PROGRESS IN MEDICAL SCIENCES, 2019
VOL 3, NO. 1, PAGE 1 – 8
DOI: doi.org/10.47363/PMS/2019(3)117

ORIGINAL ARTICLE

Open Access

Effect of *Trema guineensis* (Celtidaceae) on ethanol-induced hypertension in Wistar rats

Balakiyém Kadissoli[1], Piwèlong Aklesso Mouzou[1], Tcha Pakoussi[1], Kwashi Eklu-Gadegbeku[1], Amedonou Kodjo Aklikokou[1,2], Messanvi Gbeassor[1,2]

[1]Laboratory of Physiology/Pharmacology of Natural Substances, Faculty of Sciences, University of Lome, Lome, Togo

[2]Center for Research and Training on Medicinal Plants, University of Lome, Lome, Togo

ABSTRACT

Objective: *Trema guineensis* is a traditional medicinal plant used to treat cardiovascular diseases in Africa. Here, we evaluated the effects of its hydro-alcoholic leaves on ethanol-induced hypertension.

Method: Four groups of five rats each were used in this study. Group I, the control group, received distilled water (10 ml kg^{-1}), Group II received ethanol (35°) at 3 g/kg, and Groups III and IV received ethanol (35°) at 3 g/kg followed by 200 and 400 mg/kg extract, respectively. Ethanol solutions were administered over 8 weeks.

Results: The ethanol solutions increased body weight, blood pressure, superoxide dismutase (SOD), and malondialdehyde (MDA), and reduced glutathione (GSH) and nitric oxide (NO). As for the hematological parameters, the ethanol solutions reduced hemoglobin, red blood cells, white blood cells, platelets, polymorphonuclear cells, and lymphocytes, as well as corpuscular volume compared with the control group. The *T. guineensis* extract significantly prevented increased body weight, blood pressure, SOD, MDA, GSH, and NO. The extract also normalized hematological parameters.

Conclusion: These results suggest that this extract contains antihypertensive properties that protect against ethanol-induced hypertension.

ARTICLE HISTORY

Received November 17, 2018
Accepted January 09, 2019
Published January 17, 2019

KEYWORDS

Hypertension; *Trema guineensis;* antioxidant; oxidative stress; ethanol; hematological parameters

Introduction

Hypertension is a common disease that affects more than 972 million people in the world and this figure is expected to increase to 1.56 billion by 2025 [1]. The prevalence of hypertension in Africa reached its maximum in 2000 and has been estimated at 8%, with an average of 33.3% in Northern Africa [2]. Treatments for hypertension pose problems in developing countries. Due to mainly economic reasons, people face difficulties with care-taking, which represents a major obstacle for disease management and creates a heavy burden for developing countries [2].

Phytotherapy has become a common alternative therapy across the world [3]. Approximately 80% of Africans use traditional medicine thatbased on herbs to treat various diseases. Among them, *Trema guineensis* is the most commonly used traditional medicine to treat cough, dysentery, asthma, sore throat, and buccal infections [4]. Decoction from leaves of the plant is used to treat high blood pressure on its own or together with *Gardenia ternifolia* leaves [4]. According to Trovato *et al.* [5], *T. guineensis* leaves are used for their diuretic, anti-inflammatory and parasitic effects, and the decoction from stems of this plant is used as a bath for convulsive crises in children.

Few studies have been conducted on this plant to establish its pharmacological use for hypertension. Our previous studies demonstrated that the semi-ethanolic extract of *Trema guineensis* possesses myorelaxant properties on the smooth musculature of the rat [6].

Chronic alcohol consumption causes metabolic disorders characterized by a disruption of liver enzymes and lipid profile and a reduction in pro-oxidant/antioxidant balance in various tissues [7]. Furthermore, ethanol consumption also affects hematological parameters [8,9].

Contact Balakiyem Kadissoli ✉ kadissoliarmiss@gmail.com Laboratory of Physiology/Pharmacology of Natural Substances, Faculty of Sciences, University of Lome, Lome, Togo.

This study was conducted to evaluate the effects of *Trema guineensis* administration on an ethanol-induced hypertension model.

Materials and Methods

Plant material and extraction

Trema guineensis leaves were harvested in Badou, a town located 220 Km from Lomé (Togo) in December 2016. The Botany Department of the Sciences Faculty of Lomé University authenticated and the *Trema guineensis* voucher specimen No 15379TG was deposited. The leaves were washed in tap water and dried. Once the leaves dried, they were reduced in powder weight to approximately 250 g. This quantity was reduced through uninterrupted agitation for 72 hours in an extraction solvent of water and ethanol (20:80). After dipping, the product was filtered using absorbent cotton and filter paper. Then, the obtained filtrate was evaporated in a rotavapor R-210 (Buchi) at 45°C. We obtained 28.2 g of extract, which corresponds to 11.28%.

