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

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ABSTRACT
Aqueous leaf extract of *Lippia multiflora* was investigated for its effects on doxorubicin-induced cardiotoxicity. Wistar albino rats weighing 100-160 g were orally pretreated with resveratrol (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 7th day. 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 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 increase in the level of CK-MB, ALT, AST, ALP and white blood cells while causing reduction of red blood cells, platelets and hematocrits. Histopathological examination of rat hearts was also performed. 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 rats prior doxorubicin administration. This study suggested that *L. multiflora* extract possessed protective effect against doxorubicin-induced toxicity.

Keywords: *Lippia multiflora*, doxorubicin, resveratrol, cardiotoxicity.

INTRODUCTION
Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety of human 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 abnormalities1. 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, dextrazoxane and probucol1-3. 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.
has.

30 Wistar albino rats was boiled in traditionally, t

The leaves of this herb were used in Côte d'Ivoire to

In regard of the popular consumption of this plant against

MATERIALS AND METHODS

Extraction

The leaves were aired-dried in shade and powdered with a mechanical grinder to obtain a coarse powder. 100g opowered leaves of L. multifloras was boiled in oneliter ofdistilled water for 15-20 min. The aqueous extract was

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

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CK-MB (IU/L)</th>
<th>LDH (IU/L)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NaCl 0.9 %)</td>
<td>2052±10.29a</td>
<td>2733±127.6a</td>
<td>65.72±7.364a</td>
<td>130.3±8.642a</td>
<td>320.1±76.72a</td>
</tr>
<tr>
<td>Doxorubicin (15mg/kg)</td>
<td>2969±69.04c</td>
<td>4812±158.7d</td>
<td>104.1±5.783b</td>
<td>209.3±8.395c</td>
<td>694.9±79.66b</td>
</tr>
<tr>
<td>Resveratrol 25mg/kg+</td>
<td>2167±37.86ab</td>
<td>3137±79.11ab</td>
<td>68.30±11.25b</td>
<td>166.5±3.768bcde</td>
<td>375.6±40.67a</td>
</tr>
<tr>
<td>Doxorubicin (15 mg/kg)</td>
<td>3000mg/kg+</td>
<td>3857±129.9c</td>
<td>88.03±3.091b</td>
<td>191.1±7.839de</td>
<td>479.6±33.43ab</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (10^6/L)</th>
<th>WBC (10^3/L)</th>
<th>Platelets (10^3/L)</th>
<th>Hematocrits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NaCl 0.9 %)</td>
<td>8.28±0.0700f</td>
<td>5.0±1.313e</td>
<td>837.3±35.87f</td>
<td>39.80±4.964a</td>
</tr>
<tr>
<td>Doxorubicin (15mg/kg)</td>
<td>4.52±0.2248a</td>
<td>18.03±2.164c</td>
<td>204.7±16.83a</td>
<td>35.83±6.075a</td>
</tr>
<tr>
<td>Resveratrol 25mg/kg+</td>
<td>9.035±0.0550ef</td>
<td>6.467±1.126a</td>
<td>640.7±20.73a</td>
<td>40.35±6.250a</td>
</tr>
<tr>
<td>Doxorubicin (15 mg/kg)</td>
<td>6.157±0.4398b</td>
<td>15.10±0.4041bc</td>
<td>359.5±13.50b</td>
<td>43.05±6.145a</td>
</tr>
<tr>
<td>L. multiflora100mg/kg+</td>
<td>7.377±0.1247cdef</td>
<td>10.03±0.9871ab</td>
<td>471.0±11.00d</td>
<td>49.00±6.440a</td>
</tr>
<tr>
<td>Doxorubicin (15 mg/kg)</td>
<td>L. multiflora 300mg/kg+</td>
<td>7.797±0.09597cdef</td>
<td>7.2±0.1155a</td>
<td>518.7±29.014</td>
</tr>
<tr>
<td>L. multiflora900mg/kg+</td>
<td>130.3±8.642a</td>
<td>320.1±76.72a</td>
<td>375.6±40.67a</td>
<td>53.6±0.5431a</td>
</tr>
</tbody>
</table>

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.

Table 1: Hematological parameters at day 8

The study was carried out with 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 procedures were 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.w of 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

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The aqueous extract was filtered through Whatmann filter paper (3 mm) and dried with a vacuum evaporator below 40 °C.

Animals and Treatments

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

**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 were immediately stored at -20°C 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 hematoxylin and eosin. They were then observed with a light microscope.

**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) decreased CK-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 showed that doxorubicin decreased RBCs and platelets count and increased WBCs 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 significant decrease 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 1. Section of rat heart from normal control group showed normal myocardial fibres (Figure 1A). There was important edema enter myocytes in doxorubicinalone treated rats (Figure 1B). Normal architecture of cardiomyocytes was observed in resveratroltreated group (Figure 1C), but *L. multiflora* extract (100 mg/kg b.w.) treated group showed discreet edema enter myocytes (Figure 1D). The histopathology of the heart was improved in *L. multiflora* extract (300 and 900 mg/kg b.w.) treated groups and 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. As a result of this, CK-MB, LDH, ALT, AST and ALP were 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 lead to the damage of the myocardial cell membrane or it become permeable, that resulted in the leakage of membranes of the myocardial cell. 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 and decreasing red blood cells (RBCs) count. 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, 300 and 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 increasing severity, swelling of sarcoplasmic reticulum, cytoplasmic vacuolization, myofibrillar degeneration, myocyte disruption and fibrosis. In our study, we have observed important 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. Only heart sections of rats pretreated with *L. multiflora* extract (100 mg/kg b.w.) showed 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 potential cardioprotective effect of *L. multiflora* against doxorubicin-induced cardiotoxicity in rats. Further studies are needed to identify and purify the active phytoconstituents involved in the cardioprotection of this plant.

**ACKNOWLEDGMENTS**

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**REFERENCES**


