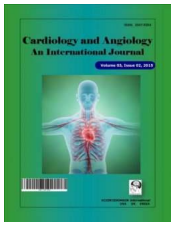

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Assessment of Cardioprotective Effects of Aqueous and Ethanolic Extracts of Stem Barks from *Trichilia emetica* against Cardiotoxicity Induced by Doxorubicin in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author DAP designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DAP and YHF managed the literature searches, analyses of the study performed the spectroscopy analysis and author YAF managed the experimental process and author DAP identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was to evaluate cardioprotective effects of extracts of stem barks from *Trichilia emetica* against cardiotoxicity induced by doxorubicin.
Methodology: This experiment was performed as follows: Group I receives some distilled water; group II receives doxorubicin only. Group III was administered 25 mg/kg b. w. of resveratrol only; Group IV received 25 mg/kg b. w. of resveratrol + doxorubicin. The groups V, VI, VII and VIII received respectively, 100 mg/kg b. w. of ethanolic extract + doxorubicin, 100 mg/kg b. w. of aqueous extract + doxorubicin, 200 mg/kg b. w. of ethanol extract + doxorubicin and 200 mg/kg b. w. of aqueous extract + doxorubicin. The other groups IX, X, XI and XII also received 100 mg/kg b. w. of aqueous extract, 200 mg/kg b. w. of aqueous extract, 100 mg/kg b. w. of ethanol extract and 200 mg/kg b. w. of ethanolic extract respectively. Distilled water, resveratrol and extracts were administered orally for 14 consecutive days. Doxorubicin was administered only on the 13th day by

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intraperitoneal route. 48 hours after administration of doxorubicin, blood samples were collected for biochemical analyzes: CK-MB, CPK, LDH, transaminases and lipid profile.
Results: Doxorubicin treated rats showed a significant ($P<0.05$) increased in activity of creatinine *kinase-MB* (CK-MB), creatinine phosphokinase (CPK), lactate deshydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, total-cholesterol and LDL-cholesterol compared to the control group. The results showed a significant decrease in HDL-cholesterol in comparison with the control group. However, pretreatment of rats with the extracts induced a significant decrease of the same enzymes, Total- Cholesterol, LDL- Cholesterol, triglycerides but also by a significant increase in HDL-cholesterol relative to the group of doxorubicin.
Conclusion: The results suggest that extracts of stem barks from *Trichilia emetica* possess cardioprotective effect.

Keywords: Cardioprotective; Trichilia emetic; doxorubicin; cardiotoxicity.

1. INTRODUCTION

Doxorubicin is a potent chemotherapeutic agent that is very effective in the treatment of patients with acute lymphoblastic leukemia, lymphomas, breast cancer, ovarian cancer, and many solid tumors [1]. However, the clinical use of this drug has been severely limited by its undesirable side effects dose-dependent in particular myocardial injuries which can be life-threatening after congestive heart failure [2]. Given this, several strategies have been followed to optimize the dosage and use analogues or combination therapy, but no promising results were found. Also, the use of several analogues of the clinically available doxorubicin did not show any stronger anti-tumor efficacy compared to doxorubicin. Antitumor activity of doxorubicin is mediated by a great number of mechanisms, but one of these activities is, the generation of free radicals, one of the major causes of cardiotoxicity. This has allowed researchers to develop strategies to reduce the toxic effects of doxorubicin without interfering with its antitumor properties [3]. Therefore, there is a need to identify other natural and safer sources of antioxidants. Today, we realize that plants can protect the heart against some heart diseases by their cardioprotective action. They possess properties such as anti-oxidant, anti-allergic, anti-inflammatory, antiviral, antiproliferative and anti-carcinogenic. Also, the cardioprotective effect of various medicinal plants and plant products have been documented [4]. That is why, the natural antioxidants, which are able to protect cells against oxidative damage, should be included in the potential antioxidant therapy [5].

