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CARDIOTOXICITY OF ETHANOL EXTRACT OF *DIALIUM GUINEENSE* STEM BARK IN RATS

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ABSTRACT

Aim: To investigate the cardiotoxicity of ethanol extract *of Dialium guineense* stem bark in rats. **Materials and Methods:** Wistar rats (n = 35) weighing 160 to 180 g were randomly assigned to seven groups (5 rats per group). One group served as control, while rats in the other groups were administered graded doses of the extract (200 - 5000 mg/kg body weight, bwt) for 28 days. Indices of cardiac function were measured. **Results:** Percentage increases in body weights of rats treated with ethanol extract of *D. guineense* stem bark were significantly reduced, relative to the control group ($p < 0.05$), but there were no significant differences in the relative heart weights among the groups ($p > 0.05$). Treatment with the extract did not elicit any significant differences in the activities of lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) as well as cardiac malondialdehyde (MDA) level among the groups ($p > 0.05$). Similarly, the basal activities of the measured indices of cardiac function were not significantly different from the values after treatment ($p > 0.05$). Moreover, the extract did not significantly alter the normal architecture of rat heart. **Conclusion:** Ethanol extract of *D. guineense* stem bark is not toxic to the heart and could be included in herbal medicine for the treatment of diseases.

KEYWORDS: Cardiotoxicity, *Dialium guineense,* Lactate dehydrogenase, Malondialdehyde, Tissue histology.

INTRODUCTION

An important goal in drug development is to determine whether drug candidates have toxic effects that could prevent their clinical use. Cardiotoxicity, defined as the toxicity that affects the heart, is of special interest.[1, 2] Cardiotoxicity continues to top safety concerns principally because of lack of sufficient knowledge of the underlying mechanisms.[3] Cardiovascular adverse effects can lead to cardiac arrhythmias.[4] Evaluation of drug-induced cardiotoxicity risk is considered a crucial component of standard preclinical assessment of new chemical entities.[5] Studies have shown that standard preclinical models do not faithfully recapitulate some important aspects of human physiology, including cardiac electrophysiology. Increased risk of ventricular arrhythmia has been involved in 28 % of drug withdrawals from the market, an indication that drug candidates that are apparently safe in preclinical models could prove to be unsafe when used by patients.[2, 3]

It has been asserted that a large population of people in developing countries use herbal medicine for some aspects of their basic health care.[6, 7] Medicinal plants have proven health benefits.[8-12] *Dialium guineense* (Velvet Tamarind) is a medicinal plant used in Traditional Medicine for the treatment of diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids.[13] It 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.[14] The plant grows naturally in West African countries, Central African Republic, and Sudan.[14] In Nigeria, it is known by different names: *Icheku* (Igbo), *Awin* (Yoruba), *Tsamiyarkurm* (Hausa) and *Amughen* (Bini).[15] Extracts of the plant are reported to be rich in important phytochemicals.[16, 17] At present little or nothing is known about the adverse effect of extracts of *D. guineense* stem bark on cardiac function. The aim of this study was to investigate the cardiotoxicity of ethanol extract of *D. guineense* stem bark in Wistar rats.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade and they were purchased 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. UBH_D330).

Plant Preparation and Extraction

The stem bark was washed and shade-dried at room temperature for two weeks and ground into powder with a mechanical blender. Exactly 500 g of the powdered stem bark was soaked in 5000 mL absolute ethanol. The resultant extract was then filtered with a muslin cloth and freeze dried using a lyophilizer.[17]

Experimental Rats

Wistar rats (n = 35) weighing 160 – 180 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 ^oC, 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. The rats were acclimatized to the laboratory environment for one week prior to commencement of the study. Standard experimental protocol was followed for this study.

Experimental Design

The rats were randomly assigned to 7 groups (5 rats per group). One group served as control, while rats in the other groups received varied doses of the extract (200 - 5000 mg/kg bwt) for 28 days. Blood samples were collected before treatment and served as basal samples. At the end of the 28th day the rats were fasted overnight and euthanized. Blood sample collected in heparin containers was centrifuged at 3000 rpm for 10 min to obtain plasma which was used for biochemical analysis.

