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Research Article
Treatment with Methanol Extract of *Ficus capensis* Stem Bark Protects Against Changes in Biomarker Levels of Carbontetrachloride-induced Cardiotoxicity of Rats

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Abstract

Background and Objective: Ficus capensis is one of the plants used in folklore medicine in Nigeria, for the treatment of various disease conditions and promotion of vascular health. This study was undertaken to investigate its cardioprotective potential against carbontetrachloride (CCl_4)-induced myocardial necrosis in rats. **Materials and Methods:** Albino rats were divided into four main groups: normal control, CCl_4 control, 1.2 mg kg^{-1} b.wt., aspirin pre-treated and *Ficus capensis* extract pre-treated groups. Extract was administered orally at doses of 100, 200, 300 and 400 mg kg^{-1} b.wt., for 7 days to evaluate their effect on CCl_4-induced cardiotoxicity. Aspirin was used as standard drug. On day 7, cardiotoxicity was induced in animals of CCl_4 control, aspirin and extract pre-treatment groups. Histopathological examination was performed to evaluate the basic action of extract and statistical differences were assessed using one-way ANOVA and Duncan's multiple range test with SPSS version 22.0. **Results:** CCl_4 intoxication in CCl_4 treated rats caused significant ($p<0.05$) increases in serum contents of cardiac biomarker enzymes and raise in lipid peroxidation whereas significant ($p<0.05$) decreases in cardiac enzymatic antioxidant and glutathione reductase contents were observed when compared to normal control. Pre-treatment with *Ficus capensis* stem bark extract expressed cardioprotective ability against CCl_4-induced oxidative changes in myocardium by significantly ($p<0.05$) altering levels of cardiac biomarker enzymes towards normal, enhancing the activity of suppressed antioxidant enzymes and lessening lipid peroxidation level. Histopathological examination of cardiac tissues, following extract pre-treatment showed correlation with serological studies. **Conclusion:** Based on these findings, *Ficus capensis* stem bark is potent against CCl_4-induced oxidative stress in heart due to its antioxidant and cell stabilization property.

Key words: Antioxidant activity, carbon tetrachloride, cardiotoxicity, *Ficus capensis*, histopathology, myocardial infarction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular related diseases are one of the most common diseases affecting a great number of people and have been reported to be one of the leading causes of death for both men and women[1]. The development of cardiovascular disease normally involves a number of cell types, injuries, loss of cardiac membrane integrity and dysfunction of vascular endothelium[1]. The development of myocardial ischemia and infarction is a dynamic process with the widespread occurrence of coronary artherosclerosis and involvement of oxidative stress in humans[2]. The principle mechanisms of cardiotoxicity are increased oxidative stress, evident from increased levels of Reactive Oxygen Species (ROS) like superoxide anion, H_2O_2 and lipid peroxidation (LPO)[3]. Carbon tetrachloride (CCl_4) has been a well known model chemical substance used in inducing tissue toxicity by production of free radicals in many tissues such as liver, kidney, heart, lung, testis, blood and brain[4]. The free radicals generated by CCl_4 biotransformation initiate lipid peroxidation of tissues[5,6]. The CCl_4 intake is followed by transformation in the liver by cytochrome P_{450} leading to the formation of trichloromethyl free radical (CCl_3^- or CCl_300*) which could modify molecular probably lipids, leading to peroxidation of polyunsaturated fatty acids in the cell membrane[7].

However, establishing a balance between natural antioxidants and free radicals is very essential to forestall oxidative damage by toxic agents[8].

Ficus capensis is one of the numerous plants used in folklore medicine in Nigeria, locally called "Akokoro" (Igbo), "Uwaryara" (Hausa), "Opoto" (Yoruba), "Rima bichehi" (Fulani) and "Obada" (Edo). It belongs to the family Moraceae and has been considered an underutilized plant. It is commonly used as vegetable in foods with a considerable blood boosting effect[9] and possesses anti-sickling effect of red blood cells[10]. The antioxidant effect of *Ficus capensis* has been reported[11]. Other pharmacological effects of *Ficus capensis* include abortfacient[12]; anti-diarrhoea[13]; immune-stimulatory and pro-fertility in treating azoospermia[14]. The effect of *Ficus capensis* stem bark extract on myocardial antioxidant defense system in CCl_4- induced myocardial necrosis has not yet being studied. Therefore, the present study was conducted to examine the beneficial effects of *Ficus capensis* stem bark extract on heart tissue in CCl_4-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Drugs and chemicals: All drugs and chemicals used were of analytical grade.

