

## Investigation of the Cardioprotective Capacity of Aqueous Extract of *Icacina Trichanta* Leaves in Rats Exposed to CCl<sub>4</sub>

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Received November 17, 2022; Revised January 08, 2023; Accepted January 19, 2023

### ABSTRACT

**Aim:** The present study investigated the cardioprotective capacity of aqueous extract of *Icacina trichanta* leaves in rats exposed to carbon tetrachloride (CCl<sub>4</sub>).

**Materials and Methods:** A total of thirty-five (35) rats weighing 160 to 180 g (mean weight = 170 ± 10 g) were divided into seven (7) groups of five (5) rats each: normal control, CCl<sub>4</sub> control, extract control, silymarin, and 200, 300 and 400 mg/kg body weight (bwt) extract groups. Cardiac injury was induced with CCl<sub>4</sub> (1.0 mL/kg but orally, thrice weekly for two weeks). Rats in the extract control were not exposed to the toxicant, but received 400 mg extract/kg bwt throughout the period of treatment. Silymarin group rats were administered 100 mg silymarin/kg bwt (standard cardioprotective drug); while those in the three treatment groups received varied doses of aqueous extract of *Icacina trichanta* leaves (200, 300 and 400 mg/kg bwt, respectively). Treatment lasted fourteen (14) days. Indices of cardiac function, oxidative stress and lipid peroxidation were measured.

**Results:** Carbon tetrachloride (CCl<sub>4</sub>) markedly elevated the levels of total protein (TP), malondialdehyde (MDA), low-density lipoprotein cholesterol (LDL-C) as well as activity of aspartate aminotransferase (AST), but these parameters were significantly reduced by extract treatment ( $p < 0.05$ ). The activities of superoxide dismutase (SOD), catalase and glucose 6-phosphate dehydrogenase (G6PDH) reduced by CCl<sub>4</sub> intoxication, were significantly increased after treatment with aqueous extract of the medicinal plant ( $p < 0.05$ ). The extract promoted the activities of SOD and catalase in a dose-dependent manner. Similarly, atherogenic index of plasma (AIP), atherogenic coefficient (AC) and cardiac risk ratio (CRR) were markedly elevated by the cardio toxic agent CCl<sub>4</sub>, but they were reduced significantly and dose-dependently after treatment with the extract ( $p < 0.05$ ).

**Conclusion:** These results indicate that the toxic cardiac injury induced by CCl<sub>4</sub> was significantly blocked by the plant extract.

**Keywords:** Atherogenic coefficient, Cardiac function, *Icacina trichanta*, Lipid peroxidation, Oxidative status

### INTRODUCTION

Heart disease is caused by a myriad of chemicals and drugs [1]. Cardiac dysfunction caused by cardiotoxic agents such as CCl<sub>4</sub> may lead to conditions like heart failure, myocardial ischemia, arrhythmias, hypertension, myocarditis, pericarditis, and thromboembolism [2]. Chemicals produce toxic effect in the heart via different mechanisms including oxidative and nitrative stress, and protein adduct formation. The formation of protein adduct within cardiomyocytes results in inflammation, altered calcium homeostasis, apoptosis, swelling, nuclear splitting, vacuolization, and alteration in signaling pathways [3]. Characterized by the hypoxic state of myocardial tissue, myocardial infarction caused by prolonged ischemia leads to permanent

myocardial cell injury or death [4,5]. It has been suggested that the accumulation of reactive oxygen species (ROS) during ischemic damage is key in the pathogenesis of heart disease with the attendant deleterious consequences [6,7].

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**Citation:** Abu OD, Umar AB & Eiremiokhae CO. (2023) Investigation of the Cardioprotective Capacity of Aqueous Extract of *Icacina Trichanta* Leaves in Rats Exposed to CCl<sub>4</sub>. J Genet Cell Biol, 6(1): 322-328.

