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Protective effect of *Trema guineensis*extracts on CCl_4-induced hepatotoxicity

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Abstract: *This study was conducted to evaluate Trema guineensis leaves efficacy in preventing CCl_4-induced hepatotoxicity. The aqueous and ethanolic extracts were obtained from 100 g of leaves powder respectively by decoction in distilled water 1 Liter and by maceration in 1 Liter of mixture ethanol-water 70 %. The animals were orally pretreated with aqueous and ethanolic extracts at doses 100 mg/kg and 200 mg/kg body weight and 100 mg/kg body weight of silymarin (reference drug) daily for seven days while CCl_4 was administered by intraperitoneal injection every 48 hours. A significant decrease ($P < 0.05$, $P < 0.01$ and $P < 0.001$) in transaminases levels (AST and ALT) and gammaglobulins concentration was observed in pretreated groups with extracts and silymarin. Also, they significantly increased ($P < 0.05$, $P < 0.01$ and $P < 0.001$) serum total protein and albumin concentration. The results showed that Trema guineensis extracts possessed a protective potential against CCl_4-induced liver damage. Trema guineensis aqueous extract at dose 200 mg / kg body weight had an equal efficacy to that silymarin.*

Keywords:*Trema guineensis, silymarin, liver, transaminases, Côte.d'Ivoire*

I. Introduction

Liver performs several functions in body. It receives, metabolizes and stores molecules resulting from digestion and then ensures detoxification and secretion functions. It is therefore essential to life [1, 2]. During its operation, the liver is under threat from bacteria, viruses, parasites, adverse drugs effects and toxic chemicals [3]. Damage to liver may alter its functions, ranging from temporary elevation of liver enzymes to liver failure [4].

Despite medical advances, various surgical and therapeutic methods available for liver disease treatment are unsatisfactory and costly, especially for patients in developing countries [5, 6]. Therefore, there is a pressing need to seek alternative drugs for treatment or prevention of liver disease to replace limited efficacy of available applications and usually associated with toxicity serious risks. Many natural products are targeted for effective and sure protection and/or liver treatment[7, 8, 9].*Trema guineensis* is a species of West Africa tropical forest. It is used traditionally in Côte d'Ivoire for several diseases treatment[10, 11, 12]. Thus, previous work has revealed phytochemical composition, curative and preventive activities of *Trema guineensis*[13, 14, 15]. However,*Trema guineensis*protective effect on liver was not invested yet. Consequently, this study aims at hepatoprotective evaluation*Trema guineensis* aqueous and ethanolic extracts leaves.

II. Material and methods

2.1 Collect and extracts preparation

Trema guineensis leaveswere collected from Abobo (Abidjan). The plant species was later identified and authenticated by Department of Botany, Felix Houphouet Boigny University of Abidjan. These leaves were dried at ambient temperature safe from light during two weeks then pulverized using an electric crusher (IKA-type MAG®). The powder was useful for various extractions.

2.2 Aqueous extraction

100 g of plant powder were boiled in distilled water one Liter for 10 minutes. The decoction was filtered twice on absorbent white cotton then once on Whatman filter paper N°3. The filtrate was concentrated and dried to 40 °C with oven and preserved to –4 °C[16].

2.3 Ethanolic extraction

100 g of *Trema guineensis* leaves powder was macerated in one Liter of 70% (70:30 ; v/v) ethanol-water mixture for 24 hours. Macerate was filtered twice on absorbent cotton and then once on Whatman N°3 filter

paper. The filtrate was concentrated under reduced pressure to 40 °C using rotary evaporator BUCHI 161 Water Bath type and then dried in oven at 40 °C[17].

2.4 Experimental animals

Wistar albino rats (*Rattus norvegicus*) healthy adults weighing between 140 and 170 g were used for the study. The animals were provided and kept in animal house of Pharmacology Laboratory of Training and Research Unit of Pharmaceutical and Biological Sciences, Felix Houphouet Boigny University, Côte d'Ivoire.Within animalery, ambient temperature was 26 °C and relative humidity was 50±5 % with 12 hours light-dark intermittence. The animals were housed in large spacious and hygienic plastic cages throughout experimentation and fed with FACI® (Fabrication d'Aliments Composés Ivoiriens) pellets and drank tap water.