Collection of stem bark of *Ficus capensis*: Fresh stem barks of *Ficus capensis* collected from Ozom Mgbagbu-Owa in Ezeagu Local Government of Enugu State were identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria.

Preparation of plant material: The stem barks were washed with distilled water, air-dried at room temperature and pulverized into powder for extraction. The powder (500 g) was extracted in 1.5 L of methanol and allowed to stand for 48 h at room temperature. The mixture was filtered with Whatman No. 4 filter paper and filtrate, concentrated using rotary evaporator to get a semi solid extract.

Phytochemical screening: The screening for some chemical constituents of the plant stem bark was carried out as described by Harborne[15] and Trease and Evans[16]. Quantitative analysis was carried out by method described by Harborne[17] and Soni and Sosa[18].

Assessment of cardioprotective and antioxidant properties

Animals: Twenty eight adult male albino rats, weighing 120-200 g were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Animals were kept in clean aluminum cages and placed in a well-ventilated house under standard laboratory condition of temperature at 25±2°C, relative humidity of 55±10% and light: dark cycle of 12 h photoperiod. All rats were allowed free access to rat pellet and water before and throughout the experiment. The rats were handled according to the guidelines of the National Institute of Health on the case and use of laboratory animals.

Treatment protocols: Twenty eight rats were randomly divided into seven main experimental groups (A-G), consisting of four animals each. After 1 week of acclimatization, 1.2 mg kg^{-1} b.wt., of aspirin and different doses of the extracts were orally administered to groups C and D-G, respectively while groups A and B received distilled water for 7 consecutive days.

Inducement of cardiotoxicity: Cardiotoxicity was induced according to the method described by Agbafor *et al.*[19]. Briefly, on the 7th day, 2 h after the extract or aspirin administration, groups B-G were treated with a single dose of 2.5 mL kg^{-1} b.wt., of CCl_4 intraperitoneally while group A received only distilled water.

Summary of groups and treatment:

Group A: Distilled water
Group B: 2.5 mL kg^{-1} b.wt., CCl_4 only
Group C: 1.2 mg kg^{-1} b.wt., aspirin+2.5 mL kg^{-1} b.wt., CCl_4
Group D: 100 mg kg^{-1} b.wt., extract+2.5 mL kg^{-1} b.wt., CCl_4
Group E: 200 mg kg^{-1} b.wt., extract+2.5 mL kg^{-1} b.wt., CCl_4
Group F: 300 mg kg^{-1} b.wt., extract+2.5 mL kg^{-1} b.wt., CCl_4
Group G: 400 mg kg^{-1} b.wt., extract+2.5 mL kg^{-1} b.wt., CCl_4

Collection of blood sample from experimental animals: Blood samples were collected from the experimental animals using the method described by Agbafor *et al.*[19]. Briefly, blood samples were collected from the animals following an overnight fast through cardiac puncture under mild anaesthesia using chloroform. The samples were put into specimen bottles without anticoagulant. Heart was also quickly excised, perfused with cold normal saline and homogenized in 0.25 M sucrose in phosphate buffer (0.2 M, pH 7.4).

Cardioprotective property: Cardioprotective effect of *Ficus capensis* stem bark extract was studied by determining the serum levels of Creatine Kinase (CK), lactate dehydrogenase (LDH) and aspartate transaminase (AST). The methods as contained in the respective kits (Randox kits, UK) of the parameters were adopted.

Preparation of heart homogenate: Heart tissues were homogenized in KCl (10 nM) phosphate buffer (1.15 %) with ethylene diamine tetracetic acid (EDTA, pH 7.4) and centrifuged at 12000 rpm for 20 min. Aliquot was used for biochemical estimation.

