Aqueous Leaf Extracts of Selected Vegetables Reduce Blood Pressure and Improve Cardiac Functions in Male Rats with Isoproterenol-Induced Myocardial Infarction

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ABSTRACT
Background: General belief of preventive and beneficial effect of vegetable consumption on cardiovascular diseases requires scientific investigation.
Objective: To investigate the effect of five selected vegetables (Basella alba, Crassocephalum crepidioides, Launaea taraxacifolia, Senecio biafrae and Solanum nigrum) on blood pressure and cardiac functions in isoproterenol-treated myocardial infarction male rats.
Materials and Methods: Dried ground leaves (350 g of each vegetable) were extracted at room temperature (26°C) with 3 L of water via maceration. Rats (150-200 g) were randomly divided into 14 groups of 6 rats each. Groups 1 and 8 served as normal and isoproterenol controls respectively, Groups 2 to 6 received 200 mg/kg body weight (b.wt.) of each extract, Groups 9 to 14 were pre-treated with each extract (200 mg/kg b.wt.) and then isoproterenol (20 mg/kg b.wt.; subcutaneously) to induce myocardial infarction (days 29 and 30). Group 7 was administered mixture of all the extracts in ratio 1:1:1:1:1, while Group 14 received extracts mixture and isoproterenol. Vegetable extracts were administered for 28 days. Blood pressure (BP) was measured via arterial cannulation under urethane and alpha-chloralose anaesthesia. C-reactive protein (CRP), lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) were measured in cardiac puncture-collected blood samples using commercially available kits.
Results: Pretreatment of the rats with vegetable extracts significantly reduced (p<0.05) BP and the plasma levels of CRP, LDH, CK, AST in isoproterenol-treated rats.
Conclusion: Findings from this study suggest that selected vegetables extract reduced BP and improved cardiac functions in isoproterenol-induced myocardial infarction male rats.

Keywords: Basella alba, Crassocephalum crepidioides, Launaea taraxacifolia, Senecio biafrae, Solanum nigrum, blood pressure, C-reactive protein, myocardial infarction.

INTRODUCTION
Cardiovascular disease (CVD) still remains the leading cause of deaths worldwide and will continue to dominate mortality trends in the future (1). Some countries classified as high-income countries have showed a significant decline in cardiovascular mortality rates over the last two decades (2). However, in low and middle-income countries, the incidence of cardiovascular disease and related deaths continues to increase at a fast rate (2, 3). The disparity in the mortality rates of cardiovascular disease in the developed and developing worlds appears to be due to different levels of prevention and treatment of cardiovascular diseases (3).

A rising trend in heart disease such as myocardial infarction (MI) has been attributed to lower or non-consumption of vegetables in most developing countries, especially amongst urban elites (4). Myocardial infarction is the rapid development of myocardial necrosis caused by a critical imbalance between oxygen supply and myocardial demand of the myocardium (5). Isoproterenol (ISO), a synthetic catecholamine that also act as a β-adrenergic agonist, has been shown to induce MI by causing severe stress in the myocardium leading to necrosis of the cardiac muscle (6). During myocardial necrosis, activities of cardiac injury markers such as C-reactive protein (CRP), lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase (AST) and blood pressure increase (7, 8).

Studies have shown that fruits and vegetables contain vital components that modulate and may prevent degenerative diseases such as heart disease. For instance, Oyebode et al. (9) reported that eating seven or more portions of fruits and vegetables a day reduced risk of heart disease by 31% compared to eating less than one portion. The reason is that many risk factors for cardiovascular disease are substantially influenced...
Extracts of Vegetables Reduce Blood Pressure and Improve Cardiac Functions

by dietary factors and preventive measures hold many promises (1). However, wild, underutilized vegetables are usually underrepresented in the studies that are designed to evaluate the effect of fruits and vegetables on the modulation of heart disease.

