Journal of Biology and Medicine: Open Access

Research| Vol 3 Iss 2

Effect of Total Saponins and Tannins Isolated From the Stem Bark of *Dialium Guineense* on Lipid Profile and CCl_4-Induced Histological Changes in Liver of Wistar Rats

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Received: July 28, 2022; **Accepted:** September 2, 2022; **Published:** September 14, 2022

Abstract

The aim of this study was to investigate the effect of total saponins and tannins isolated from the stem bark of Dialium guineense on lipid profile and carbon tetrachloride (CCl_4)-induced histological changes in liver of Wistar rats. A total of twenty-five (25) adult male Wistar rats, which weighed between 160 and 180 g were assigned to five groups of 5 rats each: normal control, CCl4 control, silymarin, total saponins and total tannins groups. Liver damage was induced in the rats using CCl_4 (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 150 mg/kg bwt of total saponins or tannins 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 after treatment with total saponins or tannins ($p < 0.05$). However, there were no significant differences in atherogenic coefficient (AC) and cardiac risk ratio (CRR) among the groups ($p > 0.05$). Carbon tetrachloride (CCl_4) markedly disrupted the structure of hepatocytes and induced steatosis (intra-hepatocyte fat in-growth and inflammation). However, treatment with total saponins and tannins of D. guineense stem bark showed marked regeneration of hepatocytes (unremarkable hepatic lobular architecture). The toxic hepatic injury induced by CCl_4 was significantly ameliorated by the phytochemicals.

Keywords: ***Cholesterol; Dialium guineense; Lipid profile; Lipid profile; Tannins; Tissue histology.***

Citation: Abu OD, Omage JI, and Ogbebor EO. Effect of total saponins and tannins isolated from the stem bark of *dialium guineense* on lipid profile and CCl_4-induced histological changes in liver of wistar rats. J Bio Med Open Access. 2022;3(2):125.

1. Introduction

The liver plays an essential role in lipid metabolism, several stages of lipid synthesis and transportation [1]. The formation and clearance of lipoproteins occur in the liver. From the diet and peripheral tissues it receives cholesterol and fatty acids and converts them into lipoprotein complexes, which are then released into the blood circulation. Liver diseases disrupt plasma lipids via different mechanisms. Plasma triacylglycerol and cholesterol levels are reduced in chronic liver disease, due to reduced biosynthetic capacity of lipoproteins [2]. Hepatic impairments caused by toxicants cause alterations in lipid metabolism [3], [4]. Changes in serum lipids are commonly found in patients with chronic liver disease [5].

Chemicals constitute an important cause of liver injury. Carbon tetrachloride is the most widely used hepatotoxic agent for the induction of liver damage [1], [6].

Plants possessing medicinal benefits have been found useful for the treatment of various diseases [7], [8]. They constitute cheap alternative to orthodox drugs since they are readily available [9], [10].

Dialium guineense is a medicinal plant used in Traditional Medicine for the treatment of different disease conditions, such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids [11], [12]. Extracts of the plant have been shown to be reservoirs of important bioactive compounds and phytochemicals [13] – [15]. 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 CCl_4. This study investigated the effect of total saponins and tannins isolated from the stem bark of *D. guineense* stem bark on lipid profile and CCl_4- induced histological changes in liver of Wistar rats.

2. Materials and Methods

2.1 Chemicals and Reagents

Analytical grade chemicals and reagents were used in this study and they were purchased from Sigma-Aldrich Ltd. (USA).

2.2 Collection of Plant Material

The plant material obtained from Auchi, Edo State, Nigeria, was identified and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330).

2.3 Plant Preparation and Extraction

The stem barks of *D. guineense* were washed and shade-dried at room temperature. The plant material was then pulverized using mechanical blender. Total saponins and tannins were isolated from the plant stem bark using standard methods as previously described [13], [16].

