Extract on AST and ALT Activities in Isoproterenol induced Cardiotoxicity

Group	AST (U/L)	ALT (U/L)
1	36.99±3.69^{b}	19.24±1.11^{b}
2	64.93±1.02^{a}	42.03±1.19^{a}
3	47.71±8.47^{ab}	27.03±1.84^{ab}
4	47.14±4.95^{ab}	28.06±3.62^{ab}
5	43.57±2.81^{b}	23.00±1.13^{b}

Each value represented Mean ± SEM (n=5). Each mean value in a row was compared with the Cardiac control with superscript 'a' and different alphabet in superscript indicates a significant difference ($p < 0.05$) between the cardiac control and a considered value.

3.6. Effect of Aqueous *C. albidum* Pulp Extract on Total Cholesterol (TC) and Triglycerides (TRIG) in Isoproterenol induced Cardiotoxicity

Isoproterenol (85mg/kg) increased the levels of total cholesterol (TC), and triacylglycerol (TRIG) when compared with the normal control group while the aqueous *C. albidum* pulp extract and propanolol reduced the concentrations of TC and TRIG (Table 5).

Table 5. Effect of Aqueous *C. albidum* Pulp Extract on Total Cholesterol and Triglycerides in Isoproterenol induced Cardiotoxicity

Group	TC (mmol/L)	TRIG (mmol/L)
1	5.92±0.87^{d}	0.86±0.11^{bc}
2	15.41±1.51^{a}	0.95±0.01^{b}
3	11.11±1.86^{b}	2.23±0.13^{a}
4	10.25±1.07^{bc}	0.65±0.10^{cd}
5	6.97±0.76^{cd}	0.54±0.05^{d}

Each value represented Mean ± SEM (n=5). Each mean value in a row was compared with Group 2; a different alphabet in superscript indicates a significant difference ($p < 0.05$) between the cardiac control and a considered value.

3.7. Effect of Aqueous *C. albidum* Pulp Extract on High Density Lipoprotein (HDL-c), Low Density Lipoprotein (LDL-c) and Very Low Density Lipoprotein (VLDL-c) in Isoproterenol induced Cardiotoxicity

Isoproterenol (85mg/kg) significantly reduce the level of HDL-C and increase the level of LDL-C when compared with the normal control group. There was no significant difference in the concentration of VLDL-C when compared with the normal control group. There was no significance difference in the levels of HDL-c and LDL-c when compared when treated with aqueous *C. Albidum* extract (Table 6). However treatment with the extract at 100 mg/kg bwt significantly reduce the concentration of VLDL-c.

Table 6. Effect of Aqueous *C. albidum* Pulp Extract on HDL-c, LDL-c and VLDL-c in Isoproterenol induced Cardiotoxicity

Group	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL-C (mmol/L)
1	2.28±0.42^{b}	4.40±0.98^{c}	0.39±0.05^{b}
2	3.01±0.10^{ab}	9.65±1.84^{a}	0.53±0.05^{bc}
3	3.63±0.42^{ab}	6.75±1.81^{abc}	1.01±0.06^{a}
4	4.45±1.00^{a}	9.14±1.67^{ab}	0.43±0.01^{b}
5	2.73±0.34^{b}	4.75±0.79^{bc}	0.24±0.02^{d}

Each value represented Mean ± SEM (n=5). Each mean value in a row was compared with the Cardiac control with superscript 'a' and different alphabet in superscript indicates a significant difference ($p < 0.05$) between the cardiac control and a considered value.

3.8. Histopathological Studies of the Heart Sections

Histopathological photomicrographs of heart are shown in plates 1a-e. In the normal control group (Plate 2a) the cardiac muscle fibres are well arranged in an orderly manner indicating that the fibres are healthy with distinct nuclei and no sign of infarction is observed. In the cardiac control group (Plate 2b), severe disruption in the arrangement of the muscle fibres (an evidence of tissue inflammation, oedema and Neutrophil infiltrations, all which are evidences of myocardial infarction is observed. In the drug control group (Plate 2c), there is also severe inflammation and oedema with disruption of normal cardiac histoarchitecture and neutrophils infiltration all which are signs of myocardial infarction as well. In group 4 (animals treated with 100 mg/kg bwt of aqueous *C. albidum* pulp extract and Isoproterenol) (Plate 2d), signs of recovery from assault is observed. The cardiac muscle fibres are realigning from oedema and inflammatory reactions and few neutrophils are present. In group 5 (animals treated with 200 mg/kg of aqueous *C. albidum* pulp extract and Isoproterenol) (Plate 2e), the photomicrograph shows greater signs of recovery. The cardiac muscle fibres are better arranged and more compact, signs of oedema are almost completely absent and no signs of inflammatory reactions. The nuclei are distinct and well arranged.

