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Research Article

Protective Property of Total Saponins and Tannins of Dialium Guineense Stem Bark in CCl_4-Induced Cardiotoxicity in Rats

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Abstract

The aim of this study was to investigate the protective property of total saponins and tannins of Dialium guineense stem bark in carbon tetrachloride (CCl_4)-induced cardiotoxicity in rats. Adult male Wistar rats (n = 25), which weighed between 170 and 190 g (mean weight = 180 ± 10 g) were divided into five groups (5 rats per group): normal control, CCl_4 control, silymarin, total saponins and total tannins groups. Except for normal control, the rats were administered a single oral dose of CCl_4 and afterwards treated with total saponins or tannins isolated from the stem bark of the medicinal plant. The rats received 150 mg/kg body weight, bwt, of total saponins or tannins orally for 28 days. Indices of cardiac function were measured. The results showed that the activity of aspartate aminotransferase (AST) as well as levels of low-density lipoprotein cholesterol (LDL-C), atherogenic index of plasma (AIP), atherogenic coefficient (AC) and cardiac risk ratio (CRR) were significantly higher in CCl_4 control group than in normal control group, but they were markedly reduced after treatment with total saponins/tannins of D. guineense stem bark ($p < 0.05$). Moreover, the phytochemicals significantly promoted the release of nitric oxide (NO) from the coronary endothelia of rats exposed to CCl4 ($p < 0.05$). Carbon tetrachloride (CCl_4) markedly disrupted the normal architecture of rat's hearts inducing fibrosis, necrosis and haemorrhage. However, treatment with total saponins/tannins of the medicinal plant led to marked regeneration of cardiomyocytes. The toxic cardiac injury induced by CCl_4 was significantly blocked by the phytochemicals.

Keywords: Cardiac function; Dialium guineens; Saponins; Tannins; Tissue histology

Introduction

The heart is a muscular organ which pumps blood through the blood vessels of the circulatory system [1]. In humans, it is located between the lungs in the middle compartment of the chest [2-4]. As a syncytium, a meshwork of cardiac muscle cells, the heart is interconnected by contiguous cytoplasmic bridges [5-7]. Heart disease is caused by a myriad of chemicals and drugs [8]. A good example of such toxic agent is CCl4 [9]. Cardiotoxic agents cause myocardial ischemic injury and damage to cardiomyocytes. The repair of damaged hearts is a huge challenge because of the limited capacity of the cardiomyocytes to regenerate post-mitotically [10]. Manifestation of cardiac injury involves biochemical, electrocardiograph, hemodynamic, and histopathological alteration of the cardiomyocytes due to the reduction of endogenous antioxidants, escape of cardiac injury marker enzymes, and lipid peroxidation [11]. Prolonged ischemia in chronic myocardial infarction (MI) ultimately leads to permanent myocardial cell injury or death [10].

Materials derived from plants have been demonstrated to be effective against a number of diseases [12]. In recent times, huge attention has been given to medicinal plants with capacity to ameliorate cardiotoxicity [13, 14]. Dialium guineense is a tall, tropical, fruit-bearing tree, belonging to the Leguminosae family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel [15]. Different parts of the medicinal plant are used against several diseases [15]. Extracts of the plant have been shown to be reservoirs of important phytochemicals [16-18]. This study investigated the protective property of total saponins and tannins of D. guineense stem bark in CCl4-induced cardiotoxicity in rats.

Method and Materials

Chemicals and reagents

Analytical grade chemicals and reagents used in this study were bought from Sigma-Aldrich Ltd. (USA).

Collection of plant material

The stem barks of D. guineense were obtained from Auchi, Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBHD330).

Plant preparation and isolation of phytochemicals

The stem barks of D. guineense were washed and shade-dried at room temperature. The plant material was then pulverized using mechanical blender. Total saponins and tannins were isolated from the plant stem bark using previously described methods [19, 20].

Experimental Rats

A total of twenty-five (25) adult male Wistar rats, which weighed between 170 and 190 g (mean weight = 180 ± 10 g) were procured from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to pelletized growers mash and clean drinking water. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for seven days. The study protocol was approved by the University of Benin Faculty of Life Sciences Ethical Committee on Animal Use.

Experimental design

The rats were divided into five groups (5 rats per group): normal control, CCl4 control, silymarin, total saponins and total tannins groups. Except for normal control, the rats were administered a single oral dose of CCl4 [21] and afterwards treated with total saponins or tannins isolated from the stem bark of the medicinal plant. The rats received 150 mg/kg bwt of total saponins or tannins orally for 28 days.