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Plants are at the center of Traditional Medicine. Their use in disease management is as old as man [8,9]. Medicinal plants serve as cheap alternative to orthodox medicine since they are readily available [10-12]. Indigenous to West and Central Africa, *Icacina trichanta* Olav grows in the Savanna areas of Senegal, Gambia, Guinea Bissau, Northern Ghana, Benin and Nigeria [13]. It is a perennial shrub with erect leafy shoot and broad elliptic simple, alternate leaves. In Nigeria, it is known as ibugo in Igbo and Esso gbegebe in Yoruba. Different parts of the plant are used for ethno medicinal purposes [13,14]. Till date, not much is known about the potential of extracts of *Icacina trichanta* to protect against CCl<sub>4</sub>-induced cardio toxicity in rats. This study was undertaken to investigate the cardioprotective capacity of aqueous extract of *I. trichanta* leaves in rats exposed to CCl<sub>4</sub>.

## MATERIALS AND METHODS

### Chemicals and Reagents

All reagents used in this study were of analytical grade. Total protein and AST assay kits were products of Randox Laboratories Limited (UK). All other chemicals were obtained from British Drug House (BDH) (England), Merck (Germany) and Sigma-Aldrich Ltd. (USA).

### Collection of Plant Material

The leaves of *Icacina trichanta* were obtained from a forest in Benin City, Nigeria. Its identification and authentication were carried out in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria. A sample was placed in the herbarium (No: UBHJ 0186). The leaves were then sun-dried, pulverized and sieved.

### Plant Extraction

Extraction of the pulverized plant material was by maceration over a 72 h period [15]. A portion (100 g) of the powdered leaf was soaked in 1000 mL distilled water. The resultant aqueous extract was filtered with a muslin cloth and freeze dried using a lyophilizer.

### Experimental Rats

A total of 35 rats weighing 160 to 180 g (mean weight = 170 ± 10 g) were obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: temperature of 25°C, 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 one week. Standard experimental protocol was followed for this study.

## Experimental Design

The rats were divided into 7 groups of 5 rats each: normal control, CCl<sub>4</sub> control, extract control, silymarin, and 200, 300 and 400 mg/kg bwt extract groups. Cardiac injury was induced with CCl<sub>4</sub> (1.0 mL/kg bwt orally, thrice weekly for two weeks) [16]. Rats in the extract control were not exposed to the toxicant, but received 400 mg extract/kg bwt throughout the period of treatment. Silymarin group rats were administered 100 mg silymarin/kg bwt, while those in the three treatment groups received varied doses of the extract (200, 300 and 400 mg/kg bwt, respectively). Treatment lasted 14 days.

## Blood and Tissue Sample Collection

At the end of the treatment period, the rats were euthanized and blood samples were collected via cardiac puncture in heparinized sample bottles. The hearts of all experimental rats were harvested, washed in ice-cold saline, blotted dry and placed in plain containers. Weighted portions of the organ were used to prepare 20 % tissue homogenate. The blood and tissue homogenate were subsequently centrifuged at 2000 rpm for 10 min to obtain plasma and supernatant, which were used for biochemical analysis.

## Biochemical Analysis

The activities of AST, catalase and SOD were measured [17-19]. Atherogenic index of plasma (AIP), LDL-C, AC, and CRR were determined [20-22]. The levels of TP and MDA were also measured [23,24]. Glucose 6-phosphate dehydrogenase (G6PDH) activity was measured as the rate of conversion of NADP<sup>+</sup> to NADPH with time in minutes.

## Statistical Analysis

Data are expressed as mean ± SEM (n = 5). Statistical analysis was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Statistical significance was assumed at p < 0.05.

## RESULTS

### Effect of Aqueous Extract of *I. trichanta* Leaves on Weight Parameters

Induction of cardiac injury with CCl<sub>4</sub> significantly reduced the body weight gained, but it increased the weight of the heart and relative organ weight (p < 0.05). However, treatment of the rats with aqueous extract of *I. trichanta* leaves led to significant improvement in the weight gained, while reducing the heart weight and relative organ weight (p < 0.05). The effect of the extract on relative organ weight was dose-dependent (**Table 1**).