Trichilia emetica, a plant of the Meliaceae family is used for multiple purposes by traditional healers. Indeed, ethnobotanical studies conducted by several authors have reported several healing properties of this plant. The powder obtained from the stem barks, is applied externally, to treat parasitic skin infections and inflammation [6]. It is also used against cough, intestinal worms, fever, syphilis; enables the stimulation of the bronchial secretions; treats gastritis, hepatitis, dyspepsia, ulcer, infertility, bloating and internal tumor. The ointment obtained from the same powder is applied externally to treat sprains and to make dressings on wounds. Some pharmacological properties such as antioxidant properties [7] as well as antibacterial activity [8] were studied. Since these studies listed above indicate the antioxidant properties of this plant, it is important to explore this property under the protection of heart cells against oxidative damage. Therefore, the aim of this study was to investigate the cardioprotective effects of aqueous and ethanolic extracts of stem barks of *Trichilia emetica* on myocardial necrosis induced by doxorubicin in rats by evaluating some biochemical markers of cardiac toxicity.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material was made up of the *Trichilia emetica* (Meliaceae) stem barks from the African pharmacopoeia. The plant was identified at the National Center of the Floristry of the Félix Houphouët Boigny University in Abidjan, Cocody. The collection of fresh stem barks was done during ethnopharmacological investigations in February 2014 in the north of Côte d'Ivoire.

2.2 Preparation of Plant Extracts

The barks of *Trichilia emetica* were harvested, dried a way from sunlight at room temperature for two weeks and made into fine powder by

grinding with a IKAMAG type mill. The aqueous and 70% ethanolic extracts were prepared according to the method of Kra and Zihiri [9]. To 100 g of this powder was added 1 liter of distilled water or the hydroalcoholic solvent (70/30, v / v). The obtained mixture was homogenized using a magnetic stirrer for 24 hours. The homogenate was subjected to successive filtration twice over cotton wool and then once over the Whatman paper. The collected filtrate was concentrated in oven at 50℃ to dryness. This was used for pharmacological activities.

3. EXPERIMENTAL ANIMAL

The animals used in this study were composed of Wistar rats. They were kept in plastic cages under standard environmental condition. The animals were fed with pellets provided by the Ivorian Animals Food Manufacturing Company (FACI®) and with tap water in bottles without discontinuity.

3.1 Experimental Design

The study of this activity was carried out on 72 rats divided into twelve batches of six. They were acclimatized at room temperature (25 ℃) at the pet shop at the Ecole Normale Supérieure (ENS) in Abidjan, Cocody. Distilled water, Resveratrol (reference cardioprotective molecule: 25 mg/kg b. w.) and the various extracts were administered orally to rats for 14 consecutive days. The single dose of doxorubicin (15 mg/kg b. w.) was administered only on the 13th day by intraperitoneal route. This experiment was performed as follows: Group I serves as a control and receives some distilled water; group II, in addition to distilled water, receives doxorubicin. Group III was administered 25 mg/kg b. w. of resveratrol only and Group IV received 25 mg/kg b. w. of resveratrol + doxorubicin. As for groups V, VI, VII, and VIII they received respectively, 100 mg/kg b. w. of ethanolic extract + doxorubicin, 200 mg/kg b. w. of ethanolic extract + doxorubicin, 100 mg/kg b. w. of aqueous extract + doxorubicin and 200 mg/kg b. w. of aqueous extract + doxorubicin. The other groups IX, X, XI and XII also received 100 mg/kg b. w. of ethanolic extract, 200 mg/kg b. w. of ethanolic extract, 100 mg/kg b. w. of aqueous extract and 200 mg/kg b. w. of aqueous extract respectively. 48 hours after administration of doxorubicin, blood samples were collected in dry tubes for the following biochemical analyzes: creatinine kinase-MB (CK-MB), creatine phoshokinase (CPK), lactate deshydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, total-cholesterol, HDL-cholesterol and LDL-cholesterol.

3.2 Statistical Analysis

The Analysis of the results used the Tukey's test, which was performed with Graph Pad Prism software 5.0 (Microsoft, USA). The average value is accompanied by the standard error of the mean (mean ± SEM). The difference between the two values is considered significant when $P < 0.05$.The Statistical analysis of these results was performed using an analysis of variance (ANOVA).

