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Nephrotoxic Evaluation of Aqueous Stem Bark Extract *of Dialium guineense* in Normal Wistar Rats

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ABSTRACT

The present study investigated the nephrotoxic effect of aqueous extract of *Dialium guineense* stem bark in normal Wistar albino rats. Thirty-five male rats were used. Graded doses of the extract ranging from 200 to 5000 mg/kg body weight, bwt, were administered to the test rats daily for twenty-eight days. The control rats received distilled water. Thereafter plasma levels of creatinine, urea, electrolytes and urease activity were determined. There were no significant differences in the concentrations of the measured renal parameters, when compared with control group ($p > 0.05$). These results appear to suggest that the extract may be safe at concentrations not exceeding 5000 mg/kg bwt. However, further studies spanning several months may be necessary.

KEYWORDS: *Dialium guineense*, Electrolytes, Graded doses, Nephrotoxic effect, Urea.

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INTRODUCTION

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. It accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system [1]. Various endocrine hormones coordinate these endocrine functions: renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others. Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron [2]. Filtration is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. It takes place in renal corpuscle. The kidney generates 180 L of filtrate a day, while reabsorbing a large percentage, allowing for the generation of only approximately 2 L of urine [3, 4]. Reabsorption is the transport of molecules from this ultrafiltrate into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine.The kidneys are responsible for the elimination of unmodified drugs and metabolites. Alterations in kidney structure and function are frequently found in severe liver disease and once liver function falls below a critical threshold, sodium retention occurs followed by ascites, associated with profound disturbances of splanchnic and systemic hemodynamic which in turn may affect renal function [5, 6]. Nephrotoxicity refers to injury to the kidneys or impairment of kidney function caused by exposure to xenobiotics such as drugs, food additives, alcohol, chlorinated solvents, peroxidized fatty acids, fungal toxins, radioactive isotopes, environmental toxicants, and even some medicinal plants [7].

With the extensive use and deepening pharmacological research on medicinal plants, the adverse effects of herbal medicines have also been determined. Plant-derived materials used in herbal medicines are considered to have slight side effects, and can be taken for a long time or at a large dose. However, in recent years, reports of adverse reactions caused by herbal medicine and its preparations have increased [8 – 10]. Nephrotoxicity is one of the main toxicities of herbal medicines [8].

Dialium guineense is a medicinal plant that is used in parts of Africa for the treatment of various ailments [11]. This plant contains bioactive substances such as alkaloids, tannins, saponins and phenolics [12, 13]. Some of these bioactive compounds may have adverse effects on the kidney. Thus assessment of renal status of Wistar rats administered extract of *Dialium guineense* may shed light on the safety of the

plant. This study was undertaken to investigate the nephrotoxic effect of aqueous extract *of D. guineense* stem bark in normal Wistar rats.

MATERIALS AND METHODS

Chemicals and Kits

Reagents used in this study were of analytical grade. Kidney function tests kits were obtained from Randox Laboratories Limited (UK). All other chemicals were purchased from British Drug House (BDH) (England) and Sigma-Aldrich Ltd. (USA).

Plant Material

Fresh 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 Extraction

Extraction of the pulverized plant material was by maceration over 72 h [14]. Exactly 500 g of the powdered stem bark was soaked in 5000 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 adult male Wistar rats, which weighed between 160 and 180 g (mean weight = 170 ± 10 g) were bought 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. Just before the 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 assigned to 7 groups (5 rats per group): One group served as control, while rats in other groups received graded doses of extract (200 - 5000 mg/kg bwt) for a period of 28 days. Blood samples were collected before treatment (basal samples) and at the end of the 28th day. Blood sample collected in plain or heparin containers was centrifuged at 3000 rpm for 10 min to obtain plasma which was used for kidney function tests.

Kidney Function Tests

Kidney function tests (KFTs) such as urea, creatinine, sodium ion, potassium ion, chloride ion and bicarbonate ion were performed in plasma [15 – 17]. Urease activity was determined as shown below:

Urease activity (U/L) = [urea]/time

Statistical Analysis

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. Statistical significance was assumed at $p < 0.05$.

