ABSTRACT

Albino Wistar rats were pretreated with 200 and 400 mg/kg of ethanol extract of Commelina diffusa leaf for 20 days and simultaneous treatment with doxorubicin (cumulative dose of 15 mg/kg i.p. in six divided doses 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th, 13th, 16th and 19th day of study). On the 20th day, parameters evaluated were, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), serum markers such as creatine kinase-MB (CK–MB), lactate dehydrogenase (LDH) and extent of lipid peroxidation viz. malondialdehyde (MDA) was estimated. Pretreatment with extract of Commelina diffusa at 200 and 400 mg/kg showed a significant decrease in the levels of triglycerides, cholesterol, LDL, VLDL, lipid peroxidation (MDA) and significant increase in the level of HDL when compared with the positive control p< 0.05. Extract treated groups also showed significant decrease in the levels of serum LDH and CK-MB, when compared with the positive control p< 0.05. The histopathological study of the extract treated rats further confirmed the cardio-protection of Commelina diffusa. In conclusion, ethanol extract of Commelina diffusa may confer cardioprotective function on doxorubicin induced cardiomyopathy in rats.

KEYWORDS: Commelina diffusa, Cardio-protective, Lactate dehydrogenase, lipid peroxidation.
INTRODUCTION

*Commelina diffusa*, sometimes known as the climbing dayflower or spreading dayflower, is a pan tropical herbaceous plant in the dayflower family. It has been introduced to the southeastern United States where it is most common in wet disturbed soils.\(^1\) It is typically an annual herb though it may be perennial in the tropics. It spreads diffusely, creeping along the ground, branching heavily and rooting at the nodes. The plant *Commelina diffusa*, was named by the Swedish taxonomist Carl Linnaeus of the 18\(^{th}\) century after the two Dutch botanists Jan commelijn and his nephew Caspar, each representing one of the showy petals of commelina communis. Recent data indicates that there are 170 species of commelina, the warmer regions of the world and with tropical Asia having the greatest diversity.\(^2\)

*Commelina diffusa* has been reportedly used for various purposes ranging from industrial and medicinal uses. The young leaf tip can be eaten directly or use fresh in salads or as a vegetable (Leonard, 2008).\(^3\) It has also been used as medicinal therapies for health disorders concerning cardiovascular, hypertension, digestive, hepatic, musculoskeletal, oncology, parasites, psychospiritual, reproductive, respiratory and kidney ailments.\(^3\) Preliminary study on phytochemical screening of *Commelina diffusa* indicated the presence of tannins, Saponins, alkaloids, flavonoids and glycosides with the absence of anthraquinones, sterols and phenols.\(^4\)

Doxorubicin is a secondary metabolite of streptomyces peucetius var, Caesius and belongs to the family of anthracyclines. This is a well-established and highly effective anti-neoplastic agent, used to treat several adult and pediatric cancers, such as solid tumors, leukemia, lymphomas and breast cancer. The successful use of doxorubicin has been hampered by toxicities such as hematopoietic suppression, nausea, vomiting, extravasation and alopecia, yet the most feared side-effect is cardio-toxicity.\(^5\) This study was carried out to determine the cardio-protective activity of the ethanol extract of the *Commelina Diffusa* leaf in doxorubicin-induced cardiomyopathy/myocardial damage.

MATERIALS AND METHOD

2.1 Animals

Adult healthy 24 male wistar albino rats weighing between 110g to 130g were obtained from the Department of Pharmacology Animal House of Niger Delta University Bayelsa State and were acclimatized for one week during which they were feed with standard feed (pellet) and distilled water. All protocols were performed in accordance with the Institutional Animal
Ethical Committee (IAEC) as per the direction of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

2.2 Chemicals/Reagents
Chloroform, 0.1M Tris-HCl buffer (pH 7.4), normal saline, absolute ethanol, thiobarbituric acid, distilled water, formaldehyde, Sodium hydroxide, 30% trichloroacetic acid (TCA). Doxorubicin (Khandelwal Laboratories Pvt Ltd, Mumbai, India). Kits from Randox Laboratories Ltd, Co. Antrim, United Kingdom. Sigma-Aldrich Ltd., U.S.A. PerkinElmer, USA, were used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

2.3 Collection of extract /extraction procedure
Fresh leaves of *Commelina diffusa* were collected from Amassoma town, Bayelsa State, Nigeria. The plant was identified by a botanist Prof. K. Ajibeshin, Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and deposited at the Herbarium of Department.