Animals

Albinos Wistar rats, between 150 and 180 g, were used in this study. The animals bred at the Science Faculty at the University of Lomé (Togo) in plastic cages under standard light (12-hour day/night natural cycle) and temperature (25°C) conditions. The rats were fed a standard diet and provided with water *ad libitum*.

Experimental design

In this study, we evaluated the effect of the extract of *Trema guineensis* on ethanol-induced hypertension. Normotensive rats were randomly divided into four groups of five animals each. Group I received distilled water (10 ml/kg) and served as a negative control. Group II received ethanol (35°) at 3 g/kg and served as a positive control. Groups III and IV received ethanol (3 g/kg) at 35° and the extract at 200 mg/kg/day and 400 mg/kg/day, respectively. The treatment lasted 8 weeks. At the end of treatment, systolic arterial blood rate was measured as previously described [10]. After blood pressure measurement, arteriovenous blood was collected in heparinized and dry tubes. The heparinized tubes were used for hematological parameters, whereas the serum obtained from dry tubes was used for biochemical parameters. The serum was separated and total cholesterol, high-density lipoproteins (HDL) cholesterol, the triglycerides, urea, Glutamo-Oxalacétique Transaminase (GOT), Glutamyl-Pyruvate-Transaminase (GTP), uric acid, and creatinine rates were determined using commercial diagnostic kits BC 3000+ Mindray. Hematological analysis was performed using an automatic hematological analyzer (Coulter STKS, Beckman). Following blood draws, the heart, aorta, liver, and the kidney were dissected and weighed. The heart and the aorta were homogenized in McEwen's solution and the liver and the kidneys were homogenized in Tris-HCl buffer solution. The serum and the homogenate were stored at −20°C. Reduced glutathione (GSH) was determined by the method of Sedlak and Lindsays [11], superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [12], nitric oxide NO was determined by the method of Tom *et al.* [13], total proteins were determined by the method of Bradford [14], and malondialdehyde (MDA) was determined using the procedure of Satoh [15].

Statistical analysis

Results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey's test was used for statistical evaluation, and $p < 0.05$ was considered significant statistically.

Results

Effects of the extract on blood pressure

Blood pressure in rats receiving only ethanol increased significantly ($p < 0.001$); however, extract treatment prevented this increase in blood pressure. At the 400 mg/kg dose, the extract has a reduction in blood pressure of 26.60 ± 2.31 mmHg compared with the positive control (Fig. 1).

Extract effects on body weight

Ethanol significantly increased body weight compared with the negative control ($p < 0.001$). However, there was no difference between the 400 mg/kg dose group compared with the negative control (Fig. 2).

Extract effects on oxidative stress markers

Chronic ethanol administration significantly increased MDA and SOD rates and reduced GSH and NO in the aorta, heart, liver, and kidney (Figs. 3–6) compared with the negative control. Extract treatment reversed the effects on MDA and SOD rates and increased GSH and NO levels compared to the positive control.

Extract effects on lipid profile

Ethanol administration significantly increased total cholesterol by 43.61%, triglycerides by 7.64%, ath-