2.5 Hepatoprotective effect

*Trema guineensis*hepatoprotective activity was evaluated usingCarbon Tetrachloride(CCl_4) intraperitoneally to induce hepatotoxicity in rats. The animals were divided into seven groups of six each according to weight and treated for 7 days with plant extracts and silymarin (reference drug) by gavage [18, 19].

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 (Silymarin): positive control received silymarin (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 IV (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 V (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 VI (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 VII (E200): rats treated with *Trema guineensis*ethanolic extract (200 mg/kg) daily and CCl_4 (2 mL / kg body weight, 1:1 v/vwith olive oil) on second, fourth and sixth days.

2.6 Biochemical study

Animals were anaesthetized with ether and blood was collected by puncturing tail vein before and after experiment [20]. The blood was centrifuged at 3000 rpm for 10 minutes (B4i centrifuge) to separate serum. The serum was stored at -20 ° C until analysis. All animals were sacrificed just after blood collection and liver was removed, rinsed in saline solution, weighed and stored in Bouin. Serum samples were used for automatic analysis (Cobas C311, Hitachi Roche) biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) andserum total protein on one hand then albumin and gammaglobulins electrophoresis (Hydrasis 2 scan, sebia) on other hand.

2.7 Statistical analysis

The values expressed as Mean ± standard deviation (SD) from 6 animals. Statistical analysis was performed using analysis of variance (ANOVA) one way followed Dunnett's test. $P < 0.05$ was considered significant.

III. Results and discussion

3.1 Results

The results showed that biochemical parameters evaluated in serum before animals treatment (Day 0) were statistically identical comparing each group to each other (Tables 1 and 2).After seven (7) days of treatment (Day 8), a significant ($P < 0.001$) increase in transaminases levels (AST and ALT) was observed in CCl_4untreated intoxicated groupcompared to Normal group (Table 1). Therefore, CCl_4 in group II involveda significant reduction ($P < 0.001$)serum total protein and albumin then a significant ($P < 0.01$) rise in gammaglobulins concentration compared to normal control (Table 2).

Pretreatment with aqueous and ethanolic extracts of *Trema guineensis* leaves and silymarin (reference drug) for seven days significantly attenuated ($P < 0.05$, $P < 0.01$ and $P < 0.001$) CCl_4 toxic effectson liver enzymesrate (AST and ALT) compared to negative control group (Tables 1). The serum AST and ALT level recorded lowest value in pretreated groups with *Trema guineensis*aqueous extract at 200 mg/kg body weight. The aqueous and ethanolic extracts of *Trema guineensis* and silymarin significantly normalized ($P < 0.05$, $P < 0.01$ and $P < 0.001$) serum total protein, albumin and gammaglobulins levels compared to CCl_4-untreated group (Table 2). However, results observed in pretreated group with ethanol extract at 100 mg/kg body weight were not statistically different compared with CCl_4negative group.

3.2 Discussion

The protective effect of *Trema guineensis*aqueous and ethanolic extracts on liver was evaluated by biochemical parameters such as transaminases (AST and ALT), total protein, albumin and gammaglobulins.The liver is responsible for detoxification of toxic chemicals present in body. Thus, it is prey during its operation [21]. AST and ALT are sensitive indicators of necrotic lesion and damage in liver [22, 23]. Intraperitoneal CCl_4 administration resulted in a significantserum transaminaseselevation inCCl_4 group indicating severe liver cell damage [24,25]. The results showed that rats-treated with*Trema guineensis* aqueous and ethanolic extracts to various doses significantly prevented hepatotoxicity. There was a reduction ($P < 0.001$) in AST and ALT levels in groups treated with these extracts compared to CCl_4-groupcorresponding to maintaining liver cell membrane functional integrity. These results are supported by previous studies plant on CCl_4-induced hepatotoxic effect, including *Corchorus olitorius*[26], *Origanum elongatum*[27] and *Nigella sativa*[28]. In addition, CCl_4 significantly decreased serum total protein and albumin inCCl_4-untreated group compared to normal group. These parameters would be cellular dysfunction indicators in liver disease. Sathesh *et al.*,[29] and Salem *et al.*, [30] reported that CCl_4 caused ribosomes rupture and dissociation on endoplasmic reticulum leading to protein biosynthesis reduction. The results were in agreement with Sadeghi *et al.*,[31] which showed that CCl_4injection reduced total protein and albumin. On the other hand, pretreatment of rats with extracts reversed total protein and albumin reductionCCl_4-induced. Results were consistent with Salem *et al.*,(30) and Al-vui *et al.*, [28] studies.Significant increase in gammaglobulins concentration is associated with liver damage caused by CCl_4 injection. Michail and Papatheodoridis [32] and Henry and Teloh [33] who revealed a high gammaglobulins level produced by reticuloendothelial liver cells during liver disease. Administration of aqueous and ethanolic extracts of *Trema guineensis* eliminated CCl_4 toxic effects by reducing gammaglobulins level. This study showed that *Trema guineensis* possesses a protective potential against CCl_4-induced hepatotoxicity due to present compounds in its extracts.