4. RESULTS

4.1 Effects of Aqueous and Ethanolic Stem Bark Extracts of *Trichilia emetic*a on Serum Level of Cardiac Enzymes of Different Groups

The obtained results show that there was no significant difference ($P>0.05$) between the groups of rats treated with both extracts at doses 100 and 200 mg/kg b. w. and the control group as regards serum values of ALT, AST, LDH, CPK and CK-MB. However, there was a significant increase ($P<0.05$) serum level of the same cardiac enzymes with the group of rats treated with doxorubicin. Also, there was a significant drop in the level of these enzymes in groups of rats pretreated with aqueous and ethanolic extracts before administration of doxorubicin compared to doxorubicin group. This decrease is much more pronounced with the ethanolic extract at a dose of 200 mg/kg b. w. (Table 1).

4.2 Effects of Aqueous and Ethanolic Stem Bark Extracts of *Trichilia emetic*a on the Level of the Lipid Profile of the Various Groups

The administration of doxorubicin to rats resulted in a significant elevation in serum level of total-cholesterol, triglycerides and LDL-cholesterol causing on the other side a significant drop in HDL cholesterol compared to the control group. Pretreatment of rats with both extracts followed by doxorubicin administration showed decreased levels of total-cholesterol, triglycerides and LDL cholesterol and increased HDL-cholesterol compared to the group receiving doxorubicin only. Furthermore, the groups of rats which were

administered only aqueous and ethanolic extracts of *Trichilia emetica* did not show any significant difference in these lipid parameters compared to the control group (Table 2).

5. DISCUSSION

This study involves the cardioprotective effects of aqueous and ethanolic extracts of the stem barks of *Trichilia emetica* against cardiotoxicity caused by doxorubicin. Indeed, *T. emetica* is a medicinal plant used in traditional medicine to treat various diseases. Therefore, this study aims to explore the cardioprotective effects of oral administration of aqueous and ethanol extracts of *T. emetica* against cardiotoxicity induced by doxorubicin in Wistar rats.

Indeed, Doxorubicin is a drug of the family of anthracyclines most commonly used to treat many forms of cancer, such as leukemia, lymphoma and solid tumors. But its clinical use is limited by its severe cardiotoxic effects [10]. Doxorubicin directly generates free radicals and also causes the reduction of the amount of endogenous antioxidants [11]. This induced oxidative damage affects the lysosomes, the microfibrils, the mitochondria and the sarcoplasmic reticulum. Finally, these intracellular changes lead to increased

Table 1. Effects of aqueous and ethanolic stem bark extracts of *Trichilia emetic*a on serum level of cardiac enzymes

	ALT (UI/L)	AST (UI/L)	LDH (UI/L)	CPK (UI/L)	CK-MB (UI/L)
GroupI	53±1.85	144±2.02	1835±20.19	2226±20.69	1081± 18.82
GroupII	79±1.49***	190±2.02***	2929±26.94***	3261±25.60***	1415±31.31***
GroupIII	52±1.70$^{###}$	140±2.50$^{###}$	1837±23.11$^{###}$	2342±35.72$^{###}$	1099±22.84$^{###}$
GroupIV	60±1.79$^{***###}$	156±1.49$^{***###}$	1954±21.59$^{***###}$	2424±21.06$^{***###}$	1237±21.03$^{***###}$
GroupV	65±1.44$^{***###}$	170±2.02$^{***###}$	1974±28.22$^{***###}$	2621±26.55$^{***###}$	1285±24.92$^{***###}$
GroupVI	63±1.69$^{***###}$	162±1.94$^{***###}$	1957±25.19$^{***###}$	2515±26.75$^{***###}$	1274±24.02$^{***###}$
GroupVII	67±1.71$^{***###}$	175±1.73$^{*###}$	1980±27.42$^{***###}$	2605±22.13$^{***###}$	1288±23.53$^{***###}$
GroupVIII	65±1.65$^{***###}$	172±2.84$^{***###}$	1963±23.38$^{***###}$	2569±26.59$^{***###}$	1283±20.63$^{***###}$
GroupIX	56±1.38$^{###}$	145±2.06$^{###}$	1872±21.82$^{###}$	2348±22.34$^{###}$	1094±22.44$^{###}$
GroupX	57±1.11$^{###}$	143±2.74$^{###}$	1848±24.36$^{###}$	2330±25.98$^{###}$	1043±20.55$^{###}$
GroupXI	53±1.25$^{###}$	143±2.18$^{###}$	1874±23.80$^{###}$	2354±33.07$^{###}$	1056±22.89$^{###}$
GroupXII	52±1.41$^{###}$	141±1.38$^{###}$	1820±21.59$^{###}$	2219±26.40$^{###}$	1074±25.12$^{###}$