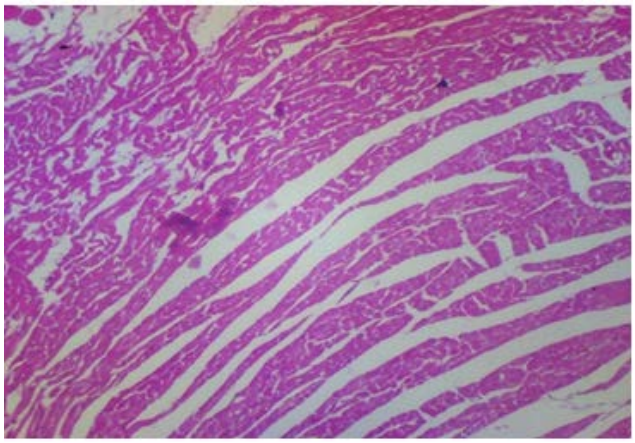

Plate 1a. Photomicroscopic Section of the Heart of Rats in Normal Control Group (X 400)

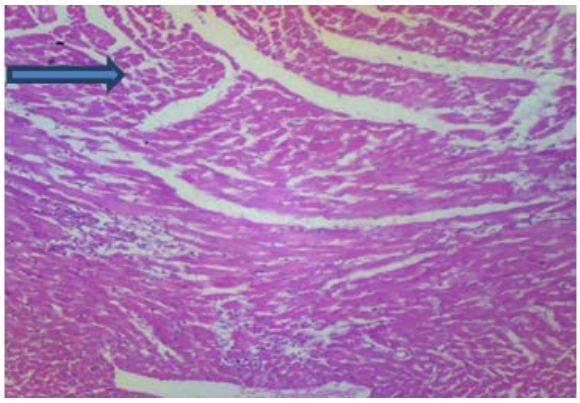

Plate 1b. Photomicroscopic Section of the Heart of Rats in Cardiac Control Group (X 400)

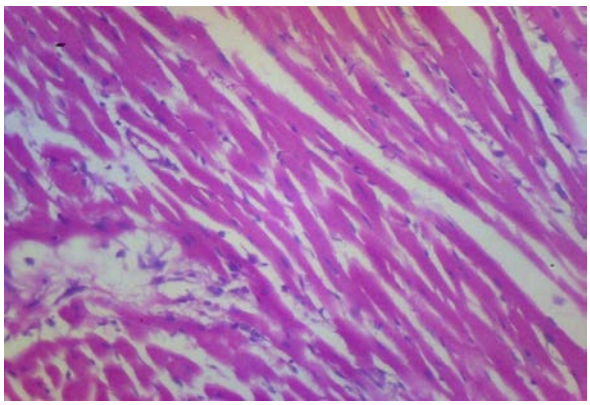

Plate 1c. Photomicroscopic Section of the Heart of Rats in Drug Control Group (X 400)

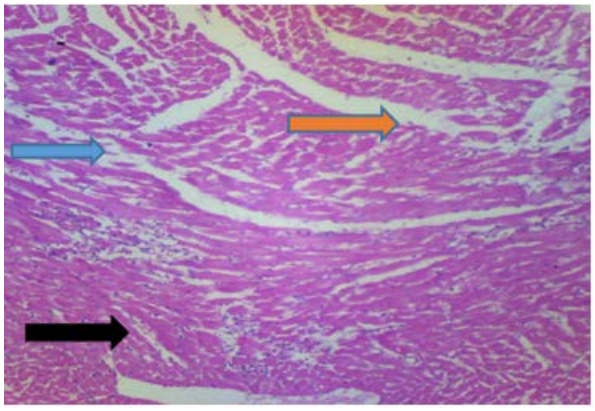

Plate 1d. Photomicroscopic Section of the Heart of Rats in (100 mg/kg bwt C. Albidiun + ISO) (X 400)

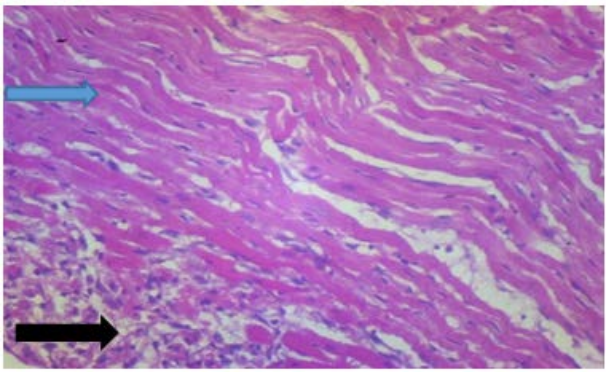

Plate 1e. Photomicroscopic Section of the Heart of Rats in (200 mg/kg bwt C. Albidiun + ISO (X 400)

Blue arrow- muscle fibre distortion; Black arrow- Oedema; Orange arrow- neutrophil infiltration. **a**-; **b**- Section of the heart of rats in Cardiac control; **c**- Section of the heart of rats in Drug control (Propanolol+ ISO); **d**- Section of the heart of rats in (100mg *C. albidum* + ISO); **e**- (200mg *C. albidum* + ISO)

4. Discussion

Despite advances in medicine, cardiovascular disease is still a public health problem with an increasing prevalence and high mortality [22]. It is becoming increasingly evident that it would be beneficial to identify high risk subjects for future cardiovascular disease check-ups. Free radicals of endogenous or environmental origin play a significant role in the genesis and progression of various forms of cardiovascular diseases. Plant derived chemicals are capable of terminating these free radical reactions preventing oxidative damage and protecting against chronic diseases such as neurodegenerative and cardiovascular disorders [23,24].