Antioxidant activity: This was evaluated in the heart homogenate by estimating the concentration of malondialdehyde (MDA), a stable product of lipid peroxidation according to the method described by Wallin *et al.*[20]. Myocardial activity of superoxide dismutase (SOD), catalase (CAT) and Glutathione Reductase (GR) were determined by the method of Fridovich[21], Aebi[22] and Goldberg and Spooner[23], respectively.

Determination of the nutrient constituents of the extract: The methods of AOAC[24] and Pearson[25] were used to determine the presence of vitamins and minerals, respectively.

Tissue preparation: Tissue sections of the heart from all the experimental groups were collected for histopathological examination. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol (60, 70, 80 and 90%). The specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 µ thickness and stained with Hematoxylin and Eosin (H and E stain) then examined under Motic™ 9.0 megapixels microscope camera (Motic Incorporation Limited, Hong Kong) at X400 magnifications[26].

Data analysis: Values were expressed as mean±standard error mean (SD) from four animals. Statistical differences were evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to detect significant differences among the mean likewise the interactions between the variables using SPSS 15.0 version. Differences were considered statistically significant at $p<0.05$[27].

RESULTS

Phytochemical constituents of the extract: The preliminary phytochemical screening of methanol extract of *Ficus capensis* stem bark revealed presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids in varied proportions (Table 1).

Nutrient compositions of *Ficus capensis* stem bark extract: The results of the nutrient concentration in *Ficus capensis* stem bark are presented in Table 2. The macroelements analyzed were calcium and magnesium. The concentration of

Table 1: Phytochemical constituents present in the extract

Phytochemical constituents (mg g^{-1})	Extract
Alkaloids	4.06±0.18
Flavonoids	4.20±0.12
Saponins	2.29±0.21
Tannins	5.93±0.40
Cardiac glycosides	3.08±0.20
Terpenoids	5.13±0.61

n: Mean±SD of triplicate determination

Table 2: Nutrients available in the extract

Nutrients	Quantity
Vitamin A (IU)	574.72±6.36
Vitamin B_1 (mg/100 g)	6.42±0.18
Vitamin B_6 (mg/100 g)	0.45±0.08
Vitamin B_9 (mg/100 g)	0.29±0.02
Vitamin C (mg/100 g)	68.34±0.47
Vitamin E (mg/100 g)	14.67±0.11
Iron (mg/100 g)	2.90±0.13
Calcium (%)	0.10±0.02
Magnesium (%)	1.12±0.04
Zinc (mg/100 g)	3.07±0.13

n: Mean±SD of triplicate determination

magnesium was higher in the stem bark than calcium. This study reports the concentrations of three microelements; vitamins (A, B_1, B_6, B_9, C and E), iron (Fe) and zinc (Zn). The result showed highest concentrations of non-enzymatic antioxidant vitamins (C and E) in the stem bark sample. Concentration of antioxidant metal ion Zn was recorded to be higher than Fe in the stem bark sample.

Effects of *Ficus capensis* stem bark extract on cardiac function tests of rats: The cardio protective activity of *Ficus capensis* stem bark extract in CCl_4-induced cardiotoxicity is shown in Table 3. Intraperitoneal administration of CCl_4 produced significant ($p<0.05$) increases in serum levels of CK, LDH and AST when compared to normal control. Pre-treatment with graded doses of *Ficus capensis* stem bark extract (100, 200, 300 and 400 mg kg^{-1}) before CCl_4 intraperitoneal administration, significantly ($p<0.05$) reduced serum concentrations of CK, LDH and AST as doses increased from 100-400 mg kg^{-1} when compared to CCl_4 toxic group. Similarly, pre-treatment with 1.2 mg kg^{-1} b.wt., aspirin also significantly ($p<0.05$) lessened the serum levels of these cardiac biomarker enzymes relative to CCl_4 control group. At highest tested dose of 400 mg kg^{-1} b.wt., extract, reduction in levels of CK, LDH and AST were significantly ($p<0.05$) greater when compared to 1.2 mg kg^{-1} b.wt., aspirin.