2.4 Experimental Rats

A total of twenty-five adult male Wistar rats, which weighed between 160 and 180 g were purchased from Anatomy Department, University of Benin, Benin City, Nigeria. The rats were subsequently housed in metal cages under standard

laboratory conditions: room temperature (25 ^{o}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. Just before the commencement of the study, the rats were acclimatized to the laboratory environment for a duration of seven days. Standard experimental protocol was followed for this study.

2.5 Experimental Design

The rats were assigned to five groups of 5 rats each: normal control, CCl_4 control, silymarin, total saponins and total tannins groups. Liver damage was induced in the rats using CCl_4 (single oral dose of 1.0 mL/kg body weight, bwt) [17]. 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 150 mg/kg bwt of total saponins or tannins orally for a duration of 28 days.

2.6 Blood Sample Collection and Preparation

At the end of the 28-day treatment period, the rats were euthanized. Blood samples were collected from the anesthetized rats through cardiac puncture in heparinized sample containers, and centrifuged at 2000 rpm for 10 min to obtain plasma. 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.

2.7 Biochemical Analysis

Lipid profile parameters were determined using Randox kits [18] – [20]. The other parameters were determined by calculations as shown below:

VLDL-C = TG/5

LDL-C = TC – (TG/5) – HDL-C [21]

AIP (rats): LDL-C + VLDL-C/HDL-C [21]

$$AC = \frac{(TC-HDL\text{-}C)}{HDL\text{-}C} \text{ [22]}$$

$$CRR = \frac{TC}{HDL\text{-}C} \text{ [23]}$$

2.8 Histological Examination of the Tissues

Sizeable portions of the liver were sectioned and fixed in 10 % formalin for 48 h, and thereafter dehydrated using varied concentrations of ethanol. Just before embedment in paraffin, the specimens were cleared thrice with xylene. Serial sections of 4 µm thickness were cut and stained with haematoxylin and eosin (H & E) according to standard protocol. Histopathological examination was carried out 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).

2.9 Statistical Analysis

Count data are expressed as mean ± standard error of mean (n = 5). The statistical analysis was performed using SPSS (version 20). The various treatment groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

3. Results

3.1 Effect of Total Saponins and Tannins of D. *guineense* Stem Bark on Relative Liver Weight

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

Table 1: Relative Liver Weights.

Group	**Relative liver weight x 10^{-2}**
Normal Control	2.98 ± 0.05
CCl_4 Control	2.86 ± 0.06
CCl_4 + Silymarin	2.84 ± 0.06
CCl_4 + T. Saponins	2.98 ± 0.05
CCl_4 + T. Tannins	2.99 ± 0.20
Data are relative liver weights and are expressed as mean ± SEM (n = 5).	

Where T. Saponins and T. Tannins = total saponins and total tannins, respectively.

3.2 Effect of Total Saponins and Tannins of D. *guineense* Stem Bark on Lipid Profile and Rats Liver Histology

The levels of TC, TG, HDL-C, VLDL-C, LDL-C as well as AIP were significantly lower in CCl_4 control group than in normal control group, but they were increased after treatment with total saponins or tannins ($p < 0.05$). There were no significant differences in AC and CRR among the groups ($p > 0.05$). Carbon tetrachloride (CCl_4) markedly disrupted the structure of hepatocytes and induced steatosis (intra-hepatocyte fat in-growth and inflammation). However, treatment with total saponins and tannins of *D. guineense* stem bark showed marked regeneration of hepatocytes (unremarkable hepatic lobular architecture). The toxic hepatic injury induced by CCl_4 was significantly ameliorated by the phytochemicals. These results are shown in Figures 1 to 4.