RESULTS

Effect of Graded Doses of Aqueous Extract of *D. guineense* Stem Bark on Weight Parameters

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

Table 1: Percentage Body Weight Increase and Relative Kidney Weight of Rats Treated with Aqueous Extract of *D. guineense* Stem Bark

Groups	% Increase in weight	Relative kidney weight (x 10^{-3})
Control	61.35 ± 4.11	3.34 ± 0.03
200 mg/kg bwt	52.60 ± 2.92[a]	3.59 ± 0.14
500 mg/kg bwt	22.63 ± 1.56[ab]	3.50 ± 0.11
1000 mg/kg bwt	21.00 ± 1.00[ab]	3.21 ± 0.07
2000 mg/kg bwt	18.30 ± 1.06[ab]	4.07 ± 0.62
3500 mg/kg bwt	17.73 ± 0.92[ab]	3.15 ± 0.11
5000 mg/kg bwt	16.80 ± 1.10[ab]	3.39 ± 0.31

Data are percentage weight increase and relative kidney 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 the 200 mg/kg bwt group.

Indices of Kidney Function

There were no significant differences in the plasma concentrations of creatinine, urea, electrolytes and urea/creatinine in rats treated with aqueous extract, when compared with the control group ($p > 0.05$). These results are shown in Tables 2 and 3.

Table 2: Concentrations of Creatinine and Urea in Rats Treated with Aqueous Extract of *D. guineense* Stem Bark

Groups		Creatinine (mg/dL)	Urea (mg/dL)	Urea /Creatinine
Control		1.70 ± 0.03	17.52 ± 6.50	10.31 ± 2.91
200 mg/kg bwt	B	1.70 ± 0.09	13.84 ± 1.02	8.14 ± 2.04
	T	1.67 ± 0.21	14.63 ± 1.79	8.76 ± 1.88
500 mg/kg bwt	B	1.38 ± 0.06	12.92 ± 1.52	9.36 ± 1.83
	T	1.70 ± 0.20	14.12 ± 1.50	8.31 ± 2.05
1000 mg/kg bwt	B	2.55 ± 0.15	21.31 ± 2.13	8.36 ± 1.57
	T	3.95 ± 1.45	23.97 ± 2.61	7.61 ± 1.18
2000 mg/kg bwt	B	2.06 ± 0.10	19.91 ± 2.07	9.67 ± 2.95
	T	2.27 ± 0.57	20.37 ± 2.46	8.97 ± 1.55
3500 mg/kg bwt	B	2.86 ± 0.22	22.97 ± 2.03	8.03 ± 0.91
	T	3.97 ± 1.13	24.37 ± 2.41	7.86 ± 1.42
5000 mg/kg bwt	B	3.02 ± 0.34	17.55 ± 2.50	5.81 ± 1.08
	T	3.40 ± 0.08	19.07 ± 2.02	5.61 ± 1.19

Data are indices of kidney function and are expressed as mean ± SEM (n = 5). B = Basal means; and T = Test means.

Table 3: Concentrations of Plasma Electrolytes in Rats Treated with Aqueous Extract of *D. guineense* Stem Bark

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)	Cl^- (mmol/L)
Control	138.50 ± 0.50	7.90 ± 0.20	18.00 ± 0.00	105.50 ± 0.50
200 mg/kg bwt	142.50 ± 0.50	5.00 ± 0.70	19.00 ± 3.00	108.50 ± 0.50
500 mg/kg bwt	144.00 ± 6.00	5.75 ± 1.55	16.50 ± 0.50	109.00 ± 4.00
1000 mg/kg bwt	136.50 ± 0.50	15.10 ± 6.40	15.50 ± 0.50	103.50 ± 0.50
2000 mg/kg bwt	137.00 ± 1.00	6.75 ± 0.85	21.00 ± 2.00	105.50 ± 1.50
3500 mg/kg bwt	139.50 ± 0.50	7.85 ± 1.05	17.00 ± 0.00	100.80 ± 5.17
5000 mg/kg bwt	127.50 ± 0.50	13.65 ± 0.65	15.00 ± 0.00	100.50 ± 0.50

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

Effect of Graded Doses of Aqueous Extract of *D. guineense* Stem Bark on Urease Activity

There were no significant differences in urease activity before and after treatment, among the groups ($p > 0.05$; Figure 1).

