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Hepatotoxicity of Graded Doses of Ethanol Extract of *Dialium guineense* Stem Bark in Wistar Rats

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ABSTRACT

Aim: To investigate the hepatotoxicity of graded doses of ethanol extract of *Dialium guineense* stem bark in Wistar rats.

Materials and Methods: Adult male Wistar rats (n = 35), which weighed between 160 and 180 g (mean weight = 170 ± 10 g) were assigned to seven (7) groups (5 rats per group). Group I rats served as control, while those in groups II - VII received graded doses of extract (200 - 5000 mg/kg body weight, bwt) for 28 days. Liver function tests (LFTs) were carried out.

Results: There were no significant differences in the activities of alanine aminotransferase (ALT) among the groups ($p > 0.05$). While there were no significant differences in the activities of aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) in groups II – V ($p > 0.05$), they were however, significantly increased in groups VI and VII, when compared with control group ($p < 0.05$). Similarly, there were no significant increases in the concentrations of albumin, total protein, globulins, bilirubin and malondialdehyde (MDA) in plasma of rats treated with graded doses of ethanol extract, relative to the control group ($p > 0.05$). In all instances, the basal activities and concentrations of the measured indices of liver function were not significantly different from the values after treatment ($p > 0.05$).

Conclusion: The graded doses of ethanol extract of *D. guineense* stem bark did not elicit any deleterious effects on liver function indices.

KEYWORDS: Aminotransferases, *Dialium guineense*, Graded doses, Hepatotoxicity, Liver function.

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INTRODUCTION

The use of medicinal plants for the treatment of diseases is common in developing countries, probably due to the cost of orthodox drugs [1]. Global drug safety depends on strong national systems that monitor the development and quality of medicines, and report their harmful effects, while providing accurate information for their safe use [2]. How well patients recover from their sicknesses/diseases depends on whether the drugs are safe or not. Toxicology is the science that deals with adverse effects of chemical, physical or biological agents on humans, animals and the environment [3]. The dose of a chemical may contribute to its toxicity. Toxic effects are generally categorized according to the site of effect. When the effect occurs at a single site, it is referred to as "specific target organ effect". However, when it occurs at multiple sites, it is referred to as "systemic toxicity". Examples of systemic toxicity are acute toxicity, sub-chronic toxicity, and chronic toxicity [3].

The liver is a vital organ in vertebrates and some other animals. Its functions include detoxification, protein synthesis, and production of biochemicals necessary for digestion [4]. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term. This organ lies below the diaphragm in the abdominal-pelvic region of the abdomen. The liver produces bile, an alkaline compound which aids digestion via emulsification of lipids. Its highly specialized tissues regulate a wide range of high-volume biochemical reactions [4]. Two major types of cells populate the liver lobes: karat parenchymal and non-parenchymal cells. About

80 % of liver volume is occupied by parenchymal cells commonly referred to as hepatocytes. Non-parenchymal cells constitute 40 % of the total number of liver cells, but only 6.5 % of its volume. Sinusoidal hepatic endothelial cells, Kupffer cells and hepatic stellate cells are some of the non-parenchymal cells that line the liver sinusoid [5]. The various functions of the liver are carried out by the hepatocytes. The liver is thought to be responsible for up to 500 different functions, usually in combination with other systems and organs [5]. The diagnosis of liver function is made by blood tests. Liver function tests (LFTs) can readily pinpoint the extent of liver damage [6]. Certain medicinal agents, chemicals and even herbal remedies may cause liver injury [7]. Hepatotoxicity refers to injury to the liver or impairment of liver 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 [8]. *Dialium guineense*, a tall, tropical, fruit-bearing tree, belonging to the *Leguminosae* family, 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 [9]. The plant grows naturally in West African countries, Central African Republic, and Sudan [10, 11]. Different parts of the medicinal plant are used against several diseases [10]. At present, not much is known about the subchronic toxicity of extracts of *D. guineense* in rats. The aim of this study was to investigate the hepatotoxicity of graded doses of ethanol extract of *D. guineense* stem bark in Wistar rats.

MATERIALS AND METHODS

Chemicals and Kits

Reagents used in this study were of analytical grade. Liver function tests kits were obtained from Randox Laboratories Limited (UK). All other chemicals were products of British Drug House (BDH) (England), Merck (Germany) 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 a 72 h period [12]. A portion (100 g) of the powdered stem bark was soaked in 1000 mL distilled water. The resultant ethanol 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 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. Standard experimental procedures were observed in this study.

Experimental Design

The rats were assigned to 7 groups (5 rats per group): Group I served as control, while rats in groups II - VII 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 liver function tests.

Organ Function Tests

Liver function tests (LFTs) such as ALT, AST, GGT, total protein, albumin, globulins and bilirubin were performed in plasma [13 – 17].