*Results are expressed as mean±SEM (n=6). ***indicates a statistically significant mean difference at p<0.05when value is compared to control; ### indicates a statistically significant mean difference at p<0.05when value is compared to doxorubicin group. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: lactate deshydrogenase; CPK: creatine phoshokinase ; CK-MB: creatinine kinase-MB*

Table 2. Effects of aqueous and ethanolic stem bark extracts of *Trichilia emetic*a on lipid profile

	Total-chol.(g/L)	TG (g/L)	HDL-c (g/L)	LDL-c (g/L)
Group I	0.62±0.02	0.50±0.03	0.50±0.05	0.04±0.01
GroupII	0.95±0.03***	0.92±0.02***	0.12±0.03***	0.5±0.03***
GroupIII	0.62±0.02$^{###}$	0.53±0.03$^{###}$	0.48±0.03$^{###}$	0.04±0.01$^{###}$
GroupIV	0.68±0.03$^{***###}$	0.65±0.04$^{***###}$	0.34±0.02$^{***###}$	0.27±0.04$^{***###}$
GroupV	0.77±0.03$^{***###}$	0.70±0.02$^{***###}$	0.30±0.04$^{***###}$	0.30±0.04$^{***###}$
GroupVI	0.72±0.03$^{***###}$	0.67±0.03$^{***###}$	0.33±0.02$^{***###}$	0.21±0.04$^{***###}$
GroupVII	0.79±0.04$^{***###}$	0.74±0.04$^{***###}$	0.29±0.02$^{***###}$	0.31±0.04$^{***###}$
GroupVIII	0.75±0.03$^{***###}$	0.71±0.02$^{***###}$	0.30±0.02$^{***###}$	0.30±0.04$^{***###}$
GroupIX	0.65±0.03$^{###}$	0.55±0.03$^{###}$	0.49±0.04$^{###}$	0.06±0.01$^{###}$
GroupX	0.56±0.03$^{###}$	0.52±0.02$^{###}$	0.48±0.04$^{###}$	0.04±0.02$^{###}$
GroupXI	0.63±0.03$^{###}$	0.54±0.03$^{###}$	0.48±0.03$^{###}$	0.06±0.01$^{###}$
GroupXII	0.64±0.02$^{###}$	0.53±0.02$^{###}$	0.48±0.03$^{###}$	0.05±0.02$^{###}$

*Results are expressed as mean±SEM (n=6). ***indicates a statistically significant mean difference at p<0.05 when value is compared to control; ### indicates a statistically significant mean difference at p<0.05when value is compared to doxorubicin group. Total chol: total- cholesterol; TG: Triglycerides; HDL-c: HDL-cholesterol; LDL-c: LDL-cholesterol*

apoptosis of cardiac myocytes [12]. Cardiac cells are most sensitive to the effects of doxorubicin [13] because it leads to acute injury of myocardial membrane. Accordingly, this results in a significantly elevated cardiac enzyme labels such as AST, ALT, LDH, CPK, CK-MB in the blood. In our study, the group of rats that received doxorubicin without being pretreated with the extracts shows elevated serum levels of LDH, CPK, CK-MB, AST and ALT compared to the control group. This is an indicator of the toxicity of this molecule for cardiac tissue because conducted studies have shown that an increase in serum levels of these enzymes may be considered as an evidence of deterioration of the heart muscle membrane [14]. In addition, the elevation of serum activity of CK-MB following the administration of doxorubicin to this group of rats is a proof of myocardial ischemia. This is beacuse creatine kinase is a more sensitive indicator of early myocardial ischemia [15]. Still according to these authors, a rough estimate of the extent of damage to myocardial tissue is given by the level of LDH. But our study shows an increase in serum level of LDH in the same group of animals showing thereby that there was actually heart tissue damage.