The leaves were plucked off their stems and air dried at room temperature for one week. The dried leaves were ground to powder using a blender. Three hundred and ten grams (310g) of the powdered materials was mixed with 500ml of absolute ethanol and 500ml of distilled water, at a ratio of 1:1. Mixture was macerated for three days (72 hours) with constant agitation (shaking) to ensure proper mixing and extraction. The mixture was filtered into 1000ml beaker using glass filter and filter paper and the filtrate was evaporated to dryness in a water bath for three days at 40°C to obtain 47.69g paste. Appropriate weights of the residue were prepared with normal saline water to obtain various concentrations that were administered to each of the rats.

2.4 Experimental design and procedures
A total of 24 adult albino rat strains grouped into four each having six rats grouped as follows;
Group 1: (Negative Control): (200ml/kg body weight saline water and pellet feed for 20 days.
Group 2: (positive control): received 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th, 13th, 16th, and 19th days.
Group 3:(test group): received 200mg/kg/bodyweight of *C. diffusa*, i.p for 20 days+ 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th, 13th, 16th, and 19th days.
Group 4: (test group): (test group): received 400mg/kg/bodyweight of C. diffusa, i.p for 20 days+ 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th, 13th, 16th, and 19th days.

Animals were sacrificed on the 20th day of study. Blood was collected into plain bottles for various biochemical analyses. The heart was harvested and fixed in 10% formalin for histological study.

2.5 Sample Collection and Biochemical Analysis
At the end of the experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. Blood was collected from each animal by cardiac puncture using sterile needle and syringe into well labeled plain tubes, allowed to stand for 2hrs before centrifuging at 3000rpm for 10 minutes. The supernatant was used for the biochemical analysis. The heart was excised using a midline abdominal incision, weighed and transferred into 10% neutral buffered formalin for histopathological examination. Heart was excised and washed in cold saline, 10% tissue homogenates were prepared in 0.1M Tris – HCL buffer (pH 7.4).

2.6 Biochemical parameters
The serum was used for estimation of creatine kinase–MB by optimized standard method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie. Lactate dehydrogenase (LDH) by kinetic method estimated according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie. Heart tissue homogenate was prepared in 0.05 M phosphate buffer pH 7.4 and homogenated in tissue homogenizer at 3000 rpm for 10 min. and extent of lipid peroxidation malondialdehyde (MDA).

Total serum cholesterol (TC), High Density Lipoprotein-cholesterol (HDL-C) and Triglycerides (TG) were estimated using Randox test kits according to the manufacturers protocol. LDL cholesterol concentration was calculated by subtraction of the sum of (HDL cholesterol + triglyceride from the total cholesterol concentration (TC) according to Friedewald et al. VLDL-cholesterol concentration was taken as one fifth of triglyceride concentrations.

2.7 Statistical Analysis
All data were expressed as Mean ± Standard deviation. The data obtained were analyzed using Two-way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social
Sciences) Version 20. The means were separated and compared by post-Hoc and Turkey method. P < 0.05 was considered as statistical significant.

RESULTS
The biochemical and histopathological studies were assessed for ethanol extract of *Commelina diffusa* on the administration of doxorubicin induced cardiomyopathy in wistar albino rats.

Preliminary phytochemical investigation
Preliminary phytochemical investigation revealed the presence of tannins, Saponins, alkaloids, flavonoids and glycosides with the absence of anthraquinones, sterols and phenols.[4]

Effect of ethanol extract of *Commelina diffusa* on the administration of doxorubicin induced cardiomyopathy

General observation
Rats in doxorubicin treated group, showed scruffy fur and developed a yellowish to reddish tinge. These rats also had red exudates around the eyes and nose, animals were sicker and lethargic when compared with the normal control. These observations were markedly reduced in the groups treated with extract of *Commelina diffusa* when compared with the doxorubicin control group.

Body weight and heart/body weight ratio
Decrease in body weight, heart weight and heart/body weight ratio was seen in doxorubicin treated rats, at the end of the study when compared with the normal. Extract of *Commelina diffusa* at 200 and 400 mg/kg body weight shows significant increase in body weight gain, increase in weight of heart when compared with doxorubicin control group (Table 1).

Biochemical study
Serum Markers: CK – MB, LDH.
Treatment with doxorubicin causes an elevation in level of CK-MB and LDH which are considered as the selective biomarkers of myocardial damage when compared with the normal p< 0.05. Extract treated groups showed significant decrease in the levels of these enzymes, when compared with the positive control (Table 3).
Lipid Profile and lipid peroxidation of heart tissue homogenate

Biochemical assessment of the lipid profile in doxorubicin treated rat showed significant increase in the levels of triglycerides, cholesterol, LDL, VLDL, lipid peroxidation (MDA) of the heart and shows significant decrease in the level of HDL when compared with the normal control. Pretreatment with extract of Commelina diffusa at 200 and 400 mg/kg showed a significant decrease in the levels of triglycerides, cholesterol, LDL, VLDL, lipid peroxidation (MDA) and significant increase in the level of HDL when compared with the positive control p< 0.05. (Table 3).