Determination of Lipid Peroxidation in Plasma

Malondialdehyde (MDA) level was measured in plasma [18]

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 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, 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 Liver Weight of Rats Treated with Ethanol Extract of *D. guineense* Stem Bark

Groups	% Increase in weight	Relative organ weight (x 10^{-2})
Control	61.35 ± 4.11	3.80 ± 0.30
200 mg/kg bwt	52.60 ± 2.92[a]	3.00 ± 0.02
500 mg/kg bwt	22.63 ± 1.56[ab]	4.70 ± 0.90
1000 mg/kg bwt	21.00 ± 1.00[ab]	3.00 ± 0.02
2000 mg/kg bwt	18.30 ± 1.06[ab]	3.40 ± 0.30
3500 mg/kg bwt	17.73 ± 0.92[ab]	3.90 ± 0.60
5000 mg/kg bwt	16.80 ± 1.10[ab]	4.10 ± 0.30

Data are percentage weight increase and relative liver 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.

Effect of Graded Doses of Ethanol Extract of *D. guineense* Stem Bark on Liver Function

There were no significant differences in the activities of ALT and AST/ALT among the groups ($p > 0.05$). While there were no significant differences in the activities of AST and GGT in groups II – V ($p > 0.05$), they were however, significantly increased in groups VI and VII, when compared with control group ($p < 0.05$). Similarly, there were no significant increases in the concentrations of albumin, total protein, globulins, bilirubin and MDA in plasma of rats treated with graded doses of ethanol extract, relative to the control group ($p > 0.05$). In all instances, the basal activities and concentrations of the measured indices of liver function were not significantly different from the values after treatment ($p > 0.05$). These results are shown in Tables 2 to 5.

Table 2: Liver Function Parameters in Rats Treated with Ethanol Extract of *D. guineense* Stem Bark

Groups		ALT (U/L)	AST (U/L)	AST/ALT	GGT (U/L)
Control		31.17 ± 2.58	27.50 ± 0.89	0.88 ± 0.04	20.32 ± 2.50
200 mg/kg bwt	B	28.17 ± 3.76	25.00 ± 4.89	0.89 ± 0.03	28.95 ± 2.79
	T	30.33 ± 4.83	29.00 ± 2.02	0.96 ± 0.04	25.80 ± 2.00
500 mg/kg bwt	B	26.33 ± 4.41	28.33 ± 3.33	1.08 ± 0.09	28.95 ± 2.79
	T	29.00 ± 2.00	34.83 ± 2.17	1.20 ± 0.09	30.63 ± 2.69
1000 mg/kg bwt	B	34.34 ± 7.28	33.33 ± 3.67	0.97 ± 0.05	26.06 ± 2.27
	T	32.35 ± 2.91	31.15 ± 2.01	0.96 ± 0.08	30.64 ± 2.48
2000 mg/kg bwt	B	24.84 ± 3.59	32.50 ± 3.50	1.31 ± 0.07	28.05 ± 3.01
	T	26.30 ± 3.93	35.00 ± 2.60	1.33 ± 0.09	33.53 ± 3.37
3500 mg/kg bwt	B	23.33 ± 2.67	32.50 ± 4.50	1.39 ± 0.06	30.64 ± 5.06
	T	29.67 ± 1.33	38.50 ± 2.47[a]	1.30 ± 0.11	43.01 ± 4.26[a]
5000 mg/kg bwt	B	26.33 ± 4.32	36.67 ± 3.43	1.39 ±0.07	35.32 ± 5.79
	T	38.83 ± 5.30	47.83 ± 2.30[a]	1.23 ± 0.11	45.06 ± 5.79[a]

Data are indices of liver function, and are expressed as mean ± SEM (n = 5). B = Basal means; and T = Test means. [a]$p < 0.05$, when compared with control group.

Table 3: Concentrations of Liver Proteins in Rats Treated with Ethanol Extract of *D. guineense* Stem Bark

Groups		T. Protein (g/dL)	Globulins (g/dL)	Albumin (g/dL)
Control		9.93 ± 2.21	7.31 ± 1.01	2.62 ± 0.22
200 mg/kg bwt	B	7.38 ± 0.72	4.01 ± 0.50	3.37 ± 0.39
	T	9.95 ± 2.45	7.50 ± 1.30	2.45 ± 0.15
500 mg/kg bwt	B	8.55 ± 1.65	4.53 ± 0.35	4.02 ± 1.10
	T	12.48 ± 5.02	10.03 ± 3.32	2.45 ± 0.15
1000 mg/kg bwt	B	6.27 ± 1.64	3.52 ± 0.71	2.75 ± 0.35
	T	10.98 ± 3.12	8.88 ± 1.72	2.10 ± 0.10
2000 mg/kg bwt	B	6.70 ± 0.60	4.50 ± 0.50	2.20 ± 0.08
	T	9.83 ± 2.51	7.63 ± 1.13	2.20 ± 0.30
3500 mg/kg bwt	B	7.86 ± 1.66	4.19 ± 0.71	3.67 ± 0.93
	T	11.43 ± 2.98	8.63 ± 0.71	2.80 ± 0.50
5000 mg/kg bwt	B	7.13 ± 1.27	3.26 ± 0.28	3.87 ± 0.91
	T	12.98 ± 2.84	10.56 ± 1.03	2.42 ± 0.16

Data are indices of liver function, and are expressed as mean ± SEM (n = 5).