Cardiac Function Tests

Cardiac function tests (CFTs) such as AST, CK and LDH were performed in plasma.[18-20]

Measurement of Lipid Peroxidation in Rat Heart

Malondialdehyde (MDA) level was measured in heart homogenate.[21]

Histological Examination of Rat Heart

Portions of the heart were sectioned and fixed in 10 % formalin for 48 h, and thereafter dehydrated using graded concentrations of ethanol. Just before embedment in paraffin, the specimens were cleared three times with xylene. Serial sections (4 µm thick) were stained with haematoxylin and eosin (H & E) according to standard protocol. Histopathological examination was performed under light microscopy. In each H and E section, exactly 25 circular tubules were measured in two axes drawn perpendicular to each other with the aid of an image analyzer (Image Proplus, version 3.0).

Statistical Analysis

Numerical data are expressed as mean ± standard error of 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 Ethanol Extract of *D. guineense* Stem Bark on Weight Parameters

As shown in Table 1, percentage increases in body weights of rats treated with ethanol extract of *D. guineense* stem bark were significantly reduced, relative to the control group ($p < 0.05$). However, there were no significant differences in the corresponding relative heart weights among the groups ($p > 0.05$).

Table 1: Comparison of the Effect of Ethanol Extract of *D. guineense* Stem Bark on Weight Parameters

Groups	% Increase in weight	Relative organ weight (x 10^{-2})
Control	61.35 ± 4.11	2.00 ± 0.03
200 mg/kg bwt	52.60 ± 2.92[a]	2.00 ± 0.02
500 mg/kg bwt	22.63 ± 1.56[b]	2.50 ± 0.02
1000 mg/kg bwt	21.00 ± 1.00[b]	2.50 ± 0.03
2000 mg/kg bwt	18.30 ± 1.06[b]	2.50 ± 0.02
3500 mg/kg bwt	17.73 ± 0.92[b]	2.50 ± 0.04
5000 mg/kg bwt	16.80 ± 1.10[b]	2.00 ± 0.10

Data are percentage weight increase and relative heart weight, and are expressed as mean ± SEM (n = 3). [a]$p < 0.05$, when compared with control group; [b]$p < 0.05$, when compared with 200 mg/kg bwt group.

Cardiac Function in Extract-Treated Rats

Treatment with ethanol extract of *D. guineense* stem bark did not elicit any significant differences in the activities of LDH among the groups ($p > 0.05$). Activities of CK in groups II and III and AST of groups II, III, IV and V were not significantly different from those of control group ($p > 0.05$), but they were significantly increased in the high dose groups ($p < 0.05$). Similarly, the basal activities of the measured indices of cardiac function were not significantly different from the values after treatment ($p > 0.05$). Moreover, there were no significant increases in the concentrations of MDA in the heart of extract-treated rats ($p > 0.05$; Tables 2 and 3).

Table 2: Effect of Aqueous Extract of *D. guineense* Stem Bark on Cardiac Function.

Groups		LDH (U/L)	CK (U/L)	AST (U/L)
Control		20.64 ± 0.00	40.45 ± 2.15	27.50 ± 0.89
200 mg/kg bwt	B	23.01 ± 0.89	37.36 ± 1.29	25.00 ± 4.89
	T	28.32 ± 5.32	39.93 ± 0.00	29.00 ± 1.02
500 mg/kg bwt	B	21.62 ± 2.06	59.01 ± 3.51	28.33 ± 3.33
	T	25.00 ± 6.50	51.85 ± 1.00	34.83 ± 1.17
1000 mg/kg bwt	B	40.81 ± 2.94	86.92 ± 5.03	33.33 ± 3.67
	T	38.59 ± 1.32	85.80 ± 2.96*	31.15 ± 1.01
2000 mg/kg bwt	B	39.45 ± 1.95	80.01 ± 2.59	32.50 ± 6.50
	T	41.61 ± 2.34	82.35 ± 0.42*	35.00 ± 0.92
3500 mg/kg bwt	B	34.72 ± 2.01	82.14 ± 6.19	32.50 ± 4.50
	T	37.27 ± 0.52	80.10 ± 1.37*	38.50 ± 2.47 *
5000 mg/kg bwt	B	41.52 ± 1.02	75.19 ± 9.81	36.67 ± 3.43
	T	43.59 ± 1.32	79.20 ± 0.44*	47.83 ± 2.30*