Effect of *Ficus capensis* stem bark extract on cardiac enzymatic antioxidant levels: The antioxidant stimulating activity of *Ficus capensis* stem bark extract is presented in Table 4. The administration of CCl_4 in rats resulted in significant ($p<0.05$) decreases in levels of cardiac CAT, SOD and GR and elevation in serum level of MDA when compared to normal control. Rats pre-treated with 1.2 mg kg^{-1} aspirin and *Ficus capensis* stem bark extract at doses of 100, 200, 300 and 400 mg kg^{-1} b.wt., showed significant ($p<0.05$) raise in levels of CAT, SOD and GR when compared with rats that received CCl_4 only. However, there was dose dependent suppression of elevated serum levels of MDA (caused by CCl_4) in rats pre-treated with different doses of extract, as well as in aspirin pre-treated groups with a significance of $p<0.05$ when compared to CCl_4 toxic group. At a dose of 400 mg kg^{-1} b.wt., the inhibitory effect of *Ficus capensis* stem bark extract on lipid peroxidation (measured by MDA serum level) was significant ($p<0.05$) when compared to 1.2 mg kg^{-1} b.wt., aspirin.

Histopathological evaluation of heart tissues: Histopathological examination of heart tissues showing degree of necrosis are shown in Fig. 1. Normal group hearts, showed normal elongated cardiomyocytes in series, with single oval to elongated pale basophilic nuclei. Group challenged with CCl_4 only, showed multifocal areas of hyaline degeneration and severe myocardial necrosis with leucocytic cellular infiltration compared to the normal group. In a dose dependent manner, extract pretreated groups (100, 200, 300 and 400 mg kg^{-1}) showed progressive normal histo-architecture of myofibrillar striations without any significant pathological changes, when compared to the

Table 3: Serum Levels of CK, LDH and AST of the animals after treatment

Groups	CK (U L^{-1})	LDH (U L^{-1})	AST (IU L^{-1})
Normal control	94.92 ± 3.49^{a}	234.18 ± 14.12^{bc}	158.0 ± 4.08^{a}
CCl_4 only	383.41 ± 7.72^{d}	300.27 ± 5.96^{d}	209.5 ± 1.77^{e}
1.2 mg kg^{-1} b.wt., aspirin+CCl_4	169.68 ± 10.33^{b}	194.77 ± 97^{a}	174.3 ± 4.06^{b}
100 mg kg^{-1} b.wt., extract+CCl_4	216.44 ± 0.71^{c}	242.64 ± 8.06^{c}	207.0 ± 1.64^{e}
200 mg kg^{-1} b.wt., extract+CCl_4	212.49 ± 8.42^{c}	222.06 ± 6.14^{b}	195.0 ± 4.65^{d}
300 mg kg^{-1} b.wt., extract+CCl_4	173.42 ± 8.80^{b}	203.14 ± 4.89^{a}	184.0 ± 1.41^{c}
400 mg kg^{-1} b.wt., extract+CCl_4	88.01 ± 8.15^{a}	198.05 ± 4.43^{a}	164.2 ± 2.38^{a}

Values are expressed in Mean±SD, n = 4. Values in the same column having different superscripts differ significantly ($p<0.05$). AST: Aspartate transaminase, CK: Creatine kinase, LDH: Lactate dehydrogenase

Table 4: Heart levels of MDA, CAT, SOD and GR of the animals

Groups	MDA (nmols g^{-1} protein)	CAT (U mg^{-1} protein)	SOD (U mg^{-1} protein)	GR (U mg^{-1} protein)
Normal control	5.05 ± 0.20^{b}	5.12 ± 0.07^{e}	10.62 ± 0.36^{cd}	28.49 ± 2.04^{d}
CCl_4 only	9.04 ± 0.10^{f}	3.41 ± 0.30^{a}	9.83 ± 0.31^{a}	19.66 ± 0.89^{a}
1.2 mg kg^{-1} b.wt., aspirin+CCl_4	5.27 ± 0.69^{b}	4.50 ± 0.13^{d}	10.61 ± 0.15^{cd}	25.33 ± 0.38^{c}
100 mg kg^{-1} b.wt., extract+CCl_4	7.88 ± 0.19^{e}	3.75 ± 0.23^{b}	10.18 ± 0.14^{b}	20.77 ± 0.23^{a}
200 mg kg^{-1} b.wt., extract+CCl_4	7.05 ± 0.04^{d}	3.99 ± 0.03^{bc}	10.33 ± 0.12^{bc}	23.48 ± 0.20^{b}
300 mg kg^{-1} b.wt., extract+CCl_4	6.51 ± 0.06^{c}	4.15 ± 0.20^{c}	10.46 ± 0.02^{bc}	24.29 ± 0.49^{bc}
400 mg kg^{-1} b.wt., extract+CCl_4	4.82 ± 0.07^{a}	4.67 ± 0.12^{d}	10.82 ± 0.07^{d}	26.52 ± 0.45^{c}