**Table 1.** Comparison of weight parameters among the groups.

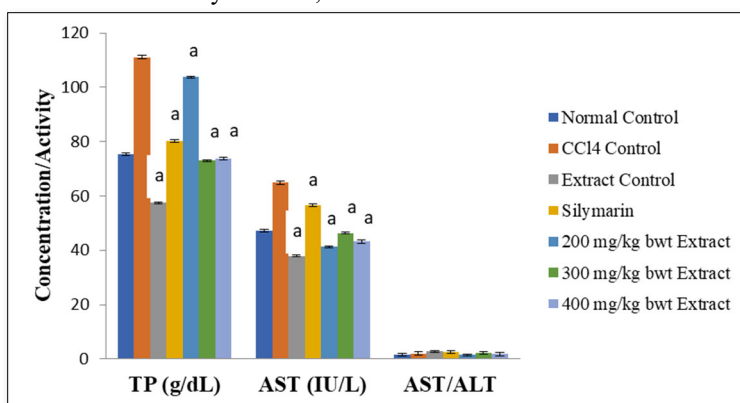
Group	Mean body weight gained (g)	% Body weight gained	Heart weight (g)	Relative organ weight (x 10 <sup>-2</sup> )
Normal Control	37.46 ± 2.78	34.12 ± 3.84	0.49 ± 0.03	1.31 ± 0.11
CCl <sub>4</sub> Control	18.01 ± 2.81	10.94 ± 1.59	0.63 ± 0.05 <sup>b</sup>	3.50 ± 0.07
Extract Control	48.74 ± 5.25 <sup>a</sup>	35.97 ± 3.07 <sup>a</sup>	0.64 ± 0.03 <sup>b</sup>	1.31 ± 0.04 <sup>a</sup>
Silymarin	23.28 ± 7.61 <sup>a</sup>	17.28 ± 2.11 <sup>a</sup>	0.61 ± 0.03 <sup>b</sup>	2.62 ± 0.13 <sup>a</sup>
200 mg/kg bwt Extract	26.13 ± 9.06 <sup>a</sup>	17.00 ± 2.92 <sup>a</sup>	0.63 ± 0.08 <sup>b</sup>	2.41 ± 0.18 <sup>a</sup>
300 mg/kg bwt Extract	28.14 ± 5.49 <sup>a</sup>	19.95 ± 1.58 <sup>a</sup>	0.61 ± 0.06 <sup>b</sup>	2.17 ± 0.10 <sup>a</sup>
400 mg/kg bwt Extract	28.57 ± 6.35 <sup>a</sup>	20.49 ± 2.19 <sup>a</sup>	0.59 ± 0.03 <sup>b</sup>	2.07 ± 0.16 <sup>a</sup>

Data are weight parameters and are expressed as mean ± SEM (n = 5). <sup>a</sup>p < 0.05, when compared with CCl<sub>4</sub> control; <sup>b</sup>p < 0.05, when compared with normal control group

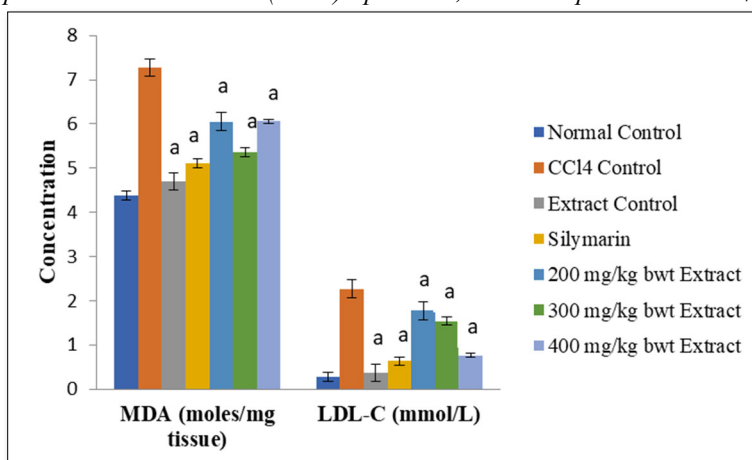
**Effect of Aqueous Extract of *I. trichanta* Leaves on Cardiac Function and Lipid Peroxidation Index**

As shown in **Figures 1 & 2**, CCl<sub>4</sub> markedly elevated the levels of TP, MDA, LDL-C as well as activity of AST, but

these parameters were significantly reduced by extract treatment (p < 0.05). The effect of the extract on LDL-C level followed a dose-dependent pattern.



