

Effect of Extracts of *Dialium guineense* Stem Bark on Lipid Profile and CCl₄- Induced Histological Changes in Liver of Wistar Rats

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Abstract

The present study investigated the effect of aqueous and ethanol extracts of *Dialium guineense* stem bark on lipid profile and CCl₄- induced histological changes in liver of Wistar rats. Adult male Wistar rats (n = 25) weighing 160 – 180 g (mean weight = 170 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl₄ control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl₄ (single oral dose of 1.0 mL/kg body weight, bwt). Silymarin group rats were administered standard hepatoprotective drug, silymarin, at a dose of 100 mg/kg bwt, while those in the

two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Lipid profile parameters were determined in plasma, while rat liver was subjected to histopathological examination. The results showed that the levels of total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), very-low density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C) as well as atherogenic index of plasma (AIP) were significantly lower in CCl₄ control group than in normal control group, but they were increased by extract treatment ($p < 0.05$). However, there were no significant differences in atherogenic coefficient (AC) and cardiac risk ratio (CRR) among the groups ($p > 0.05$). Carbon tetrachloride (CCl₄) markedly disrupted the structure of hepatocytes and induced steatosis (intra-hepatocyte fat in-growth and inflammation) which was predominantly microvesicular. However, treatment with aqueous and ethanol extracts of *D. guineense* stem bark showed marked regeneration of hepatocytes (unremarkable hepatic lobular architecture). The toxic hepatic injury induced by CCl₄ was significantly blocked by the plant extracts.

Introduction

Lipids are one of the necessary components which control cellular functions and homeostasis.

The liver plays an essential role in lipid metabolism, several stages of lipid synthesis and transportation [1]. Therefore, it is reasonable to expect an abnormal lipid profile in severe liver dysfunction [1]. Chemicals constitute an important cause of liver injury. Carbon tetrachloride is the most commonly used hepatotoxic agent for the induction of liver injuries in experimental animals [2, 3]. There is a prominent decline in plasma total cholesterol and triacylglycerol levels in patients with severe hepatitis and hepatic failure because of reduction in lipoprotein biosynthesis [4].

Today, a substantial number of drugs are developed from plants which are active against a number of diseases [5].

Dialium guineense is a medicinal plant used in Traditional Medicine for the treatment of infections such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids [6, 7]. Extracts of the plant are reported to be rich in important phytochemicals [8 - 10]. At present not much is known about the potential of extracts of *D. guineense* stem bark to alter lipid profile and histology of rats liver exposed to CCl₄. This study investigated the effect of aqueous and ethanol extracts of *Dialium guineense* stem bark on lipid profile and CCl₄- induced histological changes in liver of Wistar rats.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

Collection of Plant Material

The stem barks of *D. guineense* were obtained from Auchu, 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 Extraction

The stem bark was washed and shade-dried at room temperature for a period of two weeks and then

pulverized. Aqueous and ethanol extracts of the stem bark were obtained using cold maceration method as described previously [11].

Experimental Rats

Adult male Wistar rats (n = 25) weighing 160 - 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 rat feed (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 randomly assigned to five groups (5 rats per group): normal control, CCl₄ control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl₄ (single oral dose of 1.0 mL/kg bwt) [11]. Silymarin group rats were administered standard hepatoprotective drug, silymarin, at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days.

Blood 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, and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analysis. The liver of all experimental rats were harvested, washed in ice - cold saline, blotted dry and placed in plain containers. Weighted portions of the liver were placed in 10 % phosphosaline (pH 7.0) for histological examination.

Biochemical Analysis

Lipid profile parameters were determined using Randox kits [12 - 14]. The other parameters were determined by calculations as shown below:

$$VLDL-C = TG/5$$

$$LDL-C = TC - (TG/5) - HDL-C [15]$$

$$AIP \text{ (rats): } LDL-C + VLDL-C/HDL-C [15]$$

$$AC = (TC-HDL-C)/HDL-C [16]$$

$$CRR = TC/HDL-C [17]$$

Histological Examination of the Tissues

Portions of the liver were serially sectioned and fixed in 10 % formalin for 48 h. The specimen was then dehydrated using varied concentrations of ethanol and cleared in three changes of xylene before embedment in paraffin. Serial sections (4 μ m thick) were made and stained with haematoxylin and eosin (H & E) according to standard method. Histological assessment was performed under light microscopy. In every H and E section, a minimum of 25 circular tubules were measured in two axes drawn perpendicular to each other using an image analyzer (Image Proplus, version 3.0).

Statistical Analysis

Count data are expressed as mean \pm 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 Extracts of D. guineense Stem Bark on Relative Organ Weight

There were no significant differences in relative organ weight among the groups ($p > 0.05$; Table 1).