Phenolics and flavonoids are ubiquitous groups of plant metabolites that exhibit a wide range of physiological properties including anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective and vasodilatory effects [25]. It is also known for its improvement of endothelial function and cardiovascular protection [26]. Previous and preliminary studies on *C. albidum* plant revealed that the plant is rich in phenolics, flavonoids, saponins and some other phytochemicals that exhibit potent and appreciable antioxidant activities [27]. A confirmation of the presence of this phytochemicals in appreciable quantity was also carried out in this study.

Isoproterenol (ISO), a sympathomimetic β-adrenergic receptor agonist which causes severe stress to the myocardium resulting in an infarct-like necrosis of heart muscle and produces stimulation that increases its rate and force of contraction [6] was used for induction of cardiotoxicity in this study. Myocardial cells contain variety of cardiac enzymes (creatine kinase-MB and lactate dehydrogenases) which are employed as diagnostic markers of myocardial infarction [28]. Once myocardial cells are damaged or destroyed, the cardiac membrane becomes permeable or ruptures, resulting in leakage of cytosolic enzymes into the bloodstream with concomitant increases in their plasma concentrations [8,29,30]. Detection of cardiac injury could be done by determining the serum/plasma levels of known cardiac marker enzymes such as creatinine Kinase-MB and LDH. In this present study, ISO administration caused marked elevations of the plasma cardiac marker enzyme activities (CK-MB and LDH) [31,32]. However, pre-treatment of the animals with *C. albidum* extract at the two dose levels (100 and 200 mg/kg bwt) and the reference drug propanolol (1.8 mg/kg bwt) significantly lowered the ISO-induced elevation of plasma levels of the cardiac biomarkers. The 200 mg/kg bwt extract exhibited more potency than the 100 mg/kg bwt. However, the reference drug (propranolol) lowered the activity of the marker enzymes better. The levels of these cellular enzymes present in the serum are directly related to the intactness of the plasma membrane of the cardiac cells [33]. Thus,

the inhibition of ISO-induced elevation of marker enzymes in plasma (CK-MB and LDH) by the aqueous *C. albidum* pulp extract could probably attributed to its action in maintaining cardiac membrane integrity. The results of this investigation are consistent with earlier observations that administration of ISO elicited the release of cardiac markers and that cardioprotective compounds cause reduction of the altered biomarkers [30,31,32,34,35].

A recognized risk factor for developing atherosclerosis and other cardiovascular diseases is increased plasma total cholesterol level. It is often found in hypertension. It therefore follows that a reduction in plasma total cholesterol level will reduce the risk of cardiovascular diseases. Thus, the significantly lower plasma total cholesterol levels produced by the extract, connotes the ability of the extract to protect against cardiovascular diseases [36]. The result from total cholesterol and triglycerides showed a significant increase in the cardiac control group and a significant decrease in the drug group. The 100-200mg/kg of the extract in total cholesterol was also significantly decreased when compared to the normal control group. The triglycerides level of the propranolol group significantly increased and the 100-200mg/kg of the extract significantly decreased showing more effectiveness in reduction compared to the effectiveness of the drug (propranolol). This implies that the extract significantly protected the animals against the cardiotoxicity induced, even though it could not restore it to normal level.

High plasma concentrations of LDL and VLDL cholesterol is a risk factor for cardiovascular disease and is often found in diabetes mellitus, hypertension and obesity. Decreases in plasma LDL cholesterol have been considered to reduce risk of coronary heart disease [37]. In this study, a significantly lower plasma LDL and VLDL cholesterol levels in the treated animals was observed. High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues [38].

Histopathological observations on the heart showed that ISO induced myocardial necrosis with oedema, hypertrophy and separation of cardiac muscle fibres with inflammatory cell infiltration. Pre-treated rats with the aqueous *C. albidum* pulp extract showed improved cardiac muscle fibre architecture with reduced inflammatory infiltration. Treatment with the reference drug (Propanolol) showed improved cardiac histoarchitecture and cardiac muscle fibre. Similar findings were reported in ISO-induced cardiotoxicity in rats treated with *Gardenia gummifera* [30], lemon grass [39], *Piper guineense* Seeds [4] and Tanopati, a herbal formulation [31].

5. Conclusion

This study concludes that aqueous extract of *C. albidum* pulp may be considered as a cardioprotective agent through the free radical scavenging mechanism. This activity may be a function of certain phytochemical constituents known to possess antioxidant properties. However, the effect is dose dependent and further studies are suggested to characterize the bioactive metabolites and its toxicity profile as well as proper documentation of these findings.

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