Evaluation of effect of *Trema guineensis* extracts on serum concentrations of vitamins A, D and E during CCl$_4$ induced hepatotoxicity in rat

Droucoulia Guillaume Cyril, Okpekon Aboua Timothée, Séri Kipré Laurent, Tra Bi Irié Otis, Tchimon Dahno Perrine and Yapi Houphouët Félix

Abstract

The present study investigated the preventive effect of *Trema guineensis* on serum concentrations of vitamins A, D and E during hepatotoxicity in rat. The leaves of *Trema guineensis* collected from Abobo in the district of Abidjan were used for the preparation of the aqueous extract by decoction and ethanolic extract by maceration. Then, healthy adult Wistar albino rats were used for the experimentation. The animals were pretreated with the extracts at the doses of 100 and 200 mg/kg of body weight by oral route one hour before CCl$_4$ intraperitoneal injection for seven days. The administration of CCl$_4$ resulted in a significant decrease of vitamins A, D and E rates in the serum while the pretreatment by the extracts increased the concentrations of these vitamins. The extracts of *Trema guineensis* would prevent vitamins A, D and E deficiency in serum due to hepatic injury.

Keywords: *Trema guineensis*, Vitamins A, D and E, Hepatotoxicity, Côte d’Ivoire

1. Introduction

Liver provides several vital functions in the body grouped in exocrine and endocrine functions including metabolism and storage of vitamins [1]. This organ is generally damaged during its functioning by infections or toxic chemicals resulting in lesions associated with alteration of these functions [2]. Several studies showed serum rate of vitamins A, D and E deficiency during hepatic injury [3, 4, 5]. These vitamins can not be synthesized by the body. They are generally provided by food and are essential for life. The vitamins A, D and E deficiency is a serious public health problem [6]. The search for natural product efficient to prevent liver injury and vitamins A, D and E deficiency would be a capital interest. *Trema guineensis* is a plant consumed as food or used for the treatment of various pathologies [7, 8, 9]. Previous work revealed hepatoprotective property of this plant. In addition, it contained vitamins A, D and E then increased their serum concentration after supplementation [10, 11]. It would therefore be judicious to evaluate the effect of *Trema guineensis* on the serum concentrations of vitamins A, D and E during hepatotoxicity.

2. Materials and Methods

2.1 Collect and extracts preparation

*Trema guineensis* leaves were collected from Abobo (Abidjan). This plant was identified and authenticated by Department of Botany of Felix Houphouët Boigny University. These leaves were dried at ambient temperature safe from light during two weeks then pulverized using an electric crusher (IKAType MAG®). The powder was useful for various extractions.

2.2 Aqueous extraction

Hundred grams of plant powder was boiled in one Liter of distilled water for 10 minutes. The decoction was filtered twice on hydrophilic cotton and then once on filter paper Whatman N°3. The filtrate was dried in an oven at 40°C [12, 13].

2.3 Ethanolic extraction

Hundred grams powder of *Trema guineensis* leaves was macerated in one liter of the mixture ethanol-water 70% for 24 hours.
The macerate was filtered twice on hydrophilic cotton and then once on filter paper Whatman N° 3. The filtrate was concentrated at a temperature of 40 °C using a rotary evaporator Büchi-type and then dried in an oven at 40 °C

2.4 Animals
Healthy adult Wistar albino rats of mean weight 127.34 ± 2.43 g were used for the study. These animals came from animal house of Pharmacology Laboratory of Training and Research Unit of Pharmaceutical and Biologic Sciences, Felix Houphouët Boigny University, Côte d’Ivoire where they were kept under favorable conditions of breeding and ethics. These rats were fed a standard complete food in the form of FACI (Fabrication d’Aliments de Côte d’Ivoire) pellets and drank tap water served ad libitum and renewed daily.