The administration of doxorubicin also induced elevated level of transaminases (AST and ALT), supporting results from studies [16]. The mechanism associated with the release of these markers appears to be due to oxidative damages created by doxorubicin to cardiac tissue followed by subsequent release of its content into the bloodstream [17].

Nevertheless, pretreatment of rats with aqueous and ethanolic extracts of stem barks of *T. emetica* led to a significant reduction in serum levels of cardiac enzyme markers. But this observed decrease is greater with the ethanolic extract at a dose of 200 mg/kg b. w. Given that the concentrations of these cellular enzymes present in the blood are directly related to the integrity of the plasma membrane of cardiac cells, subsequently, the inhibition of their elevation and the reduction of their serum rate by the extracts may be due to the action of these on the maintenance of the integrity of the cardiac membrane and limiting the leakage of these enzymes [15]. This might suggest that the ethanol extract at this dose has more active phytochemicals that offer better protection to the heart tissue against the toxicity induced by doxorubicin and has cardioprotective activity similar to that resveratrol. These results could be consistence with several other research, which reported that, compounds with antioxidant properties like ethyl gammaglutamylcysteine ester and resveratrol could enhance cardiotoxicity induced by doxorubicin [18].

In addition to the measurement of known serum level of cardiac enzyme markers, serum lipid profile is also used in the detection of cardiac injuries [15]. As a matter of fact, lipids play an important role in cardiovascular diseases, not only by means of hyperlipidemia and atherosclerosis, but also by changing the composition, structure and stability of cell membranes. Hence, a lipid excess in the blood is considered as a factor in accelerating the development of atherosclerosis, which is itself the main risk factor for myocardial infarction. Thus, high levels of circulating cholesterol and its distribution in heart tissue are associated with cardiovascular damage [19]. Also, an altered lipid metabolism may impair cardiac function by modifying the cardiac membrane properties, and these changes may contribute to the death of heart cells after occlusion of the coronary artery [20] Thus, the increase in LDL-cholesterol, total-cholesterol and triglycerides and decrease HDL-cholesterol observed in the group of doxorubicin indicates interference of this drug with metabolism or lipid biosynthesis. Moreover, LDL-cholesterol is capable of carrying the highest concentration of cholesterol which would explain the increase in serum triglycerides [21] Also, high levels of LDL show a positive correlation with myocardial infarction while increasing levels of HDL-Cholesterol is a negative correlation. On the other hand, decreased serum levels of LDL-cholesterol, total- cholesterol, triglycerides with a concomitant increase in HDL-Cholesterol is found in rats pretreated with these extracts. These observed effects are also higher with the ethanolic extract at a dose of 200 mg/kg b. w. These lipid profile changes could be due to the presence of the main active components contained in extracts of *T. emetica*. These active ingredients present in the extracts would act on lipid metabolism in several levels such as inhibition of hepatic and cholesterol biosynthesis, increasing of the biliary secretion, stimulating the catabolism receptor of LDL-cholesterol or recovery of LDL-cholesterol blood by the liver [22].

6. CONCLUSION

This study reveals that the aqueous and ethanolic extracts of stem barks of *T. emetica*

possess similar protective cardioprotective activities as Resveratrol (a standard cardioprotective compound) by protecting the heart cells from the deleterious effects of doxorubicin. The results also reveals that ethanolic extract has a better cardioprotective activity as compared with aqueous extract. Additional clinical testing may further elucidate its role in protecting against the effects of agents that induce tissue damage, while other studies are needed to reveal the molecular basis of such an effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouet-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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