Blood and tissue sample collection and preparation

At the end of the treatment period, the rats were euthanized. Blood samples were collected from the anesthetized rats via cardiac puncture in heparinized sample bottles. Rats' hearts were also excised and used to prepare 20 % tissue homogenate. The plasma and homogenate were centrifuged at 2000 rpm for 10 min to obtain supernatants which were used for biochemical analysis.

Biochemical analyses

Activity of AST, and levels of NO and LDL-C were measured [22-24]. Atherogenic index of plasma (AIP), AC and CRR were also determined [25-26].

Histological examination of the tissues

Portions of the heart were serially sectioned and fixed in 10% formalin for 48h. The specimen was then dehydrated using varied concentrations of ethanol and cleared in three changes of xylene before embedment in paraffin. Serial sections (4µm thick) were made and stained with haematoxylin and eosin (H & E) according to standard method. Histological assessment was performed under light microscopy using an image analyzer (Image Proplus, version 3.0).

Statistical Analysis

Numerical data are expressed as mean ± SEM (n = 5). The statistical analysis was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Values of $p < 0.05$ were considered statistically significant.

Result

Effect of Total Saponins and Tannins of D. guineense Stem Bark on Relative Organ Weight

As shown in Table 1, there were no significant differences in relative organ weight among the groups ($p > 0.05$) (Table 1).

Table: Relative Organ Weights of Rats.

Group	Relative organ weight x 10^{-2}
Normal Control	3.34 ± 0.54
CCl_4 Control	3.02 ± 0.10
Silymarin	3.17 ± 0.16
T. Saponins	3.52 ± 0.24
T. Tannins	2.96 ± 0.14

Data are relative organ weights and are expressed as mean ± SEM (n = 5). Where T. Saponins and T. Tannins = total saponins and total tannins, respectively.

Effect of Total Saponins/Tannins of D. guineense Stem Bark on Cardiac Function

The activity of AST as well as levels of LDL-C, AIP, AC and CRR were significantly higher in CCl4 control group than in normal control group, but they were markedly reduced after treatment with total saponins/tannins isolated from the stem bark of D. guineense ($p < 0.05$). Moreover, the phytochemicals significantly promoted the release of NO from the coronary endothelia of rat's hearts exposed to CCl4 ($p < 0.05$). These results are shown in (Figures 1-4).

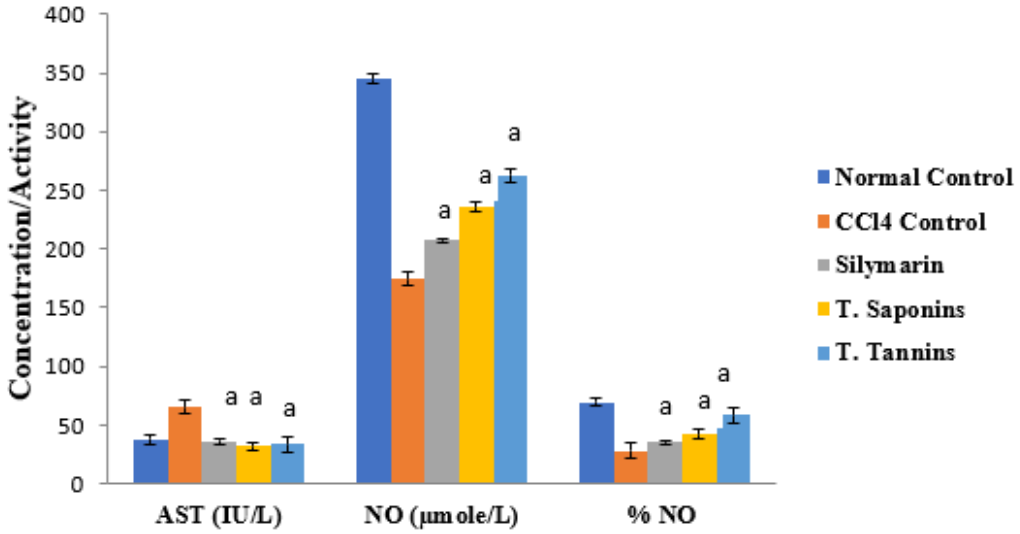

Figure 1: Effect of Total Saponins/Tannins of D. guineense Stem Bark on Markers of Cardiac Function. Data are markers of cardiac function and are expressed as mean ± SEM. ap < 0.05, when compared with CCl4 control group.