2.5 Experimental Treatment
Thirty-six healthy rats divided into five groups of six animals were used for this study. These animals received for seven days plant extracts by gavage one hour before CCl₄ intraperitoneal injection every two days [15].
Group I (Normal): normal control received distilled water every day and one hour after olive oil (1 mL/kg body weight) on second, fourth and sixth days.
Group II (CCl₄): negative control treated with distilled water every day and one hour after CCl₄ (2 mL/kg body weight, 1:1 v/v with olive oil) on second, fourth and sixth days.
Group III (A100): rats received Trema guineensis aqueous extract (100 mg/kg) daily and CCl₄ (2 mL/kg body weight, 1:1 v/v with olive oil) on second, fourth and sixth days.
Group IV (A200): rats treated with aqueous extract of Trema guineensis (200 mg/kg) daily and CCl₄ (2 mL / kg body weight, 1:1 v/v with olive oil) on second, fourth and sixth days.
Group V (E100): rats received ethanolic extract of Trema guineensis (100 mg/kg) daily and CCl₄ (2 mL / kg body weight, 1:1 v/v with olive oil) on second, fourth and sixth days.
Group VI (E200): rats treated with Trema guineensis ethanolic extract (200 mg/kg) daily and CCl₄ (2 mL / kg body weight, 1:1 v/v with olive oil) on second, fourth and sixth days.

Each animal was anesthetized with ether and blood was collected from caudal vein before and after experiment [16]. Blood was centrifuged at 3000 rpm for 10 minutes (centrifuge B4i) to obtain serum, put in an eppendorf tube and protected by aluminum foil then stored at -20 °C until fat-soluble vitamins A, D and E analysis twenty-hours after each sampling.

2.6 Vitamins Analysis
Vitamins A (retinol), D (calciferol) and E (α-tocopherol) concentrations were determined after extraction by high performance liquid chromatography (HPLC) of Waters® type in isocratic mode coupled with a fluorometric detector [17].

Fat-soluble vitamins were extracted from 0.5 mL of serum. To this quantity, 10 mL of a 10% KOH solution are added in a methanol-water mixture (1:1; v/v). Then 0.025 g of ascorbic acid was added to avoid oxidation process during saponification. Mixture is then refluxed in a water bath at 70 °C for 30 minutes. After cooling mixture, 3 x 5 mL of hexane was added. Hexanic phases were combined, dried over anhydrous sodium sulfate and evaporated to dryness. The residue obtained was taken up in methanol and 20 µl of the methanol solution were injected into the chromatographic system for the quantitative analysis of the vitamins. Standard ranges were obtained from standard vitamin initial concentrations (vitamin A concentration: 0.9 mg/mL, vitamin D concentration: 0.091 mg/mL, vitamin E concentration: 2.8 mg/mL). Fluorometric detection of vitamins A, D and E was done at respective wavelengths of 455 nm, 245 nm and 295 nm. Vitamins concentrations in sample are determined from peaks area of standard molecule and sample by following formula:

\[
CVs = \frac{SVs \times CVsr}{SVsr}
\]

CVs: concentration of vitamin in sample; SVs: peak area of the vitamin in sample; CVsr: concentration determined from standard range for each vitamin; SVsr: peak area corresponding to concentration vitamin selected from standard range.

2.7 Statistical Analysis
Values are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the software GraphPad Prism 7.0 (USA). The analysis of the variances was performed using ANOVA followed by Dunnett for comparison of vitamins serum concentrations between different groups. The value of p<0.05 was considered significant.

3. Results
Before treatment, the concentration of vitamins A, D and E in serum were evaluated statistically equal by comparing each group with every other group. After seven days of treatment, CCl₄ involved a significant decrease (p<0.01 and p<0.001) of vitamins A (0,41±0,15 mg/mL), D (4,35±0,97 µg/mL) and E (51,12±9,31 mg/mL) in untreated intoxicated group compared to Normal group (vitamin A : 0,91±0,18 mg/mL ; vitamin D : 7,91±0,69 µg/mL ; vitamin E : 71,16±8,11 mg/mL). Pretreatment with aqueous and ethanolic extracts of Trema guineensis leaves reduced the toxic effect of CCl₄ on the serum concentration of fat-soluble vitamins (A, D and E). The animals received the extracts aqueous and ethanolic at the doses of 100 and 200 mg/kg of body weight presented significantly increased (* p<0,05, ** p<0,01 and *** p<0,001) serum concentrations of vitamins A, D and E compared to those observed in the rats intoxicated by CCl₄ and untreated (Figures 1, 2 and 3).
Fig 1: Effect of Trema guineensis extracts (aqueous and ethanolic) on serum concentration of vitamin A in CCl₄-treated rats.

Values are expressed as mean ± SD (standard deviation) with n=6. *P<0.05; **P<0.01; ***P<0.001. a: mean compared to normal control group; b: mean compared to CCl₄ negative control group. Normal: distilled water + olive oil; CCl₄: distilled water + CCl₄; A 100: Aqueous extract (100 mg/kg) + CCl₄; A 200: Aqueous extract (200 mg/kg) + CCl₄; E 100: Ethanolic extract (100 mg/kg) + CCl₄; E 200: Ethanolic extract (200 mg/kg) + CCl₄.