Values are Mean±SD, n = 4. Values in the same column having different superscripts differ significantly ($p<0.05$), CAT: Catalase, GR: Glutathione reductase, MDA: Malondialdehyde, SOD: Superoxide dismutase

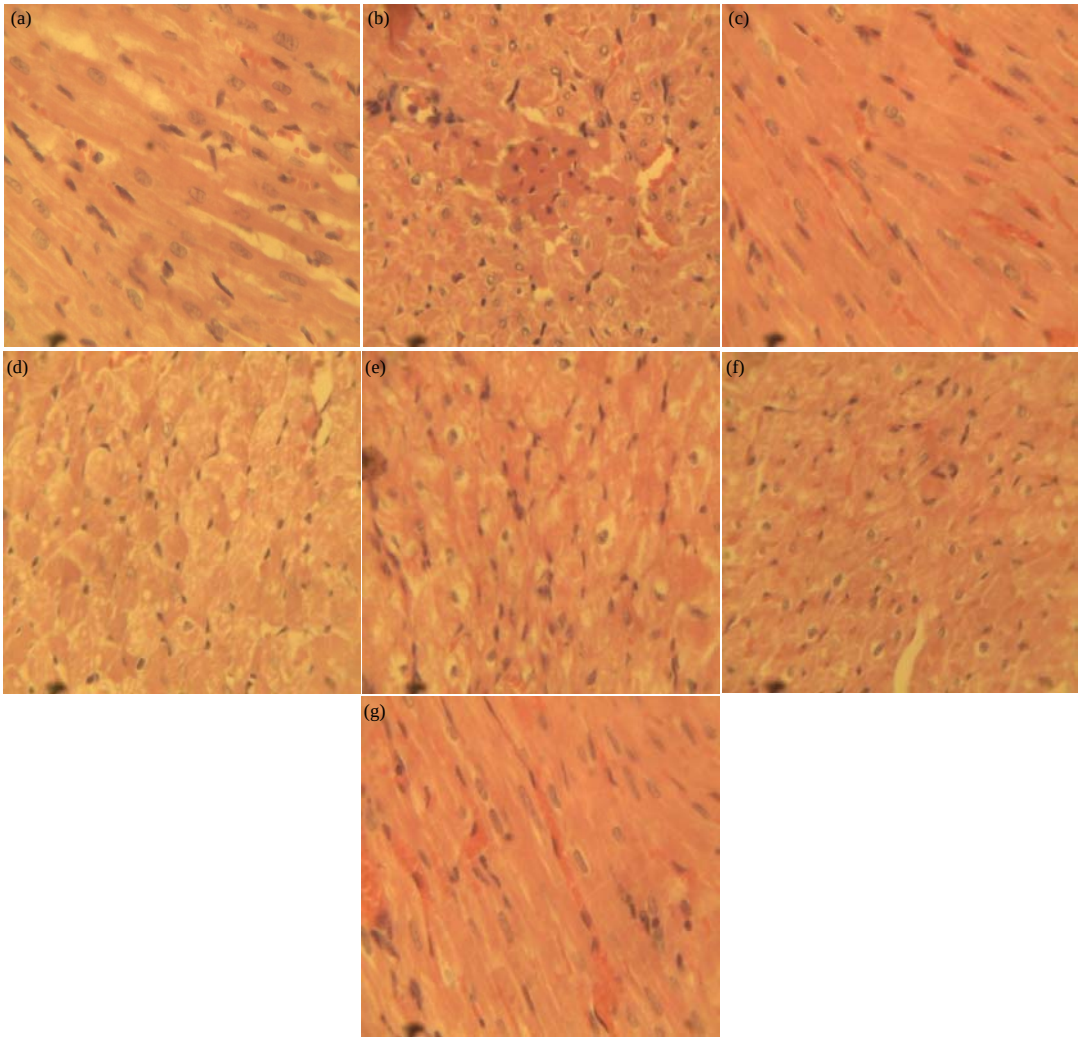

Fig. 1(a-g): Essentially similar to normal histoarchitectural structure of the heart presented in pre-treatment groups, A: Normal control, B: CCl_4 only, C: 1.2 mg kg^{-1} b.wt., aspirin+CCl_4, D: 100 mg kg^{-1} b.wt., extract+CCl_4, E: 200 mg kg^{-1} b.wt., extract +CCl_4, F: 300 mg kg^{-1} b.wt., extract+CCl_4, G: 400 mg kg^{-1} b.wt., extract

group treated with CCl_4 only. Groups treated with 400 mg kg^{-1} b.wt., extract and 1.2 mg kg^{-1} b.wt., aspirin presented features essentially similar to normal control.

DISCUSSION

This study investigated the protective effect of methanol extract of *Ficus capensis* stem bark in CCl_4-induced cardiotoxic rats. The phytochemistry of methanol extract of *Ficus capensis* stem bark revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids which complements with previous studies[28].

Carbon tetrachloride (CCl_4) is known to induce tissue toxicity and also initiate lipid peroxidation by free radicals it generates[4-6]. Since cardiac tissue has high affinity for CCl_4 due to Cytochrome P_{450}, oxidative damage to lipids and proteins of heart tissues probably occurred because of CCl_4 intoxication[29]. Damage to myocardial cells may cause decreases in the concentrations or activities of CK, AST and LDH in the heart, due to leakage of these enzymes into the extracellular fluid[3]. This leakage can be prevented by maintaining the membrane integrity of myocardium[30].

In the present study, administration of CCl_4 produced significant ($p<0.05$) increases of CK, AST and LDH levels in serum when compared to normal control, indicating possible leakage of these enzymes into the extracellular fluid due to rupture or damage of the myocardial cells containing these enzymes. Pretreatment with *Ficus capensis* stem bark extract (100, 200, 300 and 400 mg kg^{-1} b.wt.) significantly ($p<0.05$) prevented increases in serum levels of CK, AST and LDH