Data are indices of cardiac function and are expressed as mean ± SEM (n = 5).
B = basal means; and T = test means. $^{*}p < 0.05$, when compared with control group.

Table 3: Concentrations of MDA in Heart Homogenates.

Groups	MDA Concentration (mole/mg tissue) x 10^{-4}
Control	3.80 ± 1.24
200 mg/kg bwt	3.99 ± 1.71
500 mg/kg bwt	5.68 ± 2.10
1000 mg/kg bwt	5.95 ± 1.40
2000 mg/kg bwt	6.95 ± 3.90
3500 mg/kg bwt	8.68 ± 1.70
5000 mg/kg bwt	8.35 ± 3.40

Data are concentrations of cardiac MDA and are expressed as mean ± SEM (n = 5).

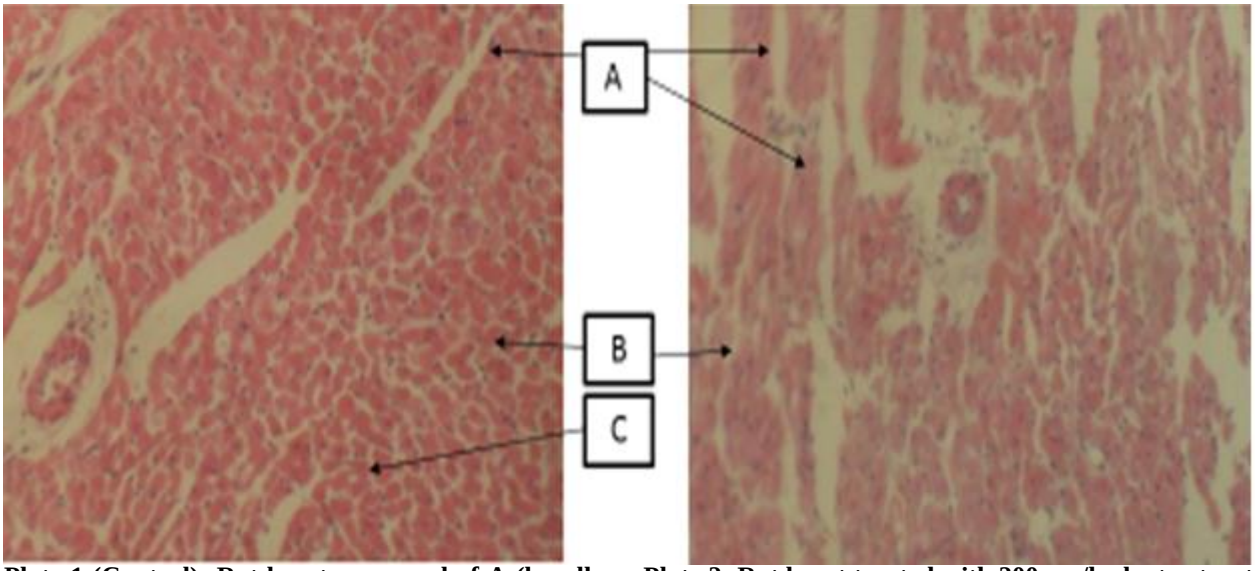

Plate 1 (Control): Rat heart composed of A (bundles of myocardial fibres); B (coronary artery); and C (interstitial space) (H & E x 100)

Plate 2: Rat heart treated with 200 mg/kg bwt extract showing A (normal myocardial fibres); and B (mild interstitial oedema) (H & E x 100)