Table 1: Effect of ethanol extract of Commelina diffusa leaf on body weight, heart weight and heart/body weight ratio by doxorubicin induced cardiomyopathy.

<table>
<thead>
<tr>
<th>Groups/Treatments</th>
<th>Mean of wt rats on day1</th>
<th>Mean wt of rats on day 21</th>
<th>Mean wt of heart</th>
<th>Mean wt of heart/body ratio x10^-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>121±8.9^a</td>
<td>193±12.1^b</td>
<td>0.99± 0.2^d</td>
<td>5.1±</td>
</tr>
<tr>
<td>With saline water</td>
<td></td>
<td></td>
<td>0.12^f</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>113±8.0^a</td>
<td>160±9.9^c</td>
<td>0.63±0.1^e</td>
<td>.9±0.01^g</td>
</tr>
<tr>
<td>With 2.5mg/kg DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>119±8.4^a</td>
<td>158±9.4^c</td>
<td>0.91± 0.1^d</td>
<td>5.8 ± 0.01^f</td>
</tr>
<tr>
<td>With 200mg/kg Extract &amp; DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>120±8.9^a</td>
<td>168±10.4^c</td>
<td>1.21±0.9^d</td>
<td>7.2± 0.87^h</td>
</tr>
<tr>
<td>With 400mg/kg Extract &amp; DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at p<0.05.

Table 2: Effect of ethanol extract of Commelina diffusa leaf on TG, HDL, CHOL and MDA levels in rats by doxorubicin induced cardiomyopathy.

<table>
<thead>
<tr>
<th>Enzyme/ Group</th>
<th>TRIGS (Mmol/l)</th>
<th>HDL (Mmol/dl)</th>
<th>CHOL (Mmol/l)</th>
<th>MDA (µmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>4.65±1.9^a</td>
<td>2.61±1.7^a</td>
<td>8.74±2.0^a</td>
<td>4.71±1.8^a</td>
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<tr>
<td>With saline water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>16.19±4.0^b</td>
<td>2.61±1.2^a</td>
<td>35.38±8.2^b</td>
<td>6.01±12.1^b</td>
</tr>
<tr>
<td>With 2.5mg/kg DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>14.74±3.7^b</td>
<td>2.91±0.9^b</td>
<td>18.65±10.5^c</td>
<td>4.88±1.6^a</td>
</tr>
<tr>
<td>With 200mg/kg Extract &amp; DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>3.76±1.8^a</td>
<td>3.11±2.2^b</td>
<td>22.02±6.1^c</td>
<td>3.56±1.2^a</td>
</tr>
<tr>
<td>With 400mg/kg Extract &amp; DOX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at p<0.05.

Table 3: Effect of ethanol extract of Commelina diffusa leaf on LDH, CK-MB, LDL and VLDL levels in rats by doxorubicin induced cardiomyopathy.

<table>
<thead>
<tr>
<th>Treatments/Groups</th>
<th>LDH (U/ L)</th>
<th>CK-MB (U/ L)</th>
<th>LDL (Mmol/l)</th>
<th>VLDL (Mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group With saline water</td>
<td>70.02±11.0\textsuperscript{a}</td>
<td>97.03±7.11\textsuperscript{a}</td>
<td>1.48±0.2\textsuperscript{a}</td>
<td>0.93±0.38\textsuperscript{a}</td>
</tr>
<tr>
<td>Positive control With 2.5mg/kg DOX</td>
<td>286.21±10.5\textsuperscript{b}</td>
<td>384.6±11.7\textsuperscript{b}</td>
<td>16.58±3.0\textsuperscript{b}</td>
<td>3.24±0.80\textsuperscript{b}</td>
</tr>
<tr>
<td>Treatment group With 200mg/kg Extract &amp; DOX</td>
<td>193.25±13.9\textsuperscript{c}</td>
<td>172.3±8.01\textsuperscript{c}</td>
<td>1.00±5.9\textsuperscript{a}</td>
<td>2.95±0.74\textsuperscript{c}</td>
</tr>
<tr>
<td>Treatment group With 400mg/kg Extract &amp; DOX</td>
<td>243.4±10.01\textsuperscript{c}</td>
<td>139.93±7.08\textsuperscript{c}</td>
<td>15.15±2.1\textsuperscript{b}</td>
<td>0.75±0.36\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at p<0.05.

Histopathological changes on Doxorubicin-induced cardiomyopathy

The heart sections obtained from doxorubicin treated animals showed abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular spaces. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed improvement in the cell integrity evidenced by absence of necrosis, less vacuolization of the cytoplasm and maintenance of normal integrity of the cardiac muscles, Fig. 1.

