Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1046-1050

ISSN: 0975-4873

Research Article

CardioprotectiveEffect of Aqueous Extract of *Lippia multiflora* Leaves against Doxorubicin-induced Toxicity in Wistar Rats

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Available Online:29th September, 2015

ABSTRACT

Aqueous leaf extract of *Lippiamultiflora*was investigated for its effects on doxorubicin-induced cardiotoxicity.Wistar albino rats weighing 100-160 g were orally pretreated withresveratrol (25 mg/kg/day) or *L. multiflora* extract (100, 300 and 900 mg/kg/day) for 7 consecutive days before receiving single intraperitoneal (i.p) dose of doxorubicin (15 mg/kg) on the 7thday. Animals were sacrificed twenty four hours after the last administration. Blood was collected and analyzed for serum marker enzymes like lactate deshydrogenase (LDH), creatine phosphokinase-MB iso enzyme (CK-MB), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and hematological parameters (number of red blood cells, white blood cells, platelets and hematocrits).Histopathological examination of rat hearts was also performed.Doxorubicin caused significant increasein the level ofCK-MB, LDH, ALT, AST, ALP and white blood cells while causing reduction of red blood cells, platelets count and hematocrits.Pretreatment with *L. multiflora* extract significantly decreased CK-MB and LDH activities and white blood cells number. Red blood cells, platelets count and hematocrits were also reduced by *L. multiflora* extract. Histopathological study of the heart showed some important edema in doxorubicin treated rats and normal architecture of myocytes in *L. multiflora* extract (300 and 900 mg/kg b.w.) treated ratsprior doxorubicin administration.This studysuggested that *L. multiflora* extractpossessedprotective effect against doxorubicin-induced toxicity.

Keywords:*Lippiamultiflora*,doxorubicin, resveratrol, cardiotoxicity.

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety ofhuman solid tumors and leukemias. However, its clinical uses are limited by seriously high incidence of cardiotoxicity.An initial acute effect includes hypotension and transient electrocardiographic abnormalities[1]. Several approaches may be taken to decrease the risk of DOX-induced cardiotoxicity while maintaining its efficacy. These include altered schedules of drug administration,modifications of the anthracycline molecule, adjunctive treatment with beta-adrenergic blockers, angiotensin-converting enzyme inhibitors, dexrazoxane and probucol[2,3].None of these have been entirely successful. A new drug to prevent or treat DOX-induced cardiotoxicity is therefore needed.Medicinal plants have recently become a focus of interest because they may play key roles in treating a majority of heart disease with minimal or no side effects.

*Lippiamultiflora*Moldenke,also known as *Lippiaadoensis*Hochst, is an herbaceous plant of thegenus *Lippia.* It belongs to the family*Verbanaceae*, which is composed of 41 generawith approximately 220 species of herbs, shrubsand small trees[4,5]. *L. multiflora*is a stoutwoody, perennial and aromatic shrub mainlydistributed throughout tropical Africa, South andCentral American countries[6]. The distributionrange of this planthas its major concentrations in Guinea Savannah, Forest Savannah and Transitional and Coastal Savannah zones. Itis commonly known as Lippia tea and commercially known as "Gambian Tea Bush", "Bush Tea" and "Healer Herb"[7]. *L. multiflora* has been used in many traditional and herbal medicines to treat bronchial inflammation, malaria fever, conjunctivitis, gastro-intestinal disturbances, enteritis, coughs and colds[6], and possesses hypotensive, fatigue relieving, anddiuretic properties[8].One of the characteristics of itsaqueous extract is its wealth in polyphenols, flavonoids and tannins. Some researchers on green teas and black teas indicate that

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Table 1: Effect of the aqueous extract of *Lippia multiflora* on enzymatic parameters after injection of doxorubicin in rats

GROUPS	Parameters (UI/L)				
	CK-MB	LDH	ALT	AST	ALP
Control (NaCl 0.9 %)	2052±10,29^{a}	2733±127,6^{a}	65,72±7,364^{a}	130,3±8,642^{a}	320,1±76,72^{a}
Doxorubicin (15mg/kg)	2969±69,04^{e}	4812±158,7^{d}	104,1±5,783^{b}	209,3±8,395^{e}	694,9±79,66^{b}
Resveratrol 25mg/kg+ Doxorubicin (15 mg/kg)	2167±37,86^{ab}	3137±79,11^{ab}	68,30±11,25^{a}	166,5±3,768^{abcd}	375,6±40,67^{a}
*L.multiflora*100mg/kg + Doxorubicin (15 mg/kg)	2654±29,51^{d}	3857±129,9^{c}	88,03±3,091^{ab}	191,1±7,839^{de}	479,6±33,43^{ab}
L. multiflora 300mg/kg + Doxorubicin (15 mg/kg)	2441±30,22^{c}	3571±50,41^{bc}	78,58±4,896^{ab}	186,2±12,21^{cde}	467,6±104,8^{ab}
L. multiflora 900mg/kg+ Doxorubicin (15 mg/kg)	2281±34,49^{bc}	3359±32,00^{abc}	72,33±10,39^{ab}	176,8±6,879^{bcde}	408,8±49,07^{ab}

The values of parameters are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P> 0.05).