Effect of Extracts of D. guineense Stem Bark on Lipid Profile of Rats

The levels of TC, TG, HDL-C, VLDL-C, LDL-C as well as AIP were significantly lower in CCl₄ control group than in normal control group, but they were increased by extract treatment ($p < 0.05$). There were no significant differences in AC and CRR among the groups ($p > 0.05$). These results are shown in Tables 2 to 4.

Histology of normal control rat liver revealed distinct centriole with the hepatocytes and well fenestrated sinusoidal with mild mononuclear cells, while that of CCl₄ control showed visible centriole with the hepatocytes nuclei appearing vacuolated. There was mild

fatty changes and visible mononuclear cells. Histopathological examination of silymarin group rats liver revealed congested centriole with fairly pyknotic nuclei hepatocytes and well fenestrated sinusoidal with mild mononuclear cells. Similarly, histological changes in aqueous extract-treated rats revealed congested centriole with thickened wall surrounded by mononuclear cells. The hepatocytes had pyknotic nuclei, while those of ethanol extract-treated rats showed congested centriole with thickened wall surrounded by mild mononuclear cells with the hepatocytes and well fenestrated sinusoidal.

Discussion

The aim of this study was to investigate the effect of aqueous and ethanol extracts of *D. guineense* stem bark on lipid profile and CCl₄- induced histological changes in liver of Wistar rats.

Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for the assessment of abnormalities in the concentrations of lipids, such as cholesterol and triacylglycerol. These tests can identify certain genetic diseases and determine approximate risks for cardiovascular diseases (CVDs), certain forms of pancreatitis, and other diseases. Lipid profile typically includes LDL-C, HDL-C, TG, TC, VLDL-C and CRR [12]. Abnormal lipid profile is seen in those with severe liver dysfunction [4]. There is a prominent decline in plasma TC and TG levels in patients with severe hepatitis and hepatic failure because of reduction in lipoprotein biosynthesis [4]. The results obtained in this study suggest that the lipids synthesis ability of the liver may be reduced with CCl₄ induction, and are in agreement with those of previous reports [18 – 20]. It is likely aqueous and ethanol extracts of *D. guineense* stem bark regulated liver secretion and uptake of plasma lipoproteins. The ability of the extracts to promote lipids biosynthesis could be due to the enhanced transport of acetate into the liver cell, resulting in increased substrate (acetate) availability. Elevated levels of serum TC and TG in CCl₄ treated rats has been reported [21, 22].

In this study, the effect produced by the extracts of the medicinal plant was comparable to that of silymarin

Table 1. Relative Organ Weights of Rats

Group	Relative organ weight x 10 ⁻²
Normal Control	2.98 ± 0.05
CCl ₄ Control	2.86 ± 0.06
Silymarin	2.84 ± 0.06
Aqueous Extract	2.98 ± 0.05
Ethanol Extract	2.99 ± 0.20

Data are relative organ weights and are expressed as mean ± SEM (n = 5).

Table 2. Comparison of Lipid Profile Parameters Among the Groups

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)
Normal Control	190.34 ± 29.43	65.27 ± 7.63	22.61 ± 2.17
CCl ₄ Control	137.36 ± 7.85	28.59 ± 3.82	15.60 ± 2.03
Silymarin	180.53 ± 17.99 ^a	46.69 ± 2.43 ^a	25.32 ± 4.07 ^a
Aqueous Extract	176.61 ± 12.62 ^a	47.17 ± 0.00 ^a	21.89 ± 0.00 ^a
Ethanol Extract	200.00 ± 5.99 ^a	66.70 ± 4.21 ^a	24.99 ± 2.16 ^a

Data are lipid profile, and are expressed as mean ± SEM. ^ap < 0.05, when compared with CCl₄ control group.

Table 3. Effect of Extracts of *D. guineense* Stem Bark on Lipid Profile of Rats

Group	VLDL-C (mg/dL)	LDL-C (mg/dL)	AIP
Normal Control	13.06 ± 3.53	154.67 ± 7.63	155.25 ± 7.21
CCl ₄ Control	5.72 ± 0.76	116.04 ± 3.82	116.41 ± 4.13
Silymarin	9.34 ± 2.29 ^a	145.87 ± 2.43 ^a	146.24 ± 4.07 ^a
Aqueous Extract	9.43 ± 0.00 ^a	145.29 ± 0.00 ^a	145.72 ± 0.00 ^a
Ethanol Extract	13.34 ± 4.29 ^a	161.67 ± 4.21 ^a	162.20 ± 6.12 ^a

Data are lipid profile parameters, and are expressed as mean ± SEM. ^ap < 0.05, when compared with CCl₄ control.