Fig 2: Effect of Trema guineensis extracts (aqueous and ethanolic) on serum concentration of vitamin D in CCl₄-treated rats.

Values are expressed as mean ± SD (standard deviation) with n=6. *P<0.05; **P<0.01; ***P<0.001. a: mean compared to normal control group; b: mean compared to CCl₄ negative control group. Normal: distilled water + olive oil; CCl₄: distilled water + CCl₄; A 100: Aqueous extract (100 mg/kg) + CCl₄; A 200: Aqueous extract (200 mg/kg) + CCl₄; E 100: Ethanolic extract (100 mg/kg) + CCl₄; E 200: Ethanolic extract (200 mg/kg) + CCl₄.

Fig 3: Effect of Trema guineensis extracts (aqueous and ethanolic) on serum concentration of vitamin E in CCl₄-treated rats.

Values are expressed as mean ± SD (standard deviation) with n=6. ***P<0.001. a: mean compared to normal control group; b: mean compared to CCl₄ negative control group. Normal: distilled water + olive oil; CCl₄: distilled water + CCl₄; A 100: Aqueous extract (100 mg/kg) + CCl₄; A 200: Aqueous extract (200 mg/kg) + CCl₄; E 100: Ethanolic extract (100 mg/kg) + CCl₄; E 200: Ethanolic extract (200 mg/kg) + CCl₄.

4. Discussion
Many plants are constantly required for their pharmacological properties. This study has been to evaluate the preventive effect of Trema guineensis on serum...
concentration of vitamins A, D and E deficiency during liver injury. The liver plays a vital role in the metabolism and performs synthesis and detoxification functions. Thus, it is damaged by toxic chemicals during its functioning [18, 19]. CCl₄ intoxication was largely widespread as an experimental model of hepatic damage [20, 21]. CCl₄ is metabolized at the liver which leads to the production of free radicals responsible for the toxic effects of this product. These metabolites bind in a covalent way to the intracellular nucleophilic structures and initiate the peroxidation of the polyunsaturated fatty acids of cellular membranes, leading to cytology and hepatocyte necrosis [22, 23, 24, 25, 26]. CCl₄ intoxication significantly reduced the serum rates of vitamins A, D and E in the untreated intoxicated group in comparison to Normal group. This decrease is due to CCl₄-induced hepatic lesions [25, 26]. The liver produces proteins such as Retinol Binding Protein and Vitamin D Binding Protein which provide the plasmatic transport of vitamins A and D [5, 27, 28]. Moreover, it ensures the activation of the retinyl palmitate stored in hepatocytes or stellate cells in rétinol where necessary of the body [6]. This organ is also involved in the hydrolysis of vitamin D in 25-hydroxyvitamin D (25(OH) vitamin D or calcifediol) [29, 30]. It also metabolizes vitamin E into α-tocopherol whose transport in blood circulation is provided by α-tocopherol transport protein (α-TTP) [31]. Hepatic cells involvement would therefore limit the metabolic functions of the liver. These results are supported by Paula et al. (2006) [32], Di Sario et al. (2007) [33], Nair (2010) [34], Tanumihardjo (2011) [35]. Chaves et al. (2015) [5] and Konstantakis et al. (2016) [36] who showed that the serum rates of vitamins A, D and E were decreased during liver diseases. The pretreatment of CCl₄-intoxicated rats led to increase serum concentrations of vitamins A, D and E. These results are corroborated by Premalatha and Parameswari (2013) [37] who indicated that chrysian increased the serum concentrations of vitamins A and E in streptozotocin-intoxicated rats. The contribution of supplements containing vitamins A, D and E in their various forms increased the serum concentrations of those and potentiated their action in the body [11, 37, 38, 39]. Also, the presence of these vitamins in the extracts of Trema guineensis would justify the maintenance of the metabolic functions of the liver, consequently the serum concentrations of vitamins A, D and E against CCl₄-induced toxicity [10, 11].