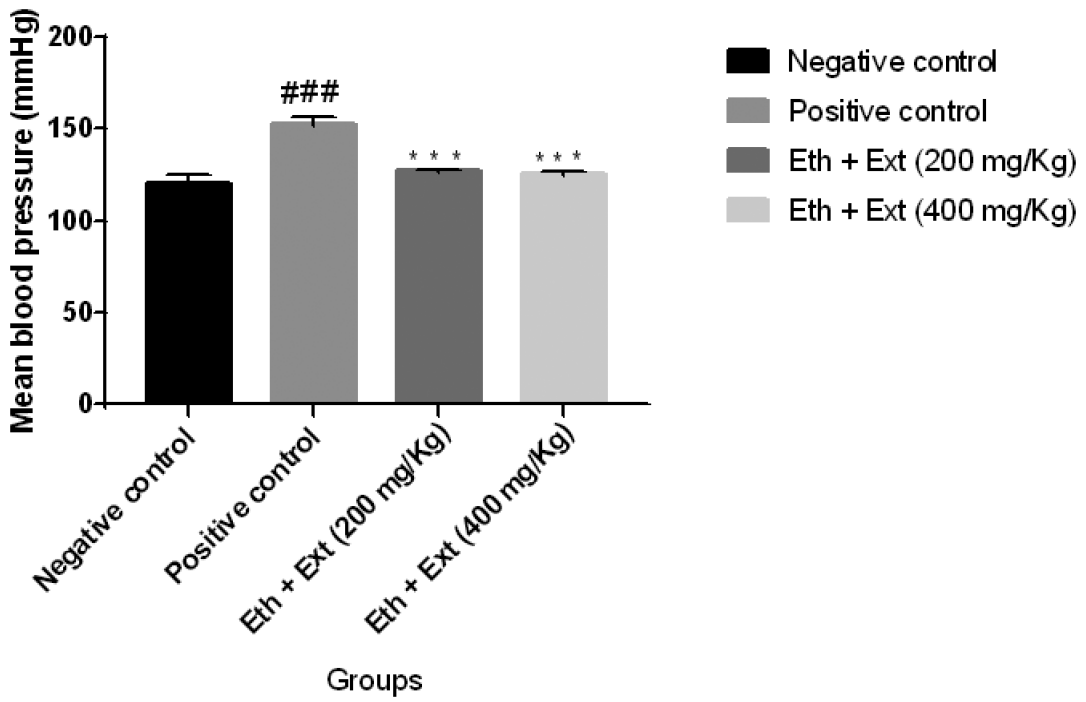

Figure 1. Effects of the extract on mean arterial blood pressure of the rats. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. ***p < 0.001 significant difference in comparison with the positive control. ###p< 0.001 significant difference in comparison with the negative control.

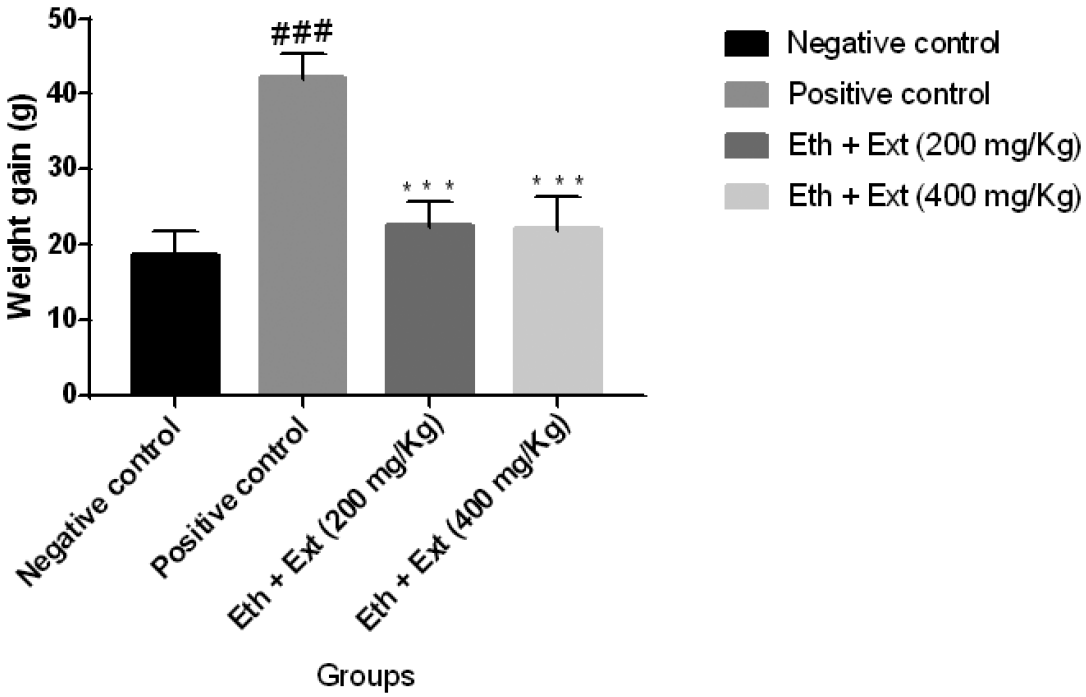

Figure 2. Effects of the extract on the weight of the rats. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. ***p < 0.001 significant difference in comparison with the positive control. ###p < 0.001 significant difference in comparison with the negative control.