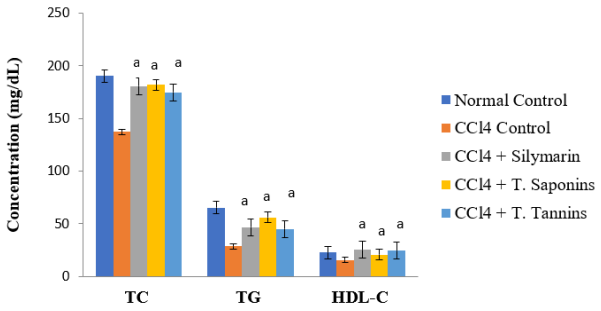

Fig. 1. Comparison of lipid profile parameters among the groups**.**

Data are lipid profile, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

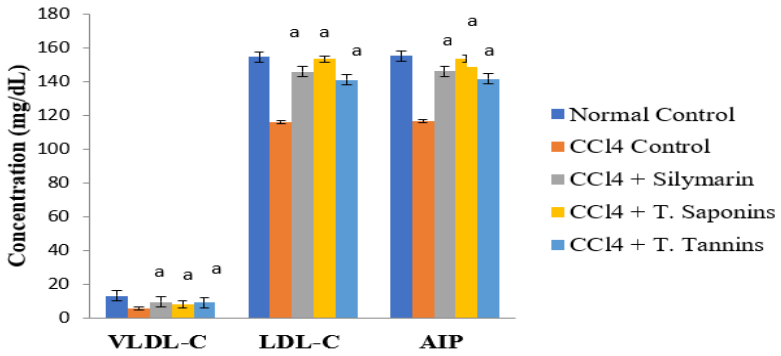

Fig. 2. Effect of total saponins and tannins of *D. guineense* stem bark

On lipid profile of rats.

Data are lipid profile parameters, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control.

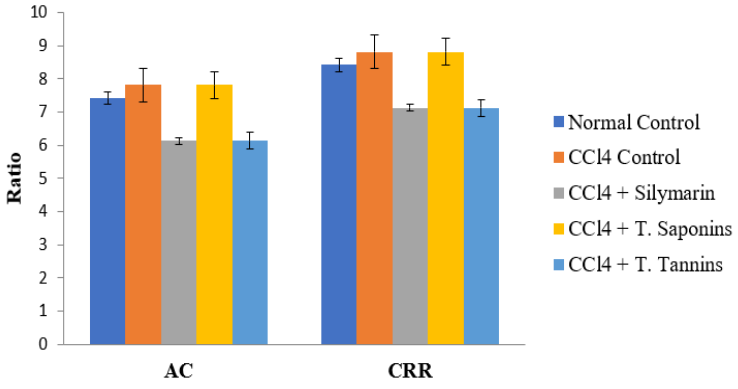

Fig. 3. Comparison of AC and CRR among the groups.

AC = atherogenic coefficient; CRR = cardiac risk ratio.

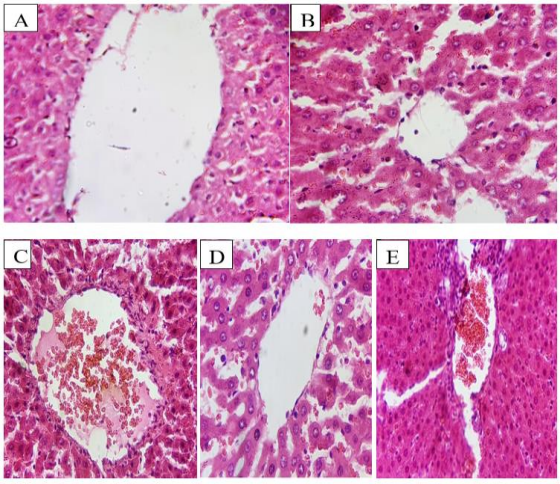

Fig. 4. Effect of total saponins and tannins of *D. guineense* stem bark on histology of rats liver.

Histology of normal control rat liver revealed distinct centriole (hepatocytes and well fenestrated sinusoidal with mild mononuclear cells), while that of CCl_4 control showed visible centriole with the hepatocytes nuclei appearing vacuolated. There were 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 total saponins-treated rats revealed visible centriole with the hepatocytes nuclei appearing vacuolated. There were visible prominent fatty changes and mononuclear cells, while those of total tannins-treated rats showed centriole with thickened wall surrounded by foci clusters of mononuclear cells with the hepatocytes having pyknotic nuclei.