**Figure 1.** Comparison of the Effect of Aqueous Extract of *I. trichanta* Leaves on Cardiac Function. Data are expressed as mean ± SEM (n = 5). <sup>a</sup>p < 0.05, when compared with CCl<sub>4</sub> control group

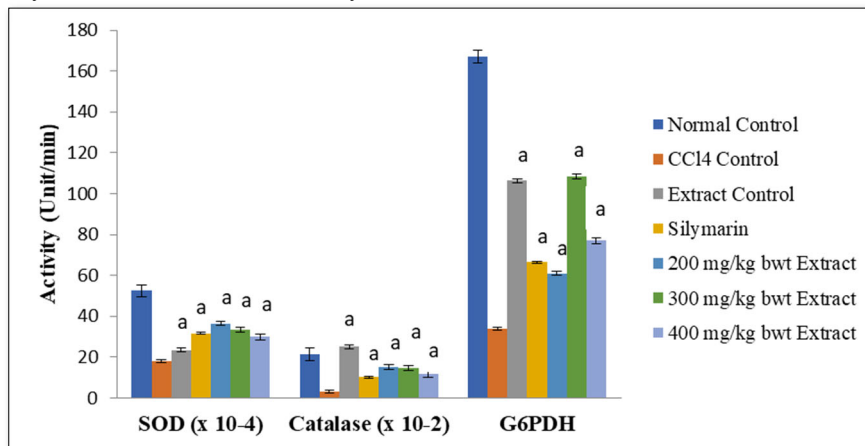


**Figure 2.** Comparison of the Effect of Aqueous Extract of *I. trichanta* Leaves on Lipid. Data are expressed as mean ± SEM (n = 5). <sup>a</sup>p < 0.05, when compared with CCl<sub>4</sub> control group

**Effect of Aqueous Extract of *I. trichanta* Leaves on Cardiac Oxidative Status**

The activities of SOD, catalase and G6PDH were significantly reduced by CCl<sub>4</sub> intoxication, but they were

increased after treatment with aqueous extract of the medicinal plant ( $p < 0.05$ ) (Figure 3). The extract promoted the activities of SOD and catalase in a dose-dependent manner.

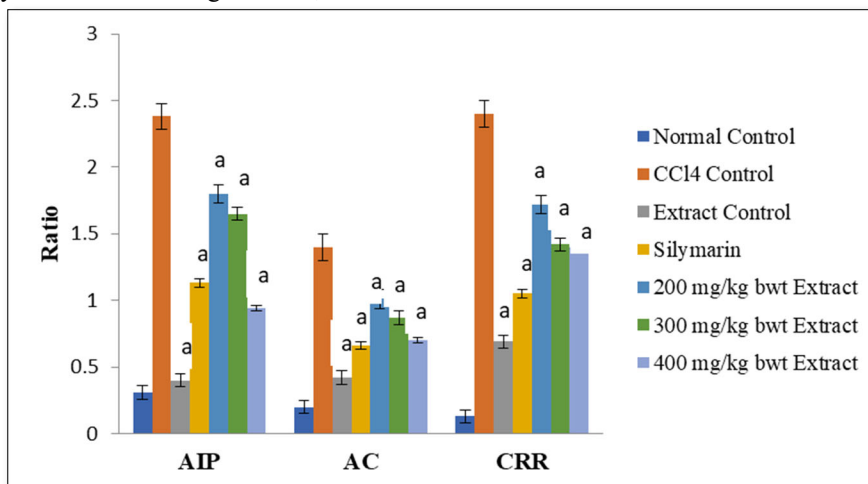


**Figure 3.** Effect of Aqueous Extract of *I. trichanta* Leaves on the Activities of Cardiac Antioxidant. Data are expressed as mean ± SEM (n = 5). <sup>a</sup>p < 0.05, when compared with CCl<sub>4</sub> control group

**Effect of Aqueous Extract of *I. trichanta* Leaves on Cardiovascular Disease Factors**

Atherogenic index of plasma (AIP), AC and CRR were markedly elevated by the cardiotoxic agent CCl<sub>4</sub>, but these

parameters were reduced significantly and dose-dependently after treatment with the extract ( $p < 0.05$ ). These results are shown in Figure 4.