Table 2: Hematological parameters at day 8

Groups	Hematological parameters			
	RBC	WBC	Platelets	Hematocrits
Control (NaCl 0.9 %)	8.280±0.07000^{f}	5.0±1.313^{a}	837.3±35.87^{f}	39.80±4.964^{a}
Doxorubicin (15mg/kg)	4.520±0.2248^{a}	18.03±2.164^{c}	204.7±16.83^{a}	35.83±6.075^{a}
Resveratrol 25mg/kg+ Doxorubicin (15 mg/kg)	8.035±0.05500^{ef}	6.467±1.126^{a}	640,7±20.73^{e}	40.35±6.250^{a}
*L. multiflora*100mg/kg + Doxorubicin (15 mg/kg	6.157±0.4398^{b}	15.10±0.4041^{bc}	359.5±13.50^{b}	43.05±6.145^{a}
L. multiflora 300mg/kg+ Doxorubicin (15 mg/kg)	7.377±0.1247^{cdef}	10.03±0.9871^{ab}	471.0±11.00^{cd}	49.00±6.440^{a}
L. multiflora 900mg/kg + Doxorubicin (15 mg/kg)	7.597±0.09597^{def}	7.2±0.1155^{a}	518.7±29.01^{d}	53.6±0.5431^{a}

The values of hematological parameter are expressed as Mean ± S.E.M. for five rats (n=5). In the same column values, the same letters are not significantly different (P> 0.05).
RBC=Red Cell Count, WBC=White Blood Cell, MCV= Mean Cell Volume.

phenolic compounds, exactly flavonoids,have antioxidant properties[9,10]. Also, literary review does not reveal that *L.multiflora* aqueous extracthascardioprotective properties.

People in West and Central African communities use savannah tea like drinker for its aroma. Traditionally, the leaves of this herbwere used in Côte d'Ivoire to treatheart diseases.In regard of the popular consumption of *L. multiflora*as a tea, the present work aimed to study the effects of the aqueous extract of this plant against doxorubicin-induced heart damages in rats. These effects were compared to resveratrol(3,4,5-trihydroxy-trans-stibene), apolyphenol compoundabundantly found in grapes and red wine, and exhibiteda large spectrum of beneficial health effects includingcardioprotective[11].For this purpose, some biochemical markers, hematological parameters and histopathological examination were investigated.

MATERIALS AND METHODS

Extraction

The leaves were air-dried in shade and powdered with a mechanical grinder to obtain a coarse powder. 100g ofpowdered leaves of *L. multiflora*was boiled in oneliter ofdistilled water for 15-20 min. The aqueous extract was filtered through Whatmann filter paper (3 mm) and dried with a vacuum evaporator below 40 °C[12].

Animals and Treatments

The study was carried outwith thirty Wistar albino rats weighing 100-160 g. They were obtained from the Animal House of the Faculty of Pharmaceutical and Biological Sciences Félix Houphouët-Boigny University of Abidjan. Animals were housed in plastic cages where they had free access to water and food, and kept at temperature of 29 ± 1°C during the day with 12 h light and 25 ± 1° C in the night with 12 h darkness. All the experimental proceduresware approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.Rats were divided into six groups of five animals each.Group 1 (control) and Group 2 (DOX) received only distilled water for 7 days. Group 3 (standard group) received resveratrol (25 mg/kgb.w.) for 7 days. Groups 4, 5 and 6 served as extract treatment groups and received respectively 100, 300 and 900 mg/kg b.wof *L. multiflora* aqueous extract for 7 days. The 7th day, all the animal groups except the control were injected intraperitoneally (i.p) by a single dose of doxorubicin (15 mg/kgb.w.)one hour after the last