4. Discussion

Major injuries to hepatocytes, such as those caused by alcohol consumption, chronic viral hepatitis or cirrhosis of the liver, produce abnormal liver function and moderate decrease in levels of total cholesterol and HDL-C [24]. Steatosis is developed in nonalcoholic fatty liver disease mainly due to altered level of hepatic lipid, particularly a decrease in polyunsaturated fatty acid (PUFA). The gene expression in liver and skeletal muscles are influenced by PUFA, therefore, reduction occurs in fatty acid synthesis, triacylglycerol storage, and fatty acid oxidation increases. The changes in tissues and PUFA contents moreover affect eicosanoid synthesis, which further promote the inflammation and steatosis [25]. 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 [26]. The aim of this study was to investigate the effect of total saponins and tannins isolated from the stem bark of *D. guineense* on lipid profile and CCl_4- induced histological changes in liver of Wistar rats. The results indicate that the lipids synthesis ability of the liver may be reduced with CCl_4 induction, and are in agreement with reports of previous studies [27 – 29]. Total saponins and tannins of *D. guineense* stem bark may have 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.

The effect produced by the isolated total saponins and tannins of the medicinal plant was comparable to that of silymarin (standard hepatoprotective drug). Silymarin protects liver against xenobiotic injury via regulation of liver secretion and uptake of plasma lipoprotein, while increasing the intracellular glutathione level [30]. 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 CCl_4-induced lipid peroxidation and hepatotoxicity in mice, first, by decreasing the metabolic activation of CCl_4 and second, by acting as a chain-breaking antioxidant [31]. In addition, silymarin is able to stimulate protein synthesis resulting in production of new liver cells to replace older and damaged ones [32].

Histopathological examination of rats liver provided supportive evidence for lipid profile analysis. Carbon tetrachloride (CCl_4) markedly disrupted the structure of hepatocytes and induced steatosis (intra-hepatocyte fat in-growth and inflammation). However, treatment with the phytochemicals showed marked regeneration of hepatocytes, 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.

5. Conclusion

The toxic hepatic injury induced by CCl_4 was significantly ameliorated by treatment with total saponins and tannins isolated from the stem bark of *D. guineense*. The dose of the phytochemicals used produced a reasonable degree of improvement in lipid profile of the rats.

REFERENCES

1. Friedman SE, Grendel JH, and McQuaid KR. Current Diagnosis and Treatment in Gastroenterology. New York. Lange Medical Books, McGraw Hill. 2003; 664-679p.
2. Mandal SK, Sil K, Chatterjee S, et al. A study on lipid profiles in chronic liver diseases. Nat J Med Res. 2012;3: 70-72.
3. Irshad M, Dube R, and Joshi YK. Impact of viral hepatitis on apo- and lipoprotein status in blood. Med Princ Pract. 2007;1(6):310-314.
4. Vere CC, Neagoe D, Streba CT, et al. Steatosis and serum lipid patterns in patients with chronic viral hepatitis: Differences related to viral etiology. Romanian J Morphol Embryol. 2010;51:509-514.
5. Su TC, Lee YT, Cheng TJ, et al. Chronic hepatitis B virus infection and dyslipidemia. J Formos Med Assoc. (Taiwan yi zhi). 2004;103:286-291.
6. Gupta AM, Mazumder UK, and Das S. Effect of alkaloid extracts *Clerodendron colebrookianum* on hematological parameters and hepatorenal function in mice. Indian J Exp. Biol. 1994;32:189-191.
7. Grabley T and Akuodor CW. Medicinal plants and traditional medicine. Watermelon Crop Information. Cucurbit breeding horticultural science, 2010.
8. Abu OD and Onoagbe IO. Biochemical effect of aqueous extract *of Dialium Guineense* stem bark on oxidative status of normal Wistar rats. Int J Clin Biol Biochem. 2019;1(2):15-18.
9. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edition. Spectrum Books Ltd., Ibadan, Nigeria. 1993; 289p.