Underutilized indigenous vegetables are naturally growing wild plant species, rarely cultivated and gradually becoming endangered vegetables in the natural ecosystem (10). These vegetables are usually collected from fallows, watercourses, field margins, disturbed fields, protected home gardens, refuse hills, abandoned areas and less included in scientific research (11). Lack of inclusion of the group of vegetables in scientific research results in their low production, processing, distribution, marketing and consumption. Their inclusion in scientific research is critical for proper integration into the World Health Organisation (WHO) global initiative for fruit and vegetable consumption promotion (12). Most of these wild vegetables are edible and have served as a basis of nutritional livelihood in the rural areas for several years. In spite of the abundance of underutilized leafy vegetables, scientists have not fully explored their potentials, especially in the prevention of heart disease. So, it is pertinent to focus attention on underutilized indigenous vegetables with a view to harnessing both their nutritive and therapeutic potentials, as well as ultimately preventing them from extinction.

Considering the increase in incidence and prevalence of cardiovascular diseases in sub-Saharan Africa and the importance of fruits and vegetables in preventing and combatting the scourge of CVD, it becomes imperative to investigate the effect of selected wild vegetables on myocardial infarction – an important and common cardiovascular disease. Therefore, this study was designed to evaluate the effect of 5 selected wild vegetables: *Basella alba* (BA), “Amunututu” (Yoruba); *Crassocephalum crepidioides* (CC), “Ebòlò” (Yoruba); *Launaea taraxacifolia* (LT), “Yánrin” (Yoruba); *Senecio biafrae* (SB), “Worowo” (Yoruba); and *Solanum nigrum* (SN), “Odu” (Yoruba) on blood pressure and cardiac functions in male rats treated with ISO for induction of MI.

**MATERIALS AND METHODS**

**Plant Materials**

Fresh tender leaves of *B. alba* (LUH5807A), *C. crepidioides* (LUH1229A), *S. biafrae* (LUH1227A), *S. nigrum* (LUH1228A) and *L. taraxacifolia* (LUH5806A) were obtained from Titi’s Home Garden, Ota, Ogun State; Ikoro market, Ikoro, Ekiti State; and Kajola, Ibadan, Oyo State, South-West Nigeria, during the rainy season (June – August, 2014). The plant samples (Plates 1 to 5) were authenticated by a Taxonomist, Dr. AB Kadiri of the Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria. A voucher specimen for each plant was deposited in the Botanical Herbarium of the Department of Botany, Faculty of Science, University of Lagos, Nigeria where voucher numbers were obtained.

**Preparation of Aqueous Leaf Extracts**

*B. alba*, *C. crepidioides*, *L. taraxacifolia*, *S. biafrae* and *S. nigrum* leaves were washed, air-dried for a week and then
coarsely pulverized using electric blender. Cold maceration technique as described by Handa (13) was employed to obtain extracts of the vegetables at the Department of Pharmacognosy Laboratory, University of Lagos, Lagos, Nigeria. The ground plant materials and mixture of all the vegetables in ratio 1:1:1:1:1 (350 g each) were macerated in 3 L of cold distilled water for 72 h (3 days) at 26°C. After 72 h, the extracts obtained were filtered using Whatman No. 1 filter paper from the marc (the damp plant sample) and freeze dried under reduced pressure (1.034 mBar). The dried extracts (24.5% yield) were stored in sterile bottles until further use.

Experimental Male Wistar Albino Rats (Rattus norvegicus)

Eighty-four (84) male Wistar albino rats (150–200 g) were obtained from the animal breeding unit of Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria. The animals were placed in large plastic cages, maintained under 12 h light/dark cycle at 28±2°C at the animal house of the Department of Cell Biology and Genetics, University of Lagos, Lagos, Nigeria, fed commercial pelleted diet (Korede Farms, Bariga, Lagos) and provided with water ad libitum. They were allowed to acclimatize to laboratory conditions for 15 days prior to the commencement of the experiment.

Ethical approval (CM/COM/08/VOL.XXV) for the research was obtained prior to commencement from the Research Grants and Ethics Experimentation Committee, College of Medicine, University of Lagos, Nigeria.