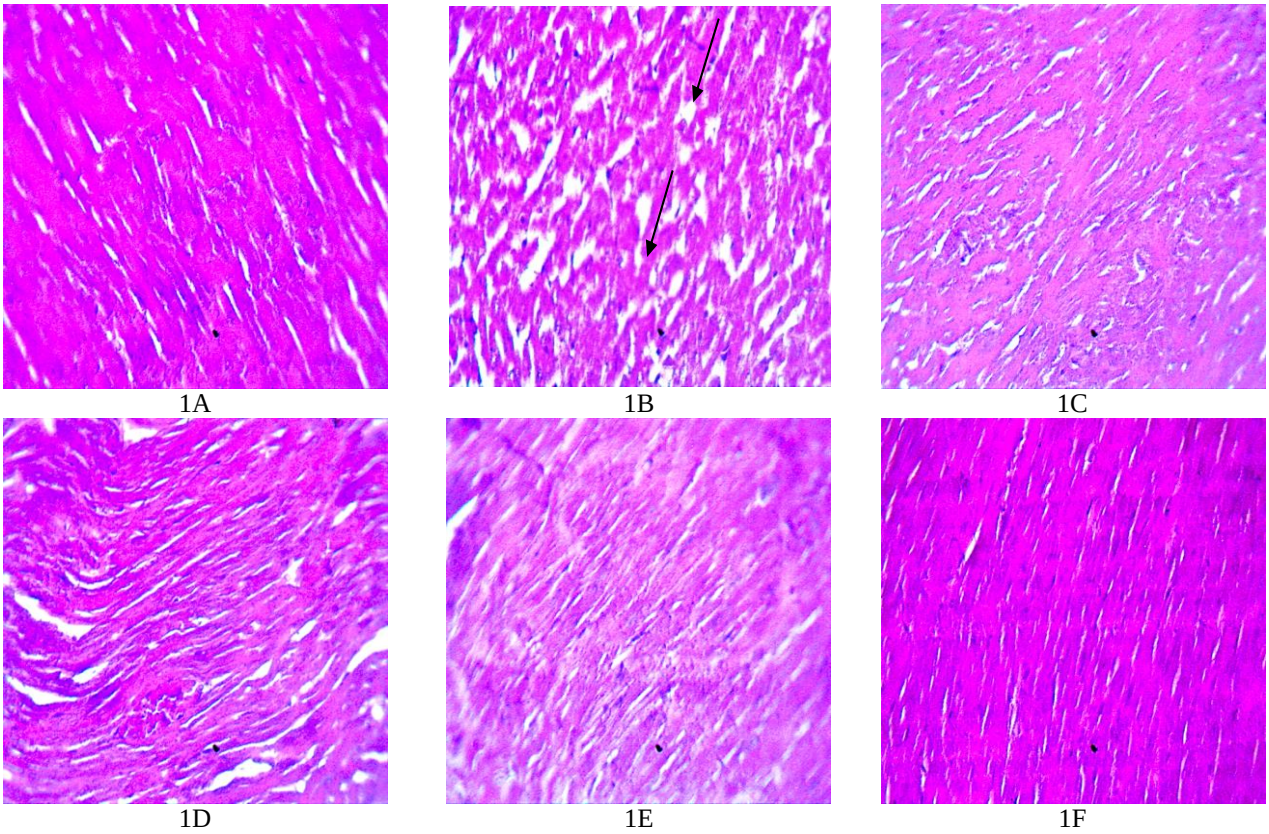

Figure 1: Representative of histological assessment of rat heart sections after 7 days of treatments. (A) Control, (B) Doxorubicin, (C) Resveratrol + Doxorubicin, (D) *L. multiflora* extract 100 mg/kg+ Doxorubicin,(E) *L. multiflora* extract 300 mg/kg+ Doxorubicin, (F) *L. multiflora* 900 mg/kg+ Doxorubicin (x400).
⟶ Important edema

treatment.Twenty four (24) hours after doxorubicin treatment, rats were anesthetized with light ether andblood wasdrawn from retro-orbital venous plexus for the estimation of marker enzymes and hematological parameters[13].

Estimation of serum marker enzymes

Blood samples were collected in non-heparinized capillary tubes and serum was separated by centrifugation at 4000rpm for 10min and stored at -20°C until analysis.CK-MB, LDH, ALT, AST and ALP activities were assayed according to standard methods using diagnostic kits and a COBAS INTEGRA 400 analyzer (Roche).

Hematological study

For the assessment of hematological parameters, blood samples were collected in ethylene diamine tetra-acetic acid (EDTA) coated bottles. Samples were immediately analyzed and the number of red blood cells (RBCs), white blood cells (WBCs), platelets and hematocrits was determined according to standard methods using a Blood Counter (Urit Coulter).

Histopathological study

After blood collection, rats were sacrificed by cervical dislocation and heart was separated, washed in ringer's solution and soaked in filter paper. Heart samples wereimmediately stored at -20ºC and used later for histological studies. For light microscopic examination, heart tissues from each animal group were fixed with 10% buffered formalin and embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4 µm thickness and stained with hematoxylinand eosin. They werethen observed with a light microscope[14].