5. Conclusion

The effect of Trema guineensis on the serum concentrations of vitamins A, D and E during hepatic toxicity was evaluated. This study showed that pretreatment with aqueous and ethanolic extracts of Trema guineensis leaves maintained elevated the serum rates of vitamins A, D and E against the serum reduction of these vitamins caused by hepatic intoxication. The aqueous extract at the dose of 200 mg/kg body weight had a better effect. Trema guineensis would therefore prevent serum concentrations of vitamins A, D and E deficiency during liver diseases.

5.1 Ethical Approval

The experimental procedures were conducted after the approval of the Ethical Guidelines of University (Côte d’Ivoire) Committee on Animal Resources. All these procedures used, were in strict accordance with the guidelines for Care and Use of Laboratory Animals and the statements of the European Union regarding the handling of experimental animals (86/609/EEC).

6. Références

1. Sujila RMS, Rajadura M, Shaiririba RM. Hepatoprotective Effect of B-Sitosterol on Lipid Peroxidation and Antioxidant Status in Ethanol-Induced Hepatotoxic Rats. International Journal of Integrative sciences, Innovation and Technology. 2014; 3(4):1-11.
2. Salem M, El-Rasheid H, Mahmoud A. Therapeutic effects of Curcumin and Royal jelly as natural antioxidants on some biochemical parameters in hepatotoxicity induced by carbon tetrachloride (CCl₄) in male albino rats. International Journal of Advanced Research. 2015; 3:520-535.
3. Di Sario A, Candelaresi C, Omenetti A, Benedetti A. Vitamin E in chronic liver diseases and liver fibrosis. Vitamins and Hormones. 2007; 76:551-573.
4. Stokes SC, Volmer AD, Gruenhage L, Lammert F. Vitamin D in chronic liver disease. Liver International. 2013; 33:338-352.
5. Chaves GV, Peres WA, Gonçalves JC, Ramalho A. Vitamin A and retinol-binding protein deficiency among chronic liver disease patients. Nutrition. 2015; 31:664-668.
6. Albahari AA, Greaves RF. Fat-Soluble Vitamins: Clinical Indications and Current Challenges for Chromatographic Measurement. Clinical Biochemist Reviews. 2016; 37:27-47.
7. Irvine FR. Woody plants of Ghana : with special reference to their uses. Oxford University Press, London (Grande Bretagne), 1961, 869.
8. Fongod AGN, Veranso MC, Libalah MN. Identification and use of plants in treating infertility in human females in Fako Division, Cameroon. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2013; 2:724-737.
9. Béné K, Camara D, Fofié NBY, Kanga Y, Yapi AB, Yapo YC et al. Étude ethnobotanique des plantes médicinales utilisées dans le Département de Transua, District du Zanzan (Côte d’Ivoire). Journal of Animal & Plant Sciences. 2016; 27:4230-4250.
10. Droucoulac GC, Okpekon AT, Kouakou SL, Miezan BAP, Djyh BN, Bla KB et al. Protective effect of Trema guineensis extracts on CCl₄-induced hepatotoxicity. IOSR Journal of Biotechnology and Biochemistry. 2017; 3(2):5-9.
11. Droucoulac GC, Tra BCO, Miezan BAP, Kouakou SL, Bla KB, Yapi HF. Concentrations of Fat-soluble Vitamins of Trema guineensis Aqueous and Ethanolic Extracts and Rats Supplemented. International Journal of Biochemistry and Biophysics. 2018; 6(1):26-31.
12. Nemlin J, Brunel JF. Travaux Pratiques de Matière Médicale (3ème année). Laboratoire de Phytologie, Université Nationale de Côte-d’Ivoire, 1995, 47.
13. Konkon NG, Adjoungoua AL, Manda P, Simaga D, N’Guessan KE, Kone BD. Toxicological and phytochemical screening study of Mitragyna Inermis (willd.) Oktze (Rubiaceae), anti diabetic plant. Journal of Medicinal Plants Research, 2008; 2:279-284.
14. Guede-guina F, Vangah-manda M, Harouna D, Bahi C. Potencies of Misca, a plant source concentrate against fungi. Journal of Ethnopharmacology. 1993; 14:45-53.
15. Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of Hygrophila auriculata (K. Schum) Heine Acanthaceae root extract. Journal of Ethnopharmacology. 2006; 104:124-128.

16. OFV. Informations sur la Protection des Animaux. Prélèvement de sang chez les rongeurs de Laboratoire et les lapins à des fins expérimentales. Office Vétérinaire Federal, 1981; 800:116-1.04.