Experimental Design

Eighty four (84) male Wistar albino rats (150-200 g) were randomized into 14 groups of 6 rats each and categorized into two: vegetables category and isoproterenol treated category. In the vegetable category, Group 1 (normal control) received water only, while Groups 2 to 7 received 200 mg/kg b.wt. of each plant extract and the mixture (1:1:1:1:1) (Table 1). In the isoproterenol treated category (Table 2), animals in Group 8 served as isoproterenol control while Groups 9 to 14 animals were administered 200 mg/kg b.wt. of each plant extract and the mixture (1:1:1:1:1). Extract administration was per oral for 28 days. Isoproterenol 20 mg/kg b.wt. was administered subcutaneously within 10 min. of preparation on day 29 and 30 according to the method of Radhika et al. (14).

Table 1: Animal Grouping in the Vegetables Category

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Rats</th>
<th>Plant Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Normal control (no extract)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>B. alba</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>C. crepidioides</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>L. taraxacifolia</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>S. biafrae</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>S. nigrum</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Mixture (1:1:1:1)</td>
</tr>
</tbody>
</table>

Table 2: Animal Grouping for 30 Days in the Isoproterenol Treated Category

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Rats</th>
<th>Plant Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>6</td>
<td>ISO (ISO control)</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>B. alba + ISO</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>C. crepidioides + ISO</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>L. taraxacifolia + ISO</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>S. biafrae + ISO</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>S. nigrum + ISO</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>Mixture (1:1:1:1) + ISO</td>
</tr>
</tbody>
</table>

Key: ISO, Isoproterenol.
Blood Pressure Measurement
The method of Oloyo et al. (15) was employed for blood pressure (BP) and heart rate measurements. A cannula (connected to a sterile, fluid-filled system) was placed in the carotid artery and connected to a pressure transducer through pressure recording catheter on 7D polygraph where a graph of pressure against time was displayed. The readings were taken at night as described by Hansen et al. (16) at the Department of Physiology, College of Medicine, University of Lagos, Lagos, Nigeria.

Sample Collection
Blood samples were collected from the cannulated carotid artery into ethylenediaminetetraacetic acid (EDTA) labelled bottles after BP measurement. The blood samples in EDTA bottles were mixed thoroughly and further centrifuged at 3000 rpm for 10 min. and the plasma collected in plain bottles (17).

Determination of Biomarkers of Cardiac Function

**Rat C-Reactive Protein Assay**
Rat C-reactive protein (CRP) was measured using commercially available kits according to the manufacturer instructions (Merck Rat CRP Enzyme-linked immunosorbent assay (ELISA) kit, USA).

**Lactate dehydrogenase Enzyme Assay**
Lactate dehydrogenase (LDH) enzyme assays were carried out using Roche/Hitachi Enzymatic colorimetric standard kits (Mannheim, Germany). Briefly, ready to use R1 solution (Phosphate buffer: 68 mmol/L, pH 7.5; pyruvate ≥ 0.73 mmol/L; stabilizers and preservatives) was mixed gently with plasma (0.25 ml) and further treated with R2 working solution (NADH ≥ 1.1 mmol/L; stabilizers and preservatives) to start reaction. LDH activity was determined photometrically.

**Determination of Creatine Kinase Enzyme**
Creatine kinase (CK) was measured using a commercially available kit according to the manufacturer instructions (Roche/Hitachi Enzymatic colorimetric diagnostic kits, Mannheim, Germany). Briefly, ready to use solution R1 (sodium hydroxide: 0.20 mol/L) was added to plasma (0.25 ml), followed by addition of R2 (Picric acid: 25 mmol/L) to commence the reaction. CK was measured photometrically using automatic analyser (902 Roche/Hitachi).