caused by CCl_4 toxicity in a dose related manner. Apparently, *Ficus capensis* stem bark extract protected the cell membrane from rupture or damage, thereby, inhibiting the efflux of the cardiac enzymes from mitochondria into the extracellular fluid. This suggested cardio protective effect of *Ficus capensis* stem bark extract on membrane integrity of myocardial cells. Possibly, the bioactive compounds in *Ficus capensis* stem bark extract interfered with Cyt P_{450} or chelated by-products of CCl_4 metabolism, intrinsically scavenging cardiotoxic free radicals reaction. Cardiac glycosides improve cardiac output and reduce distention of the heart[31], while vitamins B_6 and B_9 have been reported to lower the risk of developing cardiovascular disease[32]. The presence of cardiac glycosides, vitamins B_1, B_6 and B_9 in *Ficus capensis* stem bark extract explains cardio protective effect of *Ficus capensis* stem bark extract. Presence of these micronutrients in the extract was similar to the result obtained by Papp *et al*.[32]. Lipid peroxidation is enhanced during ischemia due to decreased antioxidant defence mechanism as reported by Suresh *et al*.[33] and MDA, its biomarker in serum, indicates the amount of membrane damaged by reactive oxygen species[3]. The inhibition of free radical generation is important in providing protection against myocardial infarction[7]. The scavenging effect on free radicals by various constituents of plants may be due to phenolic acids and flavonoids[34]. Vitamins as terpenoids, act as regulators of metabolism and play a protective role as antioxidants[35], same as tannins[19]. Iron and zinc are cofactors needed for synthesis and proper functioning of CAT and SOD[36].