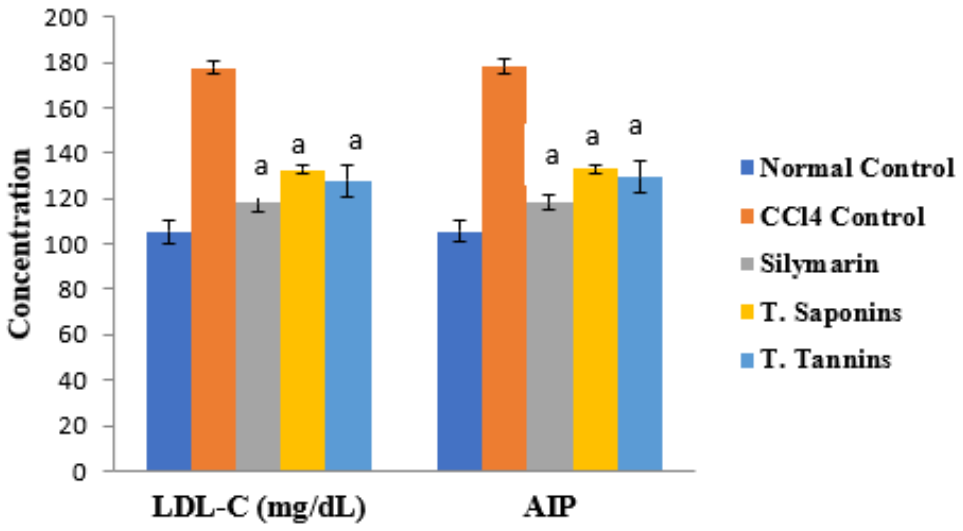

Figure 2: Comparison of the Levels of LDL-C and AIP Among the Groups. ap < 0.05, when compared with CCl4 control group.

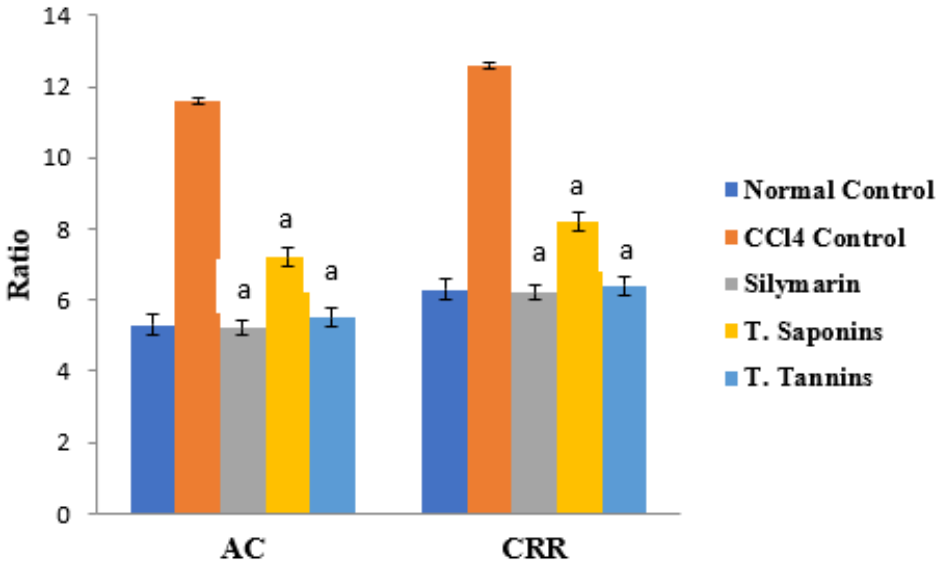

Figure 3: Comparison of AC and CRR Among the Groups. $^{a}p < 0.05$, when compared with CCl_4 control group.