Table 4. Comparison of AC and CRR Among the Groups

Group	AC	CRR
Normal Control	7.42 ± 0.53	8.42 ± 0.63
CCl ₄ Control	7.81 ± 0.76	8.81 ± 0.82
Silymarin	6.13 ± 0.92	7.13 ± 0.93
Aqueous Extract	7.07 ± 0.75	8.07 ± 0.94
Ethanol Extract	7.00 ± 0.79	8.00 ± 0.77

Data are AC and CRR, and are expressed as mean ± SEM. ^ap < 0.05, when compared with CCl₄ control.

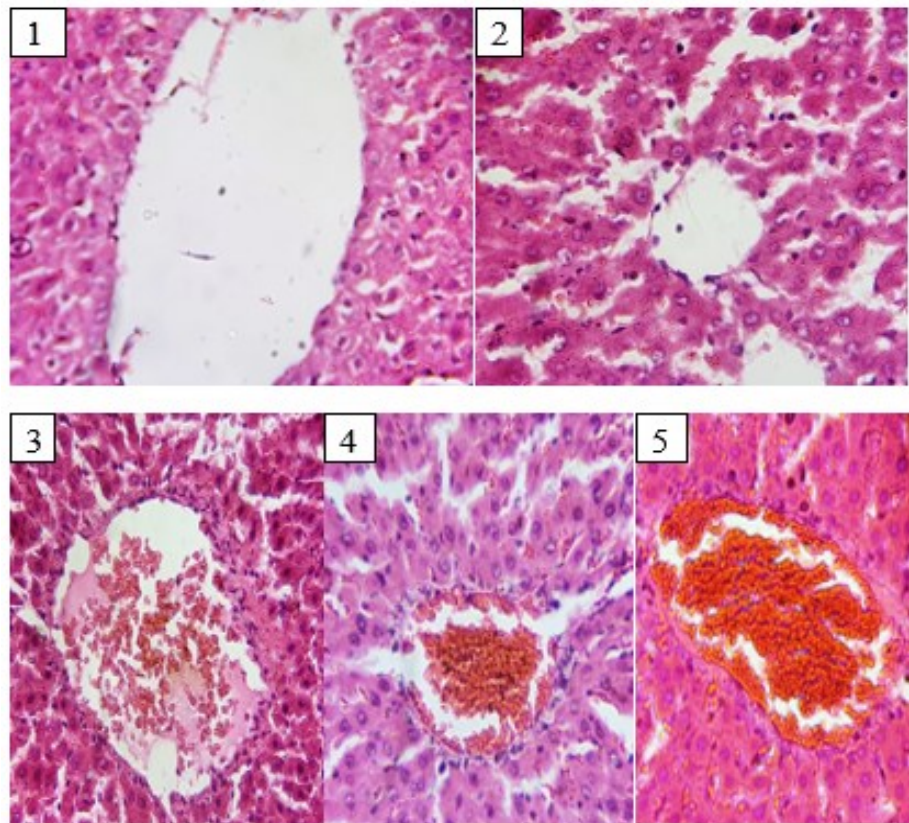


Figure 1. Effect of Extracts of *D. guineense* Stem Bark on Histology of Rats Liver.

(standard hepatoprotective drug). Silymarin protects liver against xenobiotic injury by controlling the liver secretion and uptake of plasma lipoprotein, while increasing the intracellular glutathione content [23]. Silymarin plays the role of an anti-inflammatory agent, through its ability to inhibit neutrophil infiltration and regulate the release of inflammatory mediators. It has been reported that silymarin prevents CCl₄-induced lipid peroxidation and hepatotoxicity in mice, first, by decreasing the metabolic activation of CCl₄ and second, by acting as a chain-breaking antioxidant [24]. In addition, silymarin is able to stimulate protein synthesis resulting in production of new liver cells to replace older and damaged ones [25].

Histopathological studies provided supportive evidence for lipid profile analysis. Administration of CCl₄ showed marked disruption of the structure of hepatocytes, induced steatosis (intra-hepatocyte fat in-growth and inflammation) which was predominantly microvesicular. However, treatment with aqueous and ethanol extracts of *D. guineense* stem bark showed marked regeneration of hepatocytes (unremarkable hepatic lobular architecture), which slightly affected the normal

architecture of hepatocyte cords with few areas of discontinuity. Similarly, treatment with silymarin induced mild portal congestion and dilatation without any evidence of steatosis.

Conclusion

The toxic hepatic injury induced by CCl₄ was significantly blocked by treatment with aqueous and ethanol extracts of *D. guineense* stem bark. The extent of improvement in lipid profile of the rats may not be unconnected with the dose used. Further studies are needed to assess the predictive values of measuring lipid profile as a means to estimate the extent of liver damage in cirrhotic rats.

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