The significant ($p<0.05$) increases in MDA concentration of heart tissues presented by CCl_4 treated groups when compared to normal, indicate the extent of peroxidation of polyunsaturated fatty acids in the cardiac cell membrane and hence, reflects the amount of membranes damaged by free radicals. This also buttresses the disruption of the integrity of the cardiac cell membrane observed earlier in this study. Pre-treatment with different doses of *Ficus capensis* stem bark extract significantly ($p<0.05$) lowered the CCl_4-induced increases of serum levels of MDA compared to toxic group. This reduction in lipid peroxidation shows a decrease in the amount of membrane damage by free radicals. This may be attributed to the presence of flavonoids present in the stem bark extract of *Ficus capensis*, which may account for the expressed significant ($p<0.05$) cardio protective effect of the extract when compared with aspirin (1.2 mg kg^{-1}).

Heart tissue is particularly susceptible to free radical damage because it contains low levels of detoxifying enzymes; SOD, CAT and GSH which play important role in prevention of cell injury induced by free radicals[3].

The SOD, CAT and GSH levels were significantly ($p<0.05$) decreased in the group of animals treated with CCl_4 only, when compared to the normal. This may be due to overwhelming of the antioxidant defense system by CCl_4 metabolites thus predisposing the cardiac tissue to increased free radical damage. Pretreatment with different doses of extract prior to CCl_4 administration significantly ($p<0.05$) reversed the CCl_4-induced decrease in activities of these antioxidant enzymes when compared to toxic group, except for glutathione reductase whose increase in activity was non significant at ($p>0.05$) at a dose of 100 mg kg^{-1} b.wt. Significant ($p<0.05$) increases in activities of endogenous antioxidant enzymes caused by aspirin (1.2 mg kg^{-1}) and extract suggest protective action on antioxidant defense system. This may be attributed to the antioxidant properties of flavonoids, saponins and phenols present in the extract. Thus, suggesting antioxidant activity of *Ficus capensis* stem bark extract. The presence of non-enzymatic antioxidants, vitamins C and E, in *Ficus capensis* stem bark extract may have complemented the activities of these enzymatic antioxidants. Further, the cofactors; Fe, Mg and Zn in the extract, needed for the synthesis and proper functioning of SOD and CAT, may have supported antioxidant potential of *Ficus capensis* stem bark extract.

Histological perturbation could be attributed to a decline in oxygen supply with paramount rise in wall-stress[2]. The ability of extract to show dose dependent reversal of myofibrillar necrosis towards normal histo-architectural structure of the heart, characterized by elongated cardiomyocytes in series with single oval to elongated pale basophilic nuclei, may indicate membrane stabilization and cytoprotective properties of *Ficus capensis* stem bark extract. Hence, might be comparatively safe to cardiomyocytes.

CONCLUSION

Ficus capensis stem bark methanol extract has shown significant protective effects against CCl_4-induced cardiotoxicity, possibly by its free radical scavenging mechanism and membrane stabilization property. This may be attributed to antioxidant property of the identified phytoconstituents in the extract. Consequently, *Ficus capensis* stem bark extract may be beneficial therapeutic agent in treatment and management of cardiac dysfunction. However, further experimentation is needed, using other cardiotoxicity models, to explore the exact mechanism of cardioprotection by *Ficus capensis* stem bark extract. Isolation and

characterization of the exact compounds responsible for the observed pharmacological effects are under investigation.

SIGNIFICANCE STATEMENTS

This study is a first attempt in the evaluation of *Ficus capensis* stem bark extract on myocardial antioxidant defense system and histopathology in carbontetrachloride-induced cardiotoxicity in an in vivo animal model. The findings highlight that *Ficus capensis* stem bark extract possibly possesses cardioprotective properties and may be a beneficial therapeutic agent in cardiac dysfunction due to its antioxidant and membrane stabilization activities. This research finding was able to further support the growing interest in the employment of natural antioxidants as a protective strategy against cardiovascular related diseases especially in the rural communities in Nigeria, where orthodox medicine are unaccessible.

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