Table 1 : Effect of aqueous and ethanolic *Trema guineensis* extractson transaminases (AST and ALT) in CCl4-induced rats hepatotoxicity

		NORMAL	CCl_4	SILYMARIN	A 100	A 200	E 100	E 200
AST (IU/L)	D0	259.7±11.33	248.7±21.26	252±22.34	262±12.53	267±44.77	245±13.58	256.7±36.99
	D8	242.7±20.21	$321±13^{a***}$	$229.7±12.47^{b***}$	$268±14.01^{b**}$	$227±19.33^{b***}$	$281.3±37.22^{b*}$	$278.7±24.85^{b**}$
ALT (IU/L)	D0	77±20.52	68.67±3.18	63.33±10.73	74.67±7.31	64±16.07	81.67±14.15	79.7±8.51
	D8	76±21.55	$167.7±17.17^{a***}$	$72.67±11.1^{b***}$	$87±11.36^{b***}$	$69.33±12.99^{b***}$	$133.3±11.05^{b**}$	$115±10.44^{b***}$

Values are expressed as mean ± SD (standard deviation) with n=6 in each group. D0: Day 0 (before treatment); D8: Day 8 (after treatment).* P<0.05 ; ** P<0.01 ; *** P<0.001.a: mean compared to normal control group; b: meancompared to CCl_4 negative control group.Normal: olive oil; CCl_4: negative control CCl_4; Silymarin: Silymarin 100 mg/kg/day + 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.

Table 2 : Effect of aqueous and ethanolic *Trema guineensis* extractson total protein, albumin and gammaglobulinsin CCl4-induced rats hepatotoxicity

		NORMAL	CCl_4	SILYMARIN	A 100	A 200	E 100	E 200
Total Protein (g/L)	D0	72.98±1.58	73.22±8.48	72.56±4.2	72.92±5.0	72.43±2.7	72.2±2.01	72.58±2.55
	D8	73.07±4.06	$66.74±0.56^{a***}$	$72.77±2.3^{b***}$	$70.36±1.9^{b**}$	$72.89±2.1^{b***}$	$68.4±0.99^{b}$	$69.6±2.47^{b*}$
Albumin (g/L)	D0	34.30±2.57	34.41±1.41	34.10±0.21	34.27±4.45	34.04±4.95	33.93±0.42	34.11±2.01
	D8	34.21±1.81	$26.70±1.04^{a***}$	$32.74±0.56^{b***}$	$30.95±2.47^{b**}$	$32.67±3.18^{b***}$	$29.41±1.69^{b}$	$30.24±1.94^{b*}$
Gamma-globulins (g/L)	D0	21.67±7.7	21.96±1.41	21.76±3.32	21.87±0.84	21.73±3.11	21.67±0.77	21.75±0.7
	D8	21.73±4.84	$27.75±1.13^{a**}$	$22.56±0.7^{b**}$	$23.22±3.39^{b*}$	$22.6±2.26^{b**}$	$25.99±3.96^{b}$	$23.66±1.32^{b*}$

Values are expressed as mean ± SD (standard deviation) with n=6 in each group. D0: Day 0 (before treatment); D8: Day 8 (after treatment). * 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: olive oil; CCl_4: negative control CCl_4; Silymarin: Silymarin 100 mg/kg/day + 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.

IV. Conclusion

The protective effect of aqueous and ethanolic *Trema guineensis*extracts on liver has been studied. This investigation showed that CCl_4-induced changes in biochemical parameters such as transaminases (AST and ALT), total proteins, albumin and gammaglobulins were restored by pretreatment with aqueous and ethanolic extracts of *Trema guineensis* and silymarin. The aqueous extract at dose 200 mg/kg body weight has an effective liver protection identical to silymarin.

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).

Competing interests

Authors have declared that no competing interests exist.

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