**Figure 4.** Comparison of Cardiovascular Disease Factors among the Groups. Data are expressed as mean ± SEM (n = 5). <sup>a</sup>p < 0.05, when compared with CCl<sub>4</sub> control group

**DISCUSSION**

Cardiotoxicity induced by drugs poses a serious risk to human health [25]. Carbon tetrachloride (CCl<sub>4</sub>) is highly toxic to organs/tissues such as liver, kidneys, heart, lung, testis, brain and blood [26]. Once absorbed, it is widely distributed among tissues, especially those with high lipid content. The compound is biotransformed by hepatic

microsomal cytochrome P450 (CYP2E1) to trichloromethyl radical, which initiates lipid peroxidation [27,28]. Free radicals formation, a rate limiting process in tissue peroxidative damage, is the generally accepted mechanism of CCl<sub>4</sub>-induced cardiotoxicity [29].

Reactive oxygen species (ROS) and oxidative stress have been shown to play an important role in the etiopathogenesis

of tissue injury. The role of oxidative stress in cardiac hypertrophy and remodeling has been demonstrated. In atherogenesis, increased generation of ROS in the vascular wall and a reduction in the bioavailability of nitric oxide (NO) lead to endothelial dysfunction [30,31].

Bioactive agents from natural sources have gained wide acceptability in modern medicine, as they are known to reduce the risk of cardiac ailments by either scavenging free radicals or halting their formation [32]. Most of the pharmacologically important drugs are derived from plants. Plant derivatives used as drugs play crucial role in health-care systems around the globe. They are not only used for the management of disease conditions but also for maintenance of proper health [33]. The aim of this study was to investigate the cardioprotective capacity of aqueous extract of *Icacina trichanta* leaves in rats exposed to CCl<sub>4</sub>.

Aspartate aminotransferase (AST) is a marker of cardiac function and an increase in its activity reflects the functional state of the heart. Damage to the cardiomyocytes membrane (increased permeability of the plasma membrane) results in the leakage of this enzyme into systemic circulation. Although hepatocytes also express AST, the use of AST/ALT ratio gives an indication of the source of the enzyme. Increased total protein level is seen in some disease conditions [34]. In this study, CCl<sub>4</sub> markedly elevated the levels of total protein, MDA, LDL-C as well as activity of AST, but these parameters were significantly reduced by extract treatment. An elevated LDL-C level is associated with increased risk for heart disease and stroke. Low-density lipoprotein (LDL) transports cholesterol to the arteries, and when its level is elevated, this cholesterol accumulates in blood vessel walls and contributes to the formation of plaque [35].

Superoxide dismutase (SOD) detoxifies superoxide anion (O<sup>2•-</sup>) which otherwise damage cell membrane and macromolecules [36]. Catalase plays an important role in antioxidant defense system. In animals, hydrogen peroxide is detoxified by catalase and glutathione peroxidase (GPx). Catalase protects cells from hydrogen peroxide generated within them [37].

Malondialdehyde (MDA), a commonly used biomarker of lipid peroxidation, is a breakdown product of lipid peroxyl radicals. Measured level of MDA is considered a direct index of oxidative injuries associated with lipid peroxidation [38].

In this study, G6PDH assay was employed as an indirect way of assessing the level of glutathione (reduced), since the reaction catalyzed by G6PDH in the erythrocyte membrane and cells of other sensitive tissues provides the coenzyme, NADPH which furnishes the hydride ion (or hydrogen) needed to keep or maintain glutathione in the reduced state, where it is active as a free radical scavenger. The results obtained in this study showed that the activities of SOD,

catalase and G6PDH reduced by CCl<sub>4</sub> intoxication, were significantly increased after treatment with aqueous extract of the medicinal plant. The extract promoted the activities of SOD and catalase in a dose-dependent manner. Treatment with aqueous extract of *I. trichanta* leaves significantly decreased all the cardiovascular disease risk factors, measured in this study. The effects were comparable to those of the standard cardioprotective drug, silymarin. The protective property of the extract may be attributed to its phytochemicals which act as antioxidants [10,16]. Plants rich in phenolics are reported to function as good antioxidants [39-41]. Similarly, plants containing important phytochemicals have been shown to confer protection on vital organs in animals [42-47].

## CONCLUSION

The results of this study have shown that *I. trichanta* is cardioprotective: aqueous extract of the plant leaves significantly reversed CCl<sub>4</sub>-induced cardiac injury in rats, and it compares favorably with silymarin. The effects of CCl<sub>4</sub> on rat heart were ameliorated on administration of graded doses of the extract.

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