Haematoxylin and Eosin, X 100

Fig. 1: Histopathological images of heart pretreated with ethanol extract of Commelina diffusa by doxorubicin induced cardiac toxicity. A: Normal control, B: Positive control, C: Test group – 200 mg/kg, D: Test group – 400 mg/kg.
DISCUSSION

Doxorubicin belongs to the family of anthracyclines which acts by the formation of an iron-anthracycline complex that generates free radicals, capable of causing severe damage to the plasma membrane while interfering with the cytoskeletal structure. Cardiac tissue becomes susceptible to lipid peroxidation due to the action of oxygen free radical generated by doxorubicin. This leads to a progressive irreversible loss of myofibrils, dilation of the sarcoplasmic reticulum, cytoplasmic vacuolization, swelling of mitochondria, increased number of lysosomes and myocyte necrosis, inhibition of nucleic acid as well as protein synthesis, release of vasoactive amines, change in adrenergic function, decreased activity of Na+ K+ ATPase, alteration in sarcoplasmic calcium transport, imbalance of myocardial electrolytes in response to the doxorubicin. This is evident in our present study as the formation of the free radical led to biochemical and histopathological changes in the doxorubicin treated rat groups. Cardio-protection has been defined as "all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage"

Rats in doxorubicin treated group, showed scruffy fur with significant decrease in body weight, heart weight and heart/body weight ratio when compared with the normal control. The decrease in body weight obtained in this study is in accordance with other studies which may be attributed to reduced food intake and inhibition of protein synthesis due to doxorubicin treatment compared to normal control. The present study showed a significant increase in the bodyweight gain, heart weight and heart/body weight ratio of rat groups treated with ethanol extracts of Commelina diffusa at 200 and 400mg/kg when compared with the positive control. The red exudates from eyes and nose were also reduced to normal.

Doxorubicin causes a significant increase in levels of CK-MB and LDH when compared with the normal control. Increase in the serum level of these enzymes may be attributed to deficiency of oxygen supply or glucose supply to the myocardial cell membrane that may cause damage leading to permeable and ruptures. These enzymes are specific biomarkers used in the estimation of the cardiovascular damage. Cardiotoxicity has been reported as the most feared side effect of doxorubicin in the treatment of several adult and pediatric cancers, such as solid tumors, leukemia, lymphomas and breast cancer. Treatment with extract of Commelina diffusa has shown a significant decrease in the level of these enzymes suggesting the protective or membrane stabilizing effect of the extract on the myocardium.
Oxidative stress and mitochondrial dysfunction are associated with disease and toxic process. It results from overproduction of reactive oxygen species, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes.[21] Treatments with doxorubicin cause a significant increase in the level of lipid peroxidation (MDA) when compared with the normal control. Our study demonstrated a significant decrease in the level of MDA in the Commelina diffusa extract treated groups when compared with the positive control. These results indicate the protective effect or free radical scavenging effect of the extract of Commelina diffusa in oxidative damage caused by doxorubicin. The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the free radical scavenging and antioxidant activity of the extract. Presence of tannins, saponins, alkaloids, flavonoids and glycosides has been reported during preliminary study on phytochemical screening of the plant.[4] The leaf extract of Commelina diffusa has been shown to possess strong antioxidant and anti-inflammatory properties.[22]

The lipid profile in doxorubicin treated rats showed significant increase in the levels of triglycerides, total cholesterol, LDL-cholesterol, VLDL and significant decrease in the level of HDL when compared with the normal control. Elevated plasma levels of LDL and low levels of HDL were reported to pose a major risk of development of cardiovascular diseases.[23] Our study showed that treatment with extract of Commelina diffusa at 200 and 400 mg/kg significantly decrease the levels of triglycerides, cholesterol, LDL, VLDL and cause a significant increase in the level of HDL when compared with the positive control. Elevation of these lipid indices have been used as indicators in cardiovascular diseases and therefore a beneficial role of extract of Commelina diffusa in the prevention of cardiovascular diseases may be suggested.

Increase level of serum HDL has been associated with reduced risk of cardiovascular diseases.[24,25]

Histopathological examination of myocardial tissue obtained from normal animal exhibited clear integrity of myocardial membrane. The heart sections obtained from doxorubicin treated animals showed disruption of several subcellular elements including loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm, formation of lysosomal bodies and dilation of the sarcotubular system.[26] Treatment with the extract of Commelina diffusa demonstrated less disruption of the myofibrils and less vacoulation of the cytoplasm. This further confirms the membrane stabilizing effect of the extract.
The present study clearly emphasizes the cardioprotective effect of ethanol extract of *Commelina diffusa* leaf on doxorubicin induced cardiac myopathy. *Commelina diffusa* leaf proved effective in maintaining the biochemical and histopathological parameters close to normal. Further studies focusing on the isolation, characterization and purification of the active constituent and elucidating the exact mechanism of action is to be carried out.

REFERENCES