Table 4: Concentrations of Bilirubin in Rats Treated with Ethanol Extract of *D. guineense* Stem Bark

Groups		T. Bilirubin (mg/dL)	D. Bilirubin (mg/dL)	Ind. Bilirubin (mg/dL)
Control		2.40 ± 0.20	1.10 ± 0.06	1.30 ± 0.07
200 mg/kg bwt	B	1.32 ± 0.37	0.55 ± 0.09	0.77 ± 0.07
	T	2.45 ± 0.15	1.17 ± 0.15	1.28 ± 0.13
500 mg/kg bwt	B	2.10 ± 0.08	0.65 ± 0.05	1.45 ± 0.25
	T	2.57 ± 0.17	1.42 ± 0.08	1.15 ± 0.09
1000 mg/kg bwt	B	1.95 ± 0.05	0.68 ± 0.06	1.27 ± 0.13
	T	2.78 ± 0.37	1.45 ± 0.25	1.33 ± 0.03
2000 mg/kg bwt	B	2.62 ± 0.19	1.03 ± 0.09	1.59 ± 0.09
	T	3.35 ± 0.93	1.77 ± 0.19	1.58 ± 0.08
3500 mg/kg bwt	B	3.28 ± 0.92	0.95 ± 0.08	2.33 ± 0.67
	T	3.37 ± 0.53	1.85 ± 0.10	1.52 ± 0.13
5000 mg/kg bwt	B	2.95 ± 0.50	0.75 ± 0.05	2.20 ± 0.30
	T	4.43 ± 0.93	2.82 ± 0.74	1.61 ± 0.12

Data are indices of liver function, and are expressed as mean ± SEM (n = 5). T. Bilirubin = total bilirubin; D. Bilirubin = direct bilirubin; and Ind. Bilirubin = indirect bilirubin.

Table 5: Concentrations of MDA in the Plasma of Rats Treated with Ethanol Extract of *D. guineense* Stem Bark

Groups	MDA Concentration (mole/mg protein) x 10^{-7}
Control	2.27 ± 0.92
200 mg/kg bwt	3.82 ± 0.90
500 mg/kg bwt	5.21 ± 1.53
1000 mg/kg bwt	4.10 ± 2.67
2000 mg/kg bwt	4.69 ± 0.38
3500 mg/kg bwt	4.89 ± 0.84
5000 mg/kg bwt	4.84 ± 0.22

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

DISCUSSION

Liver, as the primary organ for the detoxification and distribution of drugs is assessed to establish the safety of a substance [19]. It is a vital organ involved in metabolism, detoxication and excretion of various endogenous and exogenous substances [4]. Certain medicinal agents, chemicals and even herbal remedies may cause liver injury [7]. Liver diseases have become one of the major causes of morbidity and mortality all over the world. Drug-induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge [20]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Liver damage is associated with cellular necrosis, fibrosis, increase in tissue lipid peroxidation and depletion of tissue glutathione level [21, 22]. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market [23]. Hepatotoxicity and drug-induced liver injury also account for a substantial number of compound failures, highlighting the need for drug screening assays, capable of detecting toxicity early in the drug development process [24]. Subclinical injury to the liver caused by chemical exposure, manifests as abnormal liver enzyme levels. Drug-induced liver injury is responsible for 5 % of all hospital admissions and 50 % of all acute liver failures [25, 26].

Liver function tests (LFTs) are used to screen for the presence of liver disease, suggest the underlying cause, estimate severity, assess prognosis and monitor effectiveness of therapy [27]. These tests include liver enzymes, proteins and specific molecules. Alanine aminotransferase (ALT) is more specific to liver, and thus is a better parameter for detecting liver injury. Aspartate aminotransferase (AST) is predominantly found in mitochondria of hepatocytes. The ALT, AST and ALP activity as well as serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury [28, 29]. Elevation of enzymes such as AST, ALT, ALP and bilirubin has been attributed to damage to structural integrity of liver, because they are cytoplasmic and enter into circulation after cellular damage [30, 31]. In this study, there were no significant differences in the activities of ALT and AST/ALT in rats treated with ethanol extract. While there were no significant differences in the activities of AST and GGT in groups II – V, they were however, significantly increased in groups VI and VII, when compared with control group. There were also no significant increases in the concentrations of albumin, total protein, globulins and bilirubin in rats treated with graded doses of ethanol extract, relative to the control group. In all instances, the basal activities and concentrations of the measured indices of liver function were not significantly different from the values after treatment.

CONCLUSION

The results obtained in this study showed that graded doses of ethanol extract of *D. guineense* stem bark did not elicit any deleterious effects on liver function. The stem bark extract of the medicinal plant may be relatively safe and could be used in herbal formulations.

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