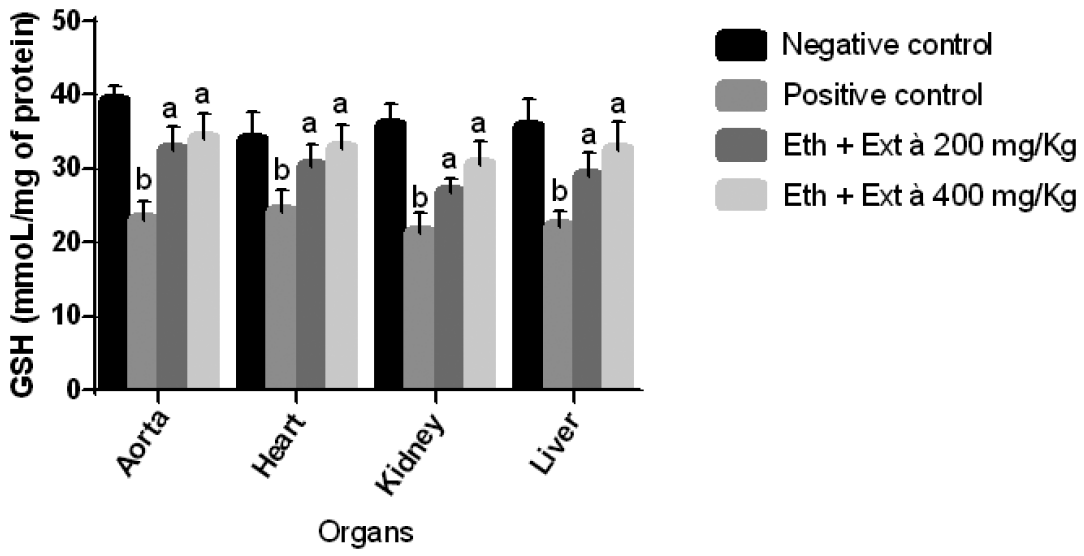

Figure 3. Effects of the extract on the rate of reduced GSH. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. [a]$p < 0.001$ significant difference in comparison with the positive control. [b]$p < 0.001$ significant difference in comparison with the negative control.

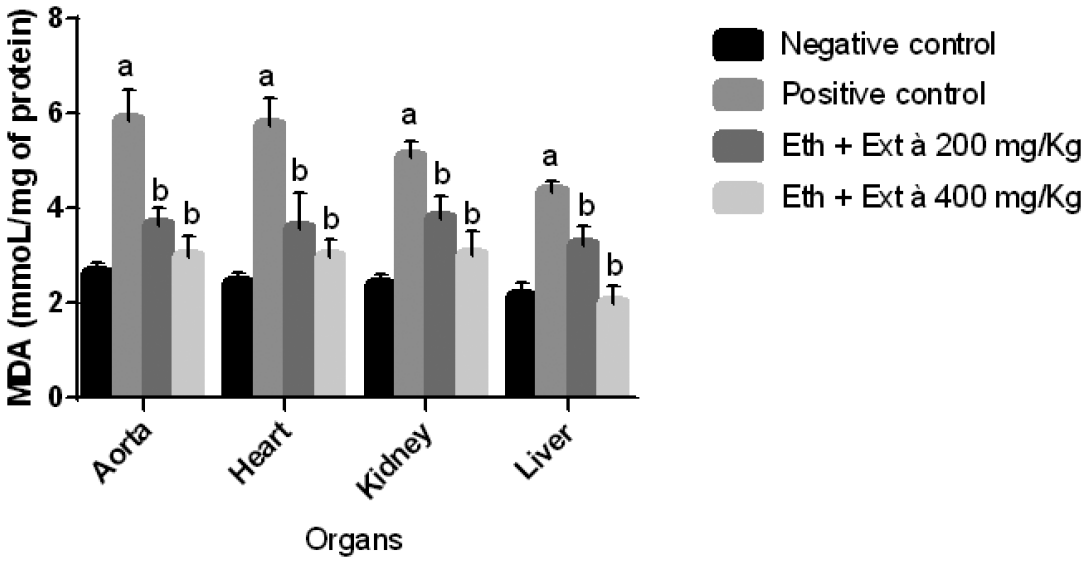

Figure 4. Effects of the extract on MDA. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. [b]$p < 0.001$ significant difference in comparison with the negative control.

erogenic index by 316.66, and low-density lipoproteins (LDL) cholesterol by 325.66% ($p < 0.001$), and reduced HDL cholesterol by 65.62% compared with the negative control. Extract treatment reversed the effects of ethanol on these parameters (Table 1).

Extract effects on the liver and kidney

Ethanol significantly increased urea (30.50%), uric acid (190.27%), GOT (13.10%), and GTP (52.74%) compared with the negative control ($p < 0.001$). Nevertheless, extract treatment significantly decreased GOT, GTP, urea, and uric acid levels compared with the negative control ($p < 0.001$). However, there was no effect on creatinine rate in the kidney (Table 2).

Extract effects on hematological parameters

Ethanol reduced hemoglobin (HB) (26.21%), red blood cell (RBC) count (55.29%), platelets (PLTs) (16.23%), polymorphonuclear cells (31.51%), lymphocytes (LYs) (56.57%), and white blood cell (WBC) count (68.57%). It also increased corpuscular