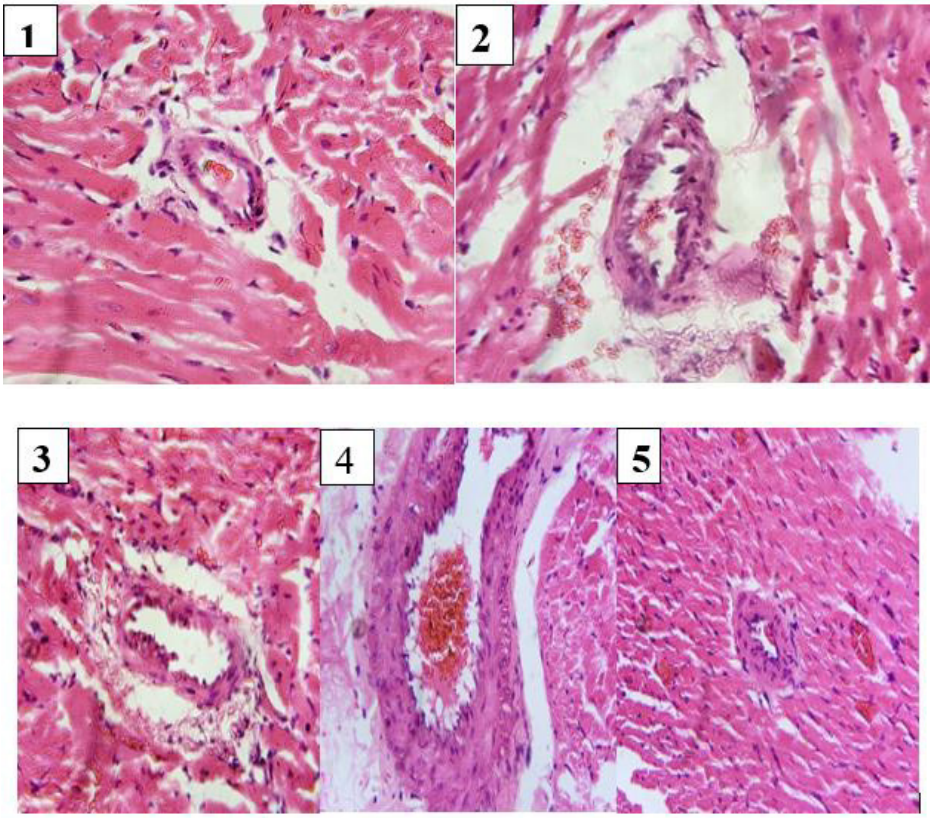

Figure 4: Effect of Total Saponins/Tannins of D. guineense Stem Bark on Histology of Rats Hearts.

Histology of normal control rat heart revealed bundles of myocardial fibres, interstitial space and coronary artery (normal cardiac architecture), while that of CCl_4 control showed congested coronary artery, mild myocyte hypertrophy and fibrosis. Interstitial inflammatory cells infiltrate, necrosis and haemorrhage were visible. Histopathological examinations of silymarin and the two treatment groups revealed markedly reduced interstitial oedema and significant reduction in cellular debris.

Discussion

The toxicity produced by CCl4 impart negatively on organs/tissues such as liver, kidneys, heart, lung, testis, brain and blood [27]. The compound is biotransformed enzymatically by liver microsomal cytochrome P450 (Cyp2E1) to trichloromethyl radical (CCl3), which in turn, starts the lipid peroxidation process [28,29]. The generally accepted mechanism by which CCl4 induces cardiotoxicity involves the generation of free radicals within cardiomyocytes [30]. The radicals and lipid peroxidation products formed result in the loss of myocardial structural integrity and reduced cardiac function [30]. This study investigated the protective property of total saponins and tannins of D. guineense stem bark in CCl4-induced cardiotoxicity in rats. The results indicated that exposure of the rats to CCl4 significantly increased their lipid profile (LDL-C and AIP) as well as cardiovascular disease risk factors, and these results are in agreement with those of previous reports [31]. The inability of the cell to package free cholesterol as high-density lipoprotein cholesterol (HDL-C) increases the amounts of cellular free cholesterol. Studies have shown that antioxidant molecules have the capacity to inhibit all these mechanisms [32]. A high LDL-C level is often linked with increased risk for heart disease and stroke. Low-density lipoprotein (LDL) transports cholesterol to arteries, and when its level is elevated, the cholesterol accumulates in blood vessel walls, thus contributing to plaque formation [33]. In this study, treatment with total saponins/tannins isolated from D. guineense stem bark markedly decreased all the parameters of cardiovascular disease risk factors. The phytochemicals produced similar effects as silymarin (standard cardioprotective drug). The cardioprotective effect of the phytochemicals may not be unconnected with their capacity to function as antioxidants [17,18, 34]. The mechanistic association of antioxidant effect with hyperlipidemic cardiomyopathy has been reported [35,36].

Histopathological assessment of rat's myocardia, valves and cardiac blood vessels provided important clues in addition to the gross examination. The various diseases of the heart are characterized by different diagnostic features. In this study, heart sections of normal control rats revealed normal structure, while rats exposed to CCl4 showed congestion, degeneration of myocardial tissue, and necrosis as a confirmation of cardiotoxicity. Reversals of such damage were observed in silymarin- and extracts-treated rats. It is likely the phytochemicals possess antithrombotic activity [37]. Platelets, platelet products and thombosis play important roles in the occurrence of acute occlusive vascular events, including myocardial infarction and ischemic stroke, since the disruption of platelet- and fibrin-rich atherosclerotic plaque may be followed by aggressive platelet deposition and, ultimately the development of a thrombus that can precipitate an acute occlusive event. Decreased platelet aggregation could be a possible mechanism for the cardioprotective effect of the phytochemicals [38].

Conclusion

The results obtained in this study suggest that total saponins and tannins of D. guineense stem bark confer some degree of protection on the heart against CCl4-induced cardiotoxicity. This investigation reaffirmed the antioxidant and antithrombotic effects of both phytochemicals.

Acknowledgement

None.

Conflicts of Interest

No conflicts of interest.

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