17. Jedlička A, Klimeš J. Determination of Water- and Fat-Soluble Vitamins in Different Matrices Using High-Performance Liquid Chromatography. Chemical Papers, 2005; 59 (3):202-222.

18. Nwaehujor CO, Nwinyi FC, Ode JO. Liver protective activity of the methanolic of Crinum jagus bulb against acetaminophen-induced hepatic damage in Wistar rats. Asian Journal of Biochemistry, 2012; 7:182-193.

19. Jaiprakash H, Narayana S, Mohanraj J. Drug-induced hepatotoxicity in a tertiary care hospital in rural south India. North American Journal of Medical Sciences, 2012; 4:90-93.

20. Li R, Guo W, Fu Z, Ding G, Zou Y, Wang Z. Hepatoprotective Action of Radix Paeniae Rubra Aqueous Extract against CCL-Induced Hepatic Damage. Molecules, 2011; 16:8684-8693.

21. Ghaffari H, Venkataramana M, Nayaka SC, Ghassam BJ, Angaswamy N, Shekar S, Kumara SKK, Prakash HS. Hepatoprotective action of Orthosiphon diffusus (Benth.) methanol active fraction through antioxidant mechanisms: An in vivo and in vitro evaluation. Journal of Ethnopharmacology, 2013; 149:737-744.

22. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World Journal of Gastroenterology, 2005; 11:6684-6688.

23. Mégarbane B, Deye N, Baud F. Toxic hepatitis: Mechanisms of toxicity and specific pharmacological agents. Réanimation, 2007; 16:632-642.

24. Amin A, Mahmoud-Ghoneim D. Zizyphus spina-christi protects against carbon tetrachloride-induced liver fibrosis in rats. Food and Chemical Toxicology, 2009; 47:2111-2119.

25. Cui CP, Wei P, Liu Y, Zhang DJ, Wang LS, Wu CT. The protective role of Hepatopoietin Cn on liver injury induced by carbon tetrachloride in rats. Hepatology Research, 2009; 39:200-206.

26. Hamdy N, El-Demerdash E. New therapeutic aspect for carvedilol: antifibrotic effects of carvedilol in chronic carbon tetrachloride-induced liver damage. Toxicology and Applied Pharmacology, 2012; 261:292-299.

27. Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J. Human plasma transport of vitamin D after its endogenous synthesis. Journal of Clinical Investigation, 1995; 91:2552-2555.

28. O’Byrne SM, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. Journal of Lipid Research, 2013; 54:1731-1743.

29. Bikle DD. Nonclassic actions of vitamin D. Journal of Clinical Endocrinology and Metabolism., 2009; 94:26-34.

30. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chemistry & Biology, 2014; 21:319-329.

31. Eggermont E. Recent advances in vitamin E metabolism and deficiency. European Journal of Pediatrics, 2006; 165:429-434.

32. Paula TP, Feres WAF, Ramalho RA, Coelho HSM. Vitamin A metabolic aspects and alcoholic liver disease. Revista de Nutrição, 2006; 19:601-610.

33. Nair S. Vitamin D Deficiency and Liver Disease. Gastroenterology & Hepatology. 2010; 6:491-493.

34. Tanumihardjo SA. Vitamin A: biomarkers of nutrition for development. American Journal of Clinical Nutrition. 2011; 94:658-665.

35. Konstantakis C, Konstantakis P, Kalafateli M, Triantos C. Vitamin D deficieny in patients with liver cirrhosis. Annals of Gastroenterology, 2016; 29:297-306.

36. Premalatha M, Parameswari CS. Effect of chrysin on nephroprotective and antioxidant status in streptozotocin induced diabetic nephropathy rats. BioMedRx, 2013; 1:484-489.

37. Wittlin L, Logomarsino VJ. Therapeutic Effects of Vitamin E Supplementation in Liver Diseases and Transplantation. Journal of Gastroenterology and Hepatology Research, 2014; 3(6):1095-1102.

38. El-Alama A, Filali H, Rahmoune I, Tazi A, Hakkou F. Impact of drugs on Vitamins. Journal of Chemical and Pharmaceutical Research. 2015; 7:1-4.

39. Komolmit P, Kimtrakool S, Sukswatamnuay S, Thanapirom K, Chattrasophon K, Thaimai P et al. Vitamin D supplementation improves serum markers associated with hepatic fibrogenesis in chronic hepatitis C patients: A randomized, double-blind, placebo-controlled study. Scientific Reports. 2017; 7:8905-8915.