**Determination of Aspartate Aminotransferase**
Aspartate aminotransferase (AST) activity was determined using commercially available kit according to the manufacturer instructions (Roche AST diagnostic test kit, Roche Diagnostics, Sandhoferstrasse, Mannheim, Germany). The only difference is that reagent for AST assay R1 comprises TRIS buffer (Tris (hydroxymethyl)-aminomethane): 100 mmol/L, pH 7.8; L-aspartate: 300 mmol/L; nicotinamide adenine dinucleotide (NADH): 0.23 mmol/L; malic dehydrogenase (MDH) ≥ 0.53 U/mL; LDH ≥ 0.75 U/mL; preservative. Ready to use working solution (R2) comprises α-ketoglutarate: 75 mmol/L; preservative. AST activity was measured photometrically by UV (ultraviolet).

Statistical Analysis
All data are presented as mean ± standard error of mean (SEM). One way Analysis of variance (ANOVA) was used to analyse the data, followed by a post-hoc multiple comparison test of Fisher’s least significance difference (LSD). The results were considered significant at p values of less than 0.05. Statistical package for Social Sciences (SPSS) software version 20 was used for the statistical analyses.

**RESULTS**

**Effect of Extracts on Blood Pressure of Male Rats Treated with Isoproterenol**
The highest BP was recorded in isoproterenol control when compared to other groups. However, feeding the isoproterenol treated rats with the vegetables singly or in combination significantly reduced the BP and restored it towards normal (Figure 1).

**Effect of Extracts on C-Reactive Protein of Male Rats Treated with Isoproterenol**
Figure 2 below shows mean concentrations of plasma C-reactive protein (CRP) in the animals. Treatment with isoproterenol significantly (p<0.05) increased the plasma concentration of CRP when compared with the control. However, pre-treatment of the rats with the wild vegetables before administration of ISO reversed the CRP elevating effect of ISO in all the rats. Administration of the vegetables’ extracts to the rats significantly (p<0.05) reduced the plasma concentration of CRP in the ISO untreated rats when compared with the control rats. After vegetables’ extracts pre-treatment, a significant decrease (p<0.05) of CRP was observed in ISO control group, however, feeding the vegetables with isoproterenol (both pre- and post-treatment) significantly reduced CRP values when compared to the control rats. While there was no significant difference in CRP concentrations in ISO control group (not pre-treated with vegetables extracts) when compared to normal control and other groups. However, feeding the isoproterenol treated rats with the vegetables singly or in combination significantly reduced the BP and restored it towards normal (Figure 1).
compared to normal and ISO controls. There was no significant difference in CRP levels \( (p>0.05) \) of CC + ISO group (pre-treated with CC and treated with ISO) in comparison to normal control. Other groups (LT, LT + ISO, SB, SB + ISO, and SN + ISO) also showed significant decrease in CRP concentrations \( (p<0.05) \) when compared to normal and ISO controls. Pre-treatment with extracts of the selected vegetables abolished the increases in CRP in a significant manner \( (p<0.05) \).

Effect of Extracts on Lactate dehydrogenase of Male Wistar Rats Treated with Isoproterenol

The highest significant \( (p<0.05) \) activities of LDH enzyme was recorded in isoproterenol control group (Figure 3). Groups in vegetable category (pre-treated with vegetables only) and groups in ISO category (pre-treated with vegetables and treated with ISO) displayed significant decrease \( (p<0.05) \) in LDH levels as compared to the controls (normal and ISO controls).

Effect of Extracts on Creatine Kinase of Male Wistar Rats Treated with Isoproterenol

Figure 4 shows mean concentrations of CK (creatine kinase) enzyme in male Wistar rats. All the groups of rats administered the extracts displayed significant \( (p<0.05) \) decrease in CK levels when compared with the control as well as the isoproterenol group. However, the ISO treated group showed a significant increase in the CK level when compared with the control group. The lowest level of CK was recorded in the mixture group when compared with the controls.