Statistical analysis

All the data were expressed as mean± S.E.M (standard error of means) and analyzed statistically by one way analysis of variance(ANOVA) followed by Tukey's Multiple Comparison. A value of $P<0.05$ was considered significant.

RESULTS

Table 1 shows effect of the aqueous extract of *L. multiflora* on enzymatic parameters. The levels of CK-MB, LDH, AST, ALT and ALP was significantly ($P<0.05$) increased in doxorubicin group compared to the control.Pretreatment with *L. multiflora* aqueous extract (100, 300 and 900 mg/kg b.w.)significantly ($P<0.05$) decreasedCK-MB and LDH activities. *L. multiflora* aqueous extract also reduced the levels of AST, ALT and ALP but not significantly when compared to the values in Doxorubicin group.However, effect of *L. multiflora* aqueous extract (300 and 900 mg/kg b.w.) on enzyme activity was comparable to that of the standard resveratrol.

Concerning hematological parameters, results showedthat doxorubicindecreased RBCs and platelets count and increasedWBCs number compared to the control group.

Pretreatment with *L. multiflora* aqueous extract (100, 300 and 900 mg/kg b.w.) significantly (P<0.05) increased RBCs and platelets count while extract (300 and 900 mg/kg b.w.) showed a significantdecrease in the number of WBCs. Also, there were no significant differences between hematocrits level in all the animal groups (Table 2).

Histopathological examinations of the heart sections were shown in figure 1Section of rat heart from normal control group showed normal myocardial fibers(figure1A). There was important edema enter myocytes in doxorubicinalone treated rats (figure1B).Normal architecture of cardiomyocyteswas observedinresveratroltreated group (Figure 1C), but *L. multiflora*extract (100 mg/kg b.w.)treated group showed discreet edemaenter myocytes(Figure 1D).The histopathology of the heart was improved in*L. multiflora*extract (300 and 900 mg/kg b.w.) treated groupsand showed a normal shape, size and configuration of cardiac muscle fibers(Figures 1E and 1F).

DISCUSSION

Doxorubicin is a well-known cardiotoxic agent due to its ability for the destruction of myocardial cells as well as oxidative damage[15, 16]. As a result of this, CK-MB, LDH, ALT, AST and ALPwere released into blood stream and served as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in the blood reflects the alteration in plasma membrane integrity and/or permeability.Our experiment reveals an increase in the activities of these enzymes in doxorubicin alone treated rats.Administration of DOX may leads to the damage of the myocardial cell membrane or it become permeable, that resulted in the leakage of CK-MB, LDH, ALT, AST and ALPin the blood. This probably accounts for the increase in the level of these marker enzymes in the serum. Treatment with *L. multiflora* (100, 300 and 900 mg/kg b.w.) restored the activities by reducing these enzymes level toward normal in serum. This may be due to the protective role of *L. multiflora* on the myocardium, reducing the myocardial damage, thereby restricting the leakage of these enzymes in serum.

Doxorubicin also seems to affect blood cells by increasing white blood cells (WBCs) number[17]and decreasing red blood cells (RBCs)count[18]. Results obtained in DOX alone treated animals showed significant changes on WBCs, RBCs and platelets count as well as those reported by previous authors.Effect of *L. multiflora* extract (100, 300and 900 mg/kg b.w.) on these parameters was indicatedby the significant reduction in WBCs and increase of RBCs,platelets count and hematocrits compared to DOX group.

The histopathological changes of DOX induced cardiotoxicity, consistin order of increasingseverity, swelling of sarcoplasmic reticulum, cytoplasmic vacuolization, myofibrillar degeneration, myocyte disruption and fibrosis[19,20].In our study, we have observedimportant edema enter myocytes.Rats pretreated with resveratrol and *L. multiflora* extract (300 and 900 mg/kg b.w.) showed cardiac muscle fibers of normal shape, size and configuration.Onlyheart sections ofrats pretreated with *L. multiflora* extract (100 mg/kg b.w.)showeda discreet edemaenter myocytes. These results confirmed the capacity of *L. multiflora* extract to reduce the harmful effects caused by doxorubicin and to restore the normal cardiac physiology that has been disrupted by this anthracycline. In conclusion, the present study has demonstrated a potentialcardioprotective effect of *L. multiflora* against doxorubicin-induced cardiotoxicity in rats.Further studies are neededto identify and purify the active phytoconstituents involved in the cardioprotection of this plant.

ACKNOWLEDGMENTS

The authors thank staff members of the National Floristic Center of Félix Houphouet-Boigny University of Cocody-Abidjan for identification of *Lippia multifora* (Voucher of this specimen with thy corresponding number 6718 was deposited in the herbarium of the University).

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