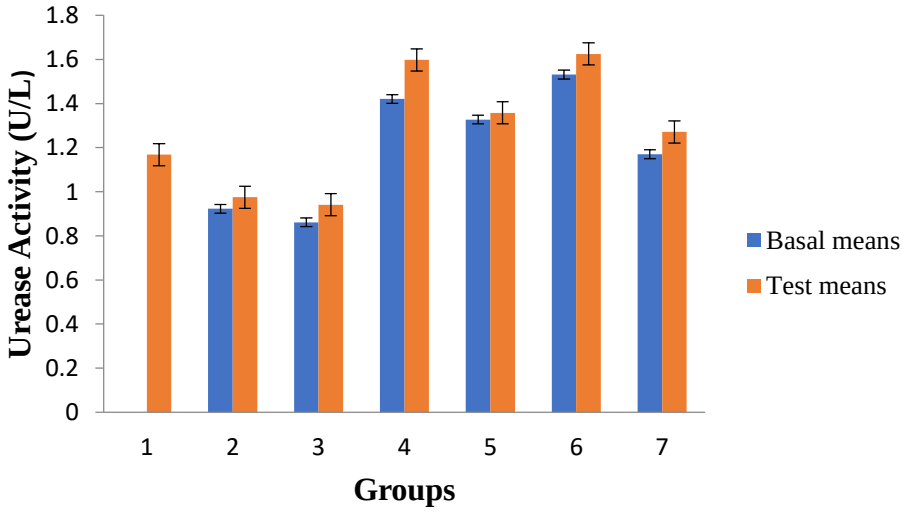

Figure 1: Comparison of Urease Activity Among the Groups

DISCUSSION

The kidney is one of the organs susceptible to damage by harmful substances [7]. Nephrotoxicity may be caused by drugs, food additives, alcohol, chlorinated solvents, peroxidized fatty acids, fungal toxins, radioactive isotopes, environmental toxicants, and even some medicinal plants [7]. Herbal medicine has been widely used to treat diseases for thousands of years and has greatly contributed to the health of human beings. Many new drugs have been developed from medicinal plants, such as artemisinin. However, artemisinin has adverse effects, such as renal toxicity. The nephrotoxicity of plant extracts has attracted worldwide attention [18 - 21]. In 1993, a study conducted in Belgium reported for the first time that the root extracts of *Aristolochia obliqua* led to progressive interstitial renal fibrosis [8].

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 [11]. Different parts of the medicinal plant are used against several diseases [11]. Extracts of the plant have been shown to be reservoirs of important phytochemicals [12, 13]. The plant contains bioactive substances such as alkaloids, tannins, saponins and phenolics [12]. Some of these bioactive compounds may have adverse effects on the kidney. This study investigated the nephrotoxic effect of aqueous extract *of Dialium guineense* stem bark in normal Wistar rats.

Kidney, an organ that metabolizes harmful substances besides liver, is constantly perfused with huge volume of blood carrying different kinds of compounds, thereby making it at high risk of toxicity [22, 23]. High levels of blood creatinine are found in renal dysfunction or muscle injury [24]. Levels of specific ions such as sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO^{3-}) are used as biomarkers of electrolyte imbalance [25]. Electrolytes promote fluid balance via maintenance of blood volume, fluid absorption and generation of impulses. In pathological conditions, electrolyte imbalance occurs with increased sodium and chloride, and decreased potassium levels [26, 27]. Electrolyte balance is crucial for normal cellular function. Decreased electrolyte levels affects nerve conduction, as well as cell function [28]. Blood urea and creatinine are considered traditional indices of kidney function. Urea is a by-product of protein catabolism. About 90 % of urea produced is excreted through the kidneys [29]. Creatinine, a waste product of muscle catabolism, is excreted exclusively via the kidneys [28]. Therefore, renal damage reduces the kidney's capacity to excrete both urea and creatinine, thereby making them to accumulate in the blood. The results of this study showed that graded doses of aqueous extract *of D. guineense* stem bark did not significantly alter the levels of electrolytes, urea and creatinine, an indication that it may not be toxic upon prolonged use.

CONCLUSION

The results obtained in this study show that aqueous extract *of D. guineense* stem bark is not nephrotoxic and may be used in traditional medicine at doses not exceeding 5000 mg/kg bwt.

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