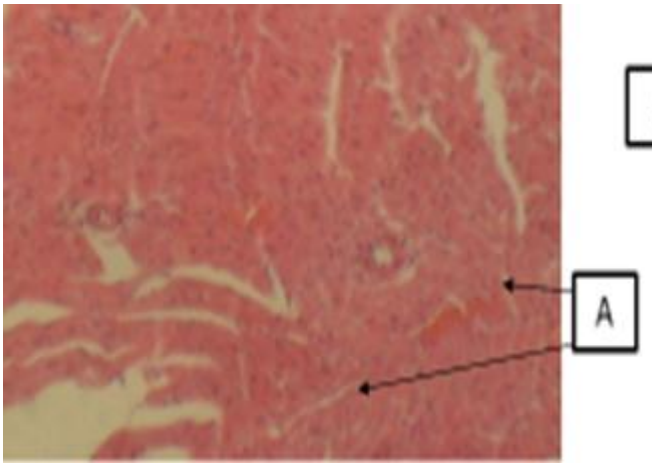

Plate 3: Rat heart treated with 500 mg/kg bwt extract showing A (normal myocardial architecture) (H & E x 100)

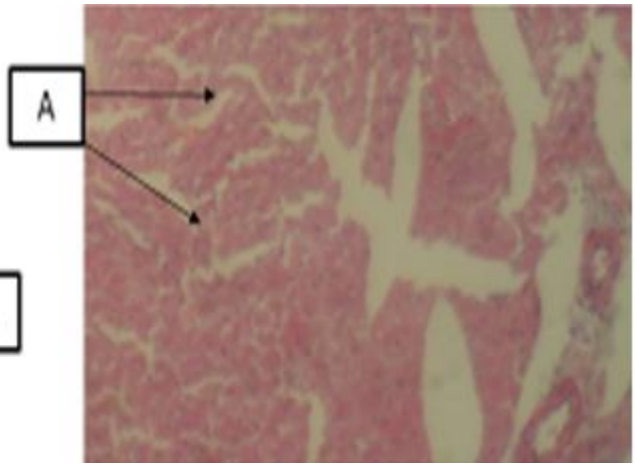

Plate 4: Rat heart treated with 1000 mg/kg bwt extract showing A (normal myocardial architecture) (H & E x 100)

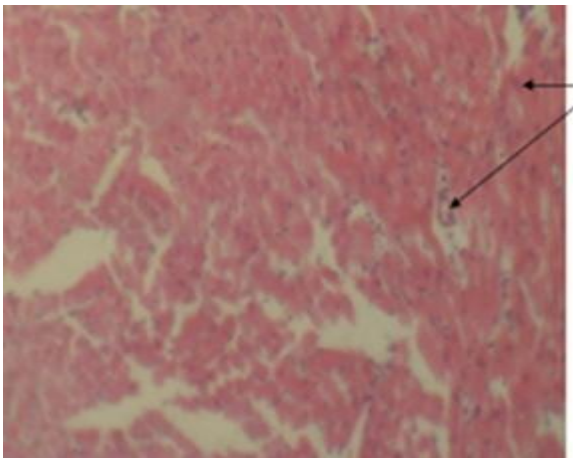

Plate 5: Rat heart treated with 2000 mg/kg bwt extract showing A (normal myocardial architecture) (H & E x 100)

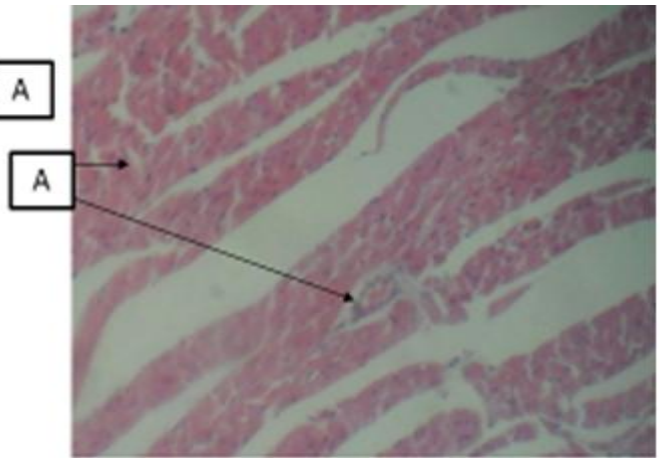

Plate 6: Rat heart treated with 3500 mg/kg bwt extract showing A (normal myocardial architecture) (H & E x 100)