Effect of Extracts on Aspartate Aminotransferase of Male Wistar Rats Treated with Isoproterenol

Figure 5 shows mean concentrations of aspartate aminotransferase in experimental rats. A significant \( (p<0.05) \) increase was recorded in isoproterenol group when compared to normal control and other groups. All vegetables pre-treated groups in both categories (vegetables and ISO categories) had significantly decreased \( (p<0.05) \) AST values in comparison with ISO control group. However, lowest significant decrease \( (p<0.05) \) in AST activities was observed in LT, LT + ISO, SB and mixture groups when compared to both controls (normal and ISO controls).
DISCUSSION

Myocardial infarction in the rat is an ideal model to study cellular modifications after cardiac injury and preventative dietary intervention (18). In this study, the effect of five wild selected edible vegetables (Basella alba, Crassocephalum crepidioides, Launaea taraxacifolia, Senecio biafrae and Solanum nigrum) on cardiac function in male Wistar rats following administration of isoproterenol to induce myocardial infarction was investigated. Blood pressure measurements at night showed a significant increase (highest systolic blood pressure and lowered diastolic blood pressure) in the isoproterenol control compared to other groups. This observation disagrees with a previous study which reported a significant decrease in blood pressure of isoproterenol treated rats (19). Evidence pointing to the absence of the normal fall in blood pressure in rats at night is a stronger risk of cardiovascular disease than day-time blood pressure (16). This significant increase in blood pressure recorded in the ISO control may be as a result of its stimulating action on the heart, resulting in increased cardiac output and excitability. It may also cause peripheral vasodilatation to produce a fall in diastolic blood pressure and an increase in systolic blood pressure (6). This contrast may be as a result of 20 mg/kg of ISO used to induce MI in rats as opposed to 85 mg/kg of ISO used by Adeoye et al. (19). However, other vegetable pre-treated groups (singly or in combination) had significantly reduced BP which was reinstated towards normal. This result affirms the efficacy of these vegetables in preventing one of the most important risk factors for CVD, therefore improving cardiac health. The blood pressure lowering effect of the vegetable extracts could also be due to their antioxidant properties as it has been reported that antioxidants scavenge vascular reactive oxygen species and therefore improve vascular function (20). Fathiazad et al. (8) opined that vegetables are beneficial in reducing the risk of heart disease.

The myocardium contains bountiful concentrations of diagnostic markers of myocardial infarction and once metabolically injured, these substances are released into the extracellular fluid (21). One of such diagnostic markers for myocardium damages is CRP. A cytosolic enzyme marker such as CRP has been used widely as a sensitive predictor of acute cardiovascular events (22). Previous studies have reported that serum CRP concentrations are inversely associated with dietary intake of vegetables which are rich in polyphenolic antioxidants (23, 24). This is in accordance with the present findings as a significant reduction of CRP concentration was elicited in singly and combined vegetable pre-treated groups. These findings suggest that the extracts retard or prevent the exacerbation of cardiac tissue damage in ISO-induced MI or ameliorate the deleterious effect of MI on the heart. The fact that the lowest level of CRP was recorded in the mixture group suggests additive and synergistic effects of the vegetable mixture. These effects could be attributed to the antioxidant efficacy of the extracts as it has been reported that these vegetables are very rich in natural antioxidants (25). This study also conforms with marked elevated CRP concentration in isoproterenol control as reported by Adeoye et al. (19).

A higher significant LDH activity was shown in ISO control than vegetable pre-treated groups which is consistent with earlier reports (26, 27). Elevated LDH activity indicates an inflammation of heart tissue. When the myocardial cells are impaired due to deficient oxygen supply, the cell membrane ruptures and becomes porous and results in the leakage of enzymes such as LDH. The extent of prophylactic effect offered by pre-treatment with the extracts is associated with significant attenuation of plasma LDH activity. This is indicative of significant cardio-preventive activity and maintenance of the myocardial membrane architecture (26).

Creatine kinase (CK) is a cytosolic enzyme and a sensitive marker of ischemic myocardial injury. Enzyme levels of CK also showed an elevation in isoproterenol administered