10. Akah PA and Nwabie AI. Evaluation of Nigerian traditional medicinal plants used for rheumatic inflammatory disorders. J Ethnopharmarcol. 1994;42:179-182.
11. Abu OD, Imafidon KE, and Iribhogbe ME. Biochemical effect of aqueous leaf extract of *Icacina trichanta* Oliv. on urea, creatinine and kidney oxidative status in CCl_4-induced Wistar rats. Nigerian J Life Sci. 2015;5(1):85-89.
12. Bero J, Ganfon H, Jonville MC, et al. In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J Ethnopharmacol. 2009;122(3):439-444.
13. Hostettmann K and Marston A. Saponins. Cambridge: Cambridge University press. Pp. 3.
14. Kar, A. (2007). Pharmacognosy and Pharmaco-biotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers, New Delhi. 1995, 332-600p.
15. Abu OD, Onoagbe IO, and Obahiagbon O. Qualitative phytochemical screening and proximate analysis of *Dialium guineense* stem bark. IAR J Agri Res Life Sci. 2020;1(4):108-112.
16. Haslam E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J Nat Prod. 1996;59:205-215.
17. Abu OD, Imafidon KE, and Iribhogbe ME. Aqueous leaf extract of *Icacina trichanta* Oliv. ameliorates CCl_4-induced liver toxicity in Wistar rats. J Nigerian Soc Exp Biol. 2017;17(3):107-111.
18. Reiser R, Probstfield JL, Silvers A, et al. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. Am J Clin Nutr. 1985;42(2):190-197.
19. Tietz NW, Finley PR, and Pruden EL. Clinical guide to laboratory tests. 2nd Ed, W.B. Saunders, Philadelphia. 1990; 304-306p.
20. Virella L, Stone MF, Ellis P, et al. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem. 1977;23(5):882-884.
21. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 18(6):499-502.
22. Ikewuchi CJ and Ikewuchi CC. Alteration of Plasma Lipid Profiles and Atherogenic Indices by *Stachytarpheta jamaicensis* L. (Vahl). Biokemistri. 2009;21(2):71-77.
23. Frohlich J and Dobiášová M. Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography. Clin Chem. 2003;49 (11):1873-1880.
24. Hoover-Plow J and Huang M. Lipoprotein (a) metabolism: Potential sites for therapeutic targets. Metabolism. 2013;62:479-491.
25. Arendt BM, Mohammed SS, Aghdasasi E, et al. Hepatic fatty acid compission differs between chronic hepatitis C patients with and steatosis. J Am Soc Nutr. 2009;139:691 –695.
26. Halsted CH. Nutrition and alcoholic liver disease. Semin Liver Dis. 2004;24(3):289-304.
27. Siagris D, Christofidou M, Theocharis GJ, et al. Serum lipid pattern in chronic hepatitis C: histological and virological correlations. J Viral Hepat. 2006;13(1):56 – 61.
28. Selimoglu MA, Aydogdu S, and Yagci RV. Lipid parameters in childhood cirrhosis and chronic liver disease. Pediatr Int. 2002;44(4):400-403.
29. Sposito AC, Vinagre CG, Pandullo FL, et al. Apolipoprotein and lipid abnormalities in chronic liver failure. Braz J Med Biol Res. 1997;30(11):1287-1290.

30. Toklu HZ, Akbay T, Velioglu-Ogunc A, et al. Silymarin, the Gedik, antioxidant component of *Silybum marianum*, prevents sepsis-induced acute lung and brain injury. J Surg Res. 2008;145(2):214-222.
31. Letteron P, Labbe G, Degott C, et al. Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice: Evidence that silymarin acts as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochem Pharmacol.1990;39(12): 2027-2034.
32. Cecilia LB, Enrique JS, Aldo DM, et al. Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. Chem.-Biol. Interact. 2009;179:297-303.

Citation: Abu OD, Omage JI, and Ogbebor EO. Effect of total saponins and tannins isolated from the stem bark of *dialium guineense* on lipid profile and CCl4-induced histological changes in liver of wistar rats. J Bio Med Open Access. 2022;3(2):125.