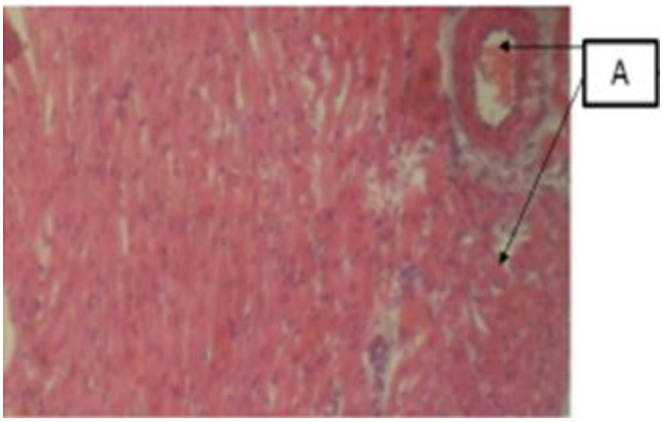

Plate 7: Rat heart treated with 5000 mg/kg bwt extract showing A (normal myocardial architecture) (H & E x 100)

Figure 1: Effect of Ethanol Extract of D. guineense Stem Bark on the Histology of Rat Heart.

DISCUSSION

In drug development, a crucial step is the screening of potential drug candidates for possible toxicity.[1] Cardiotoxicity is of special concern. Cardiotoxicity became a phenomenon to worry about since the advent of cancer chemotherapy. It is characterized by abnormality of cardiac electrical activity and contractile dysfunction, ultimately leading to heart failure. A proper understanding of the pathogenesis of cardiotoxicity is key to improve pharmaceutical development for effective

treatment without cardiac side effects.[22] Due to the complex nature of muscle structure and the heterogeneity of cardiomyocyte populations, it is very difficult to identify the primary cause of the condition at the cellular level.[23] Cultured cardiomyocytes are best suited for studies involving the evaluation of direct effects of xenobiotics on the heart at cellular, subcellular, and molecular levels.[23] Drug-induced cardiotoxicity, in the form of cardiac muscle dysfunction that may progress to heart failure, represents a major adverse effect of some common traditional antineoplastic agents, biological monoclonal antibodies, tyrosine kinase inhibitors, antiretroviral drugs, and illicit drugs such as alcohol, cocaine, methamphetamine, ecstasy, and synthetic cannabinoids.[24 – 26] Cardiac toxicity of antineoplastic agents includes left ventricular failure, myocardial ischemia, QT prolongation, arrhythmias, pericarditis, myocarditis, hypertension, and thromboembolism. Asymptomatic diastolic dysfunction, which is a common feature observed in many cancer survivors, has been shown to be the earliest noticeable cardiac abnormality.[22, 26] Subclinical cardiotoxicity is commonly defined on cardiac imaging as clinically asymptomatic left ventricular systolic dysfunction with a fall in left ventricular ejection fraction by > 10 % points to a value of less than < 50 %.[2]

This study investigated the cardiotoxicity of ethanol extract of *D. guineense* stem bark in rats. The results showed that percentage increases in body weights of rats treated with ethanol extract of the medicinal plant were significantly reduced, relative to the control group, but there were no significant differences in the relative heart weights among the groups. Treatment with the extract did not elicit any significant differences in the activities of LDH and cardiac MDA level among the groups. Similarly, the basal activities of the measured indices of cardiac function were not significantly different from the values after treatment. These results are in agreement with those of previous reports.[33-35]

CONCLUSION

This study has shown that ethanol extract of *D. guineense* stem bark is not toxic to the heart and may be used in Traditional Medicine for the treatment of diseases. However, further studies will be needed to ascertain the long-term effect of the extract on other systems in animal models.

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