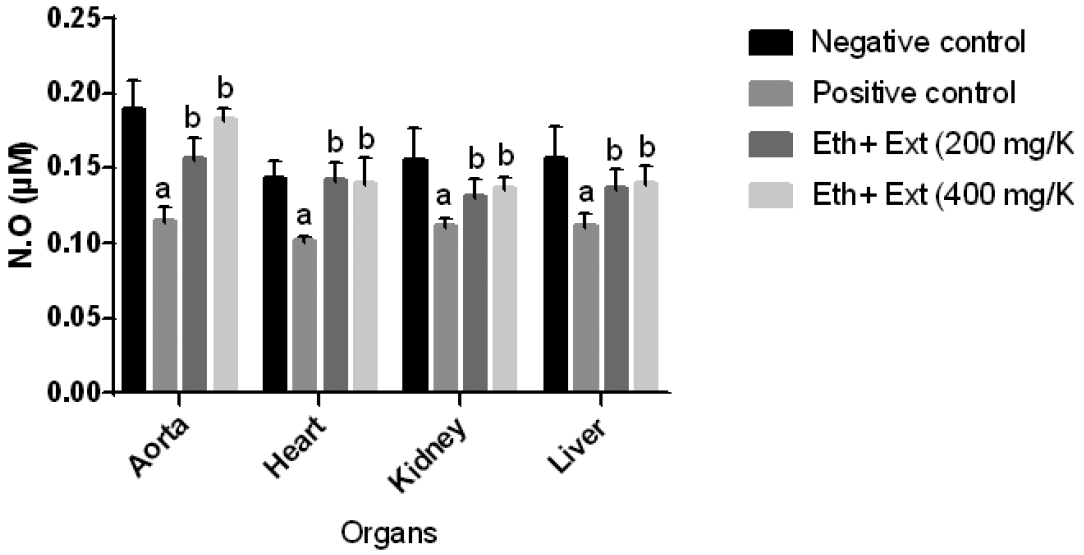

Figure 5. Effects of the extract on the NO of the rats. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. [b]$p < 0.001$ significant difference in comparison with the positive control. [a]$p < 0.001$ significant difference in comparison with the negative control.

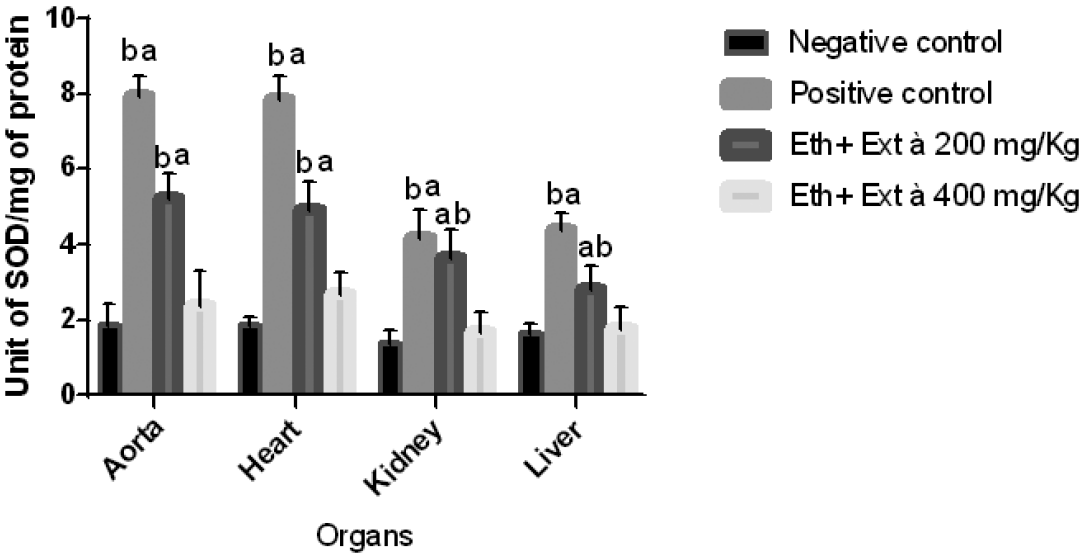

Figure 6. Effects of *Trema guineensis* extract on SOD levels. Every value represents mean ± SEM (n = 5). Data analysis is performed by one-way ANOVA followed by Tukey's post-hoc test. [a]$p < 0.001$ significant difference in comparison with the positive control. [b]$p < 0.001$ significant difference in comparison with the negative control.

volume (VGM) (43.72%) compared with the negative control (Table 3).

Discussion

Traditional practitioners in Togo treat cardiovascular diseases such as hypertension using *Trema guineensis*. In the current study, we evaluated the antihypertensive activities of *Trema guineensis* extract on ethanol-induced hypertension in rats.

We found that significantly increased blood pressure in the rats treated with ethanol was reversed by extract treatment. This effect may be due to the vasodilator action of the *Trema guineensis* extract as demonstrated *in vitro* on the isolated aorta [6].

Ethanol induces hypertension at high doses by depleting antioxidants and increasing oxidative tissue lesions [16]. Increases in reactive oxygen species and alteration of antioxidant defense status are a parameter of cardiovascular dysfunction

Table 1: Effects of extract on the lipid profile of the rats.

Parameters	Negative control	Positive control	Eth + Ext (200 mg/kg)	Eth + Ext (400 mg/kg)
Triglycerides (mg/dl)	38.20 ± 2.24	56.40 ± 3.69 ##	37.80 ± 3.23 **	34.80 ± 2.20 ***
Cholesterol (mg/dl)	56.40 ± 1.43	81.00 ± 3.36 ###	59.40 ± 3.60 **	55.00 ± 4.30 ***
HDL (mg/dl)	35.20 ± 2.31	12.10 ± 0.21 ###	30.20 ± 1.35 ***	32.40 ± 2.50 ***
LDL (mg/dl)	13.56 ± 2.35	57.72 ± 2.84 ###	21.16 ± 3.54 ***	15.64 ± 3.45 ***
Atherogenic index	1.62 ± 0.12	6.75 ± 0.26 ###	1.97 ± 0.15 ***	1.72 ± 0.14 ***

Every value represents mean ± SEM, with $n = 5$. $^{**}p < 0.01$, $^{***}p < 0.001$, significant difference in comparison with the positive control. $^{\#\#}p < 0.05$, $^{\#\#\#}p < 0.001$, significant difference in comparison with negative control.

Table 2: Effects of extract on liver and kidney of the rats.

Parameters	Negative control	Positive control	Eth + Ext at 200 mg/kg	Eth + Ext at 400 mg/kg
GOT (U.I)	175.60 ± 7.40	198.61 ± 3.58 #	170.60 ± 1.56 ***	165.20 ± 4.74 ***
GTP (U.I)	165.80 ± 2.87	254.84 ± 3.92 ###	162.20 ± 1.35 ***	154.60 ± 4.43 ***
Urea (mg/dl)	43.70 ± 2.12	57.03 ± 3.18 ##	42.81 ± 0.83 **	42.23 ± 2.40 **
Uric acid (mg/l)	21.09 ± 0.43	61.22 ± 4.69 ###	25.46 ± 2.80 ***	20.92 ± 2.00 ***
Creatinine (mg/dl)	9.14 ± 0.43	7.80 ± 0.25	8.56 ± 0.57	9.0 ±0.2

Every value represents mean ± SEM, with $n = 5$. $^{**}p < 0.01$, $^{***}p < 0.001$, significant difference in comparison with the positive control. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$, significant difference in comparison with treated with the negative control.

Table 3: Effects of the extract on hematological parameters of the rats.

	Negative control	Positive control	Ethanol + Ext (200 mg/kg)	Ethanol + Ext (400 mg/kg)
HB (g/dl)	16.40 ± 0.58	12.10 ± 0.43 ###	15.64 ± 0.31 **	15.60 ± 0.70 **
RBC ($\times 10^{12}$/l)	10.20 ± 0.60	4.56 ± 0.25 ###	9.62 ± 0.13 ***	9.78 ± 0.278 ***
Hematocrit (%)	39.12 ± 2.16	43.00 ± 1.12	43.94 ± 1.19	44.06 ± 1.83
VGM (fl)	43.32 ± 0.18	53.180 ± 0.38 ###	44.80 ± 1.24 ***	44.34 ± 0.90 ***
Average content in hemoglobin (pg)	18.88 ± 0.21	20.02 ± 0.68	19.78 ± 0.27	19.42 ± 0.34
Average hemoglobin concentration (g/dl)	35.72 ± 0.13	33.10 ± 1.94	34.50 ± 1.07	35.34 ± 0.29
PLT ($\times 10^9$/l)	734.40 ± 4.28	615.20 ± 6.79 ###	742.6 ± 4.00 ***	760.80 ± 3.72***
P.N (%)	42.64 ± 1.87	29.20 ± 2.05 ###	40.20 ± 1.71 **	42.20 ± 1.75 ***
LY (%)	45,60 ± 1.69	19.80 ± 2.33 ###	39.00 ± 1.87 ***	41.00 ± 4.02 ***
WBC ($\times 10^9$/l)	12.60 ± 0.67	3.96 ± 0.36 ###	9.06 ± 0.34 **##	11.00 ± 0.78 ***

Every value represents mean ± SEM, $n = 5$. $^{**}p < 0.01$, $^{***}p < 0.001$, significant difference in comparison with the positive control. $^{\#\#\#}p < 0.001$, significant difference in comparison with negative control.

development in alcohol-induced hypertension [7]. In our studies, chronic ethanol administration resulted in a significant increase in SOD activity in the aorta, liver, heart, and kidney, which were reversed by extract treatment. Juan *et al.* [17] showed that increased SOD activity results from increased O^{2+} production and, therefore, increased oxidative stress, which is involved in the pathogenesis of hypertension. Thus, SOD reduction observed in the extract-treated groups may reflect a total reduction in oxidative stress.

Our results revealed a significant increase in MDA, as well as a decrease in GSH rate, in the rats that only received ethanol, consistent with previous studies [16,18–20]. The plant extract reduced MDA and prevented a reduction in GSH. Thus, extract treatment demonstrated antioxidant properties by preventing lipid peroxidation of tissues and GSH rate. The extract also proved beneficial for tissue parameters of oxidative stress caused by ethanol consumption.

Chronic alcohol consumption leads to various metabolic disorders due to liver enzyme disruption, and chronic ethanol administration in rats is a source of hepatotoxicity, causing high rates of serous transaminase. Our results showed increased GTP and GOT in the positive control, consistent with previous work [7]. Concomitant alcohol and extract significantly reduced transaminase rates compared to rats exclusively treated with alcohol, suggesting

that the extract protects the liver from ethanol toxicity [21,22].

Following 8 weeks of treatment, there was a significant increase in triglycerides and total cholesterol in the positive control, which is consistent with previous work [23]. Ethanol deteriorates fat oxidation, stimulates lipogenesis, and contributes to fat development in the liver. Therefore, this extract could protect animals against hypercholesterolemia and hypertriglyceridemia caused by ethanol.

Our work has also shown a decrease in the NO in the aorta, kidney, heart, and liver. Endothelial dysfunction caused by ethanol can alter relaxing abilities of the endothelium by reducing the release of NO [24,25]. Since the extract corrected this deficit at the NO level, it could also prevent endothelial dysfunction.

Ethanol treatment reduced red and WBC counts, HB, PLTs, LYs, and neutrophil levels, and increased VGM levels, consistent with previous studies [8,9]. Extract treatment also regularized these blood parameters.

Alcohol abuse has several deleterious effects on hematopoietic system functioning, such as macrocytosis with or without anemia, leukopenia, and thrombocytopenia. Some studies have indicated that ethanol is toxic to blood cells due to its amphiphilic properties. Ethanol can diffuse into blood cells and affect their membrane fluidity through permeability changes and can increase membrane lipid solubility by allowing xenobiotics to reach different organs [26]. Our results suggest that the extract reversed these toxic effects on rat hematological parameters, preventing anemia, thrombocytopenia, and leukopenia.

Phytochemical studies of the extract have revealed polyphenols' presence such as flavonoids and tannins. The main characteristic of polyphenols is that they are very powerful antioxidants [27]. Indeed, flavonoids are also known to have a preventive role against cardiotoxicity, their inhibition of lipid peroxidation and their ability to prevent various hematological disorders [28]. Indeed, flavonoids provide protection against free radicals by preventing their binding with cell membrane lipids, which results in a decrease in MDA (lipid peroxide) and in the hematological composition's protection by allowing good erythrocyte regeneration and prevention against leukopenia and thrombocytopenia observed in the presence of free radicals [29]. Flavonoids have the ability to capture and deactivate free radicals. Flavonoids are known for their protective effect on cardiovascular health by modifying several pathological processes involved in cardiovascular development diseases by inhibition of the oxidation of LDL cholesterol (bad cholesterol) by free radicals. These results obtained during our experiment would be due to the polyphenols contained in the extract and, in particular, the flavonoids.

This study indicates that *Trema guineensis* has significant beneficial effects on some aspects of ethanol-induced hypertension. Therefore, this plant can be used to treat essential hypertension caused by alcohol consumption. Further studies are needed to determine the bioactive molecule that causes these effects and establish therapeutic doses to avoid possible toxicity.

Conclusion

These results suggest that the *Trema guineensis* extract prevents ethanol-induced lipid peroxidation and oxidative stress caused by chronic ethanol consumption in rats. Moreover, the extract has beneficial effects on tissue lesions leading to kidney and liver function alteration.

Conflicts of Interest

The authors declare that there was no conflict of interest.

Acknowledgment

None.

References

[1] Kearney PM. Global burden of hypertension analysis of worldwide data. Lancet 2005; 365:217–23.

[2] Adeloye D, Basquill C. Estimating the prevalence and awareness rates of hypertension in Africa: a systematic analysis. PLoS One 2014; 9:e104300–16.

[3] Soncinia R, Santiagoa M, Orlandia L, Moraesb G, Pelosoa A, Santosb M. Hypotensive effect of aqueous extract of *Averrhoa carambola L*. (Oxalidaceae) in rats: an in vivo and in vitro approach. J Ethnophar 2011; 133:353–7.

[4] Adjanohoun, Ahyi M, Ake AL, Akpagana K, Chibon P, El-Hadj Watara A, et al. Contribution aux études ethnobotaniques et floristiques du Togo. Rapport de l'ACCT, Paris, France, 1986.

[5] Trovato, A, Forestierri AM, Barbera R, Galati EM. Effect of *Trema guineensis* decoction on some metabolic activities in the rat. Pharmacol *Res* Comm 1988; 20:665.

[6] Kadissoli B, Mouzou AP, Tekou E, Aklikokou A, Gbeassor M. Effet de l'extrait semi-éthanolique des feuilles de *Trema guineensis* (Celtidacea) sur la musculature lisse du rat Wistar. Int J Biol Chem Sci 2012; 6(3):985–94.

[7] Kasdallah-Grissa A, Mornagui B, Aouani E, Hammani M, Gharbi N, Kamoun A, et al. Protective effect of resveratrol on ethanol-induced lipid peroxidation in rats. Alco Alcohol 2006; 41:236–9.

[8] Paquot NJ, De Flines, Scheen AJ. L'alcoolisme, un modèle d'addiction aux complications somatiques multiples. Rev Med Liège 2013; 68:272–80.

[9] Nakao S, Harada M, Kondo K. Reversible bone marrow hypoplasia induced by alcohol. Am J Hematol 1991; 37:120.

[10] Bopda Mtopi OS, Dimo T, Nguelefack TB, Dzeufiet Djomeni PD, Rakotonirina SV, Kamtchouing P. Effects of Brillantaisia nitens Lindau Acanthaceae methylene chloride/methanol leaf extract on rat arterial blood pressure and heart rate. Pharmacology Online 2007; 1:495–510.

[11] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25:192–205.

[12] Misra, Fridovich. Determination of the level of superoxide dismutase in whole blood pp 101–109. Yale University Press, New Haven, 1972.

[13] Tom ENL, Demougeot COB, Mtopi T, Dimo PDD, Djomeni CD, Bilanda C, et al. The aqueous extract of *Terminalia superba* (Combretaceae) prevents glucose-induced hypertension in rats. J Ethnopharmacol 2011; 133:828–33.

[14] Bradford M. A rapid and sensitive method for quantification of microgram quantities of protein utilising the principle of protein-dye bindind. Anal Biochem 1976; 72:248–54.

[15] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta 1978; 90(1):37–43.

[16] Husain K, Mejia J, Lalla J, Kasim S. Dose response of alcohol induced changes in BP, nitric oxide and antioxidants in rat plasma. Pharmacol Res 2005; 51:337–43.

[17] Juan CC, Fang VS, Hsu YP, Huang YP, Hsia DB, Kwok CF, et al. Over expression of vascular endothelin-1 and endothelin-A receptors in a fructose-induced hypertensive rat model. J hypert 1998; 16:1775–82.

[18] Panchenco LF, Pirozhkov SV, Popova SV. Effect of chronique ethanol treatment on peroxidation acyl-CoA oxidase activity and lipid peroxidation in rat liver and heart. Experientia 1987; 43:580–1.

[19] Ribère C, Hininger I, Rouach H. Effects of chronic ethanol administration on free radical defence in rat myocardium. Biochem Pharmacol 1992; 44:1495–1500.

[20] Albano E, French S, Ingelmen-Sundberg M. Hydroxyethyl radicals in ethanol hepatotoxicity. Front Biosci 1999; 4:533–40.

[21] Yun JW, Kim YK, Lee BS, Kim CW, Hyun JS, Baik JH, et al. Effect of Dietary Epigallocatechin-3-gallate on cytochrome P450 2E1-dependent alcoholic liver damage: enhancement of fatty acid oxidation. Biosci Biotechnol Biochem 2007; 71:2999–3006.

[22] Calivarathan L, Laura EN, Thomas MM. Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation, Free Radic Biol Med 2014; 8:403–16.

[23] You M, Grabb DW. Recent advances in alcoholic liver desease. II. Minireview: molecular mechanism of alcoholic fatty liver. Am J Physiol Gastrol Int Liver physiol 2004; 287:G1–G6.

[24] Panza JA, Quyyumi AA, Brush Jr JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. Nat Eng J Med 1990; 323:22–7.

[25] De Cort P, Phillips H, Govaerts F, Van Royen P. Recommandations de bonne pratique: l'hypertension. Société Scientifique de Médecine Générale 2004; 40:28–33.

[26] Gulati A, Chandishawar N, Shanker K. Effect of alcohols on the permeability of bloodbrain barrier. Pharmacol Res 1985; 17:85–93.

[27] Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K. Antioxidant tannins from Rosaceae plant roots. Food Chem 2007; 100(2):579–83.

[28] Sadzuka Y, Sugiyama T, Shimoi K, Kinae N, Hirota S. Protective effect of flavonoids on doxorubicin induced radiotoxicity. Toxicoll Lett 1997; 92(1):1–7.

[29] Chandana Venkateswara R, Vijayakumar M. Effect of quercetin, flavonoids and α- tocopherol, an antioxidant vitamin on experimental reflux oesophagitis in rats. Eur J Pharmacol 2008; 589(1–3):233–8.