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Evaluation of effect of *Trema guineensis* extracts on serum concentrations of vitamins A, D and E during CCl_4 induced hepatotoxicity in rat

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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 Houphouet 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 [14].

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 Houphouet 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 Cote 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 CCl_4 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 (CCl_4): negative control treated with distilled water every day and one hour after CCl_4 (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 CCl_4 (2 mL/kg body weight, 1:1v/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 CCl_4 (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 CCl_4 (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 CCl_4 (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:

$$CVs = \frac{SVs \times CVsr}{SVsr} \quad (1)$$

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, CCl_4 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 CCl_4 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 CCl_4 and untreated (Figures 1, 2 and 3).

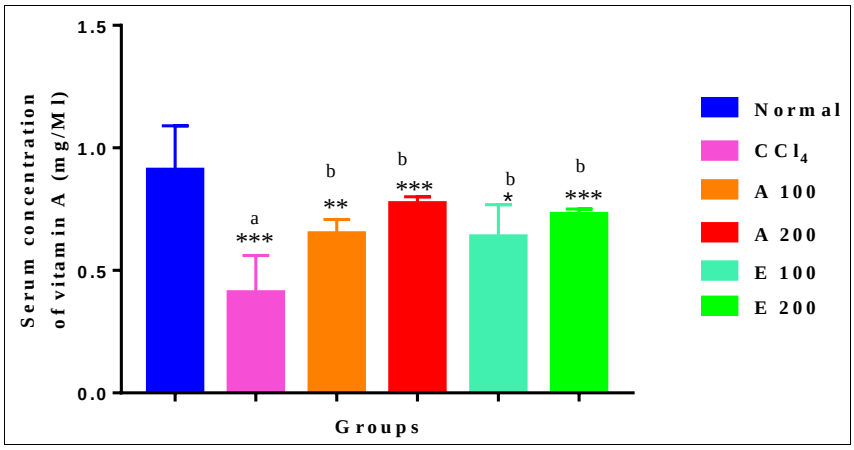

Fig 1: Effect of *Trema guineensis* extracts (aqueous and ethanolic) on serum concentration of vitamin A in CCl_4-treated rats.

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 CCl_4 negative control group. Normal: distilled water + olive oil; CCl_4: distilled water + CCl_4; A 100: Aqueous extract (100 mg/kg) + CCl_4; A 200: Aqueous extract (200 mg/kg) + CCl_4; E 100: Ethanolic extract (100 mg/kg) + CCl_4; E 200: Ethanolic extract (200 mg/kg) + CCl_4.

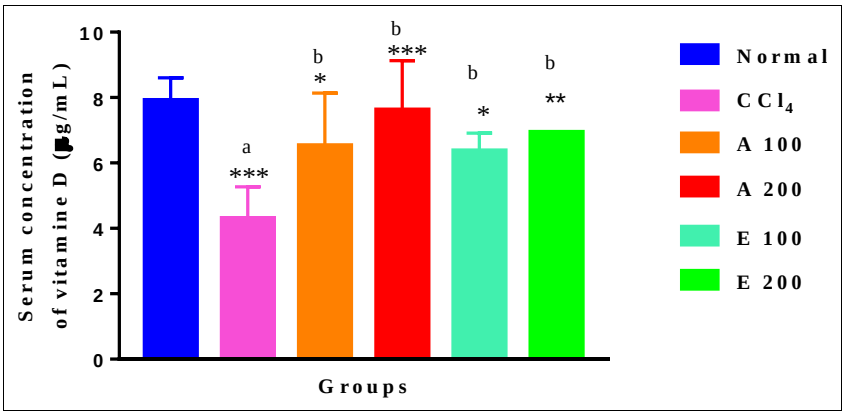

Fig 2: Effect of *Trema guineensis* extracts (aqueous and ethanolic) on serum concentration of vitamin D in CCl_4-treated rats.

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 CCl_4 negative control group. Normal: distilled water + olive oil; CCl_4: distilled water + CCl_4; A 100: Aqueous extract (100 mg/kg) + CCl_4; A 200: Aqueous extract (200 mg/kg) + CCl_4; E 100: Ethanolic extract (100 mg/kg) + CCl_4; E 200: Ethanolic extract (200 mg/kg) + CCl_4.

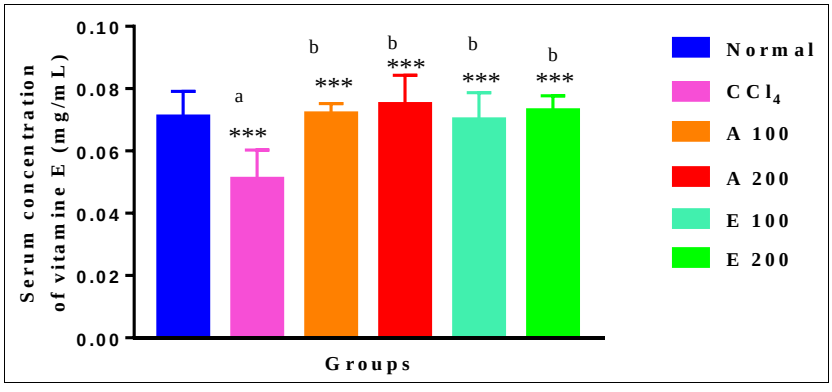

Fig 3: Effect of *Trema guineensis* extracts (aqueous and ethanolic) on serum concentration of vitamin E in CCl_4-treated rats.

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 CCl_4 negative control group. Normal: distilled water + olive oil; CCl_4: distilled water + CCl_4; A 100: Aqueous extract (100 mg/kg) + CCl_4; A 200: Aqueous extract (200 mg/kg) + CCl_4; E 100: Ethanolic extract (100 mg/kg) + CCl_4; E 200: Ethanolic extract (200 mg/kg) + CCl_4.

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 detoxication functions. Thus, it is damaged by toxic chemicals during its functioning [18, 19]. CCl_4 intoxication was largely widespread as an experimental model of hepatic damage [20, 21]. CCl_4 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 cytolysis and hepatocyte necrosis [22, 23, 24, 25, 26]. CCl_4 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 CCl_4-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) [3], Nair (2010) [33], Tanumihardjo (2011) [34], Chaves *et al*. (2015) [5] and Konstantakis *et al*. (2016) [35] who showed that the serum rates of vitamins A, D and E were decreased during liver diseases. The pretreatment of CCl_4-intoxicated rats led to increase serum concentrations of vitamins A, D and E. These results are corroborated by Premalatha and Parameswari (2013) [36] who indicated that chrysin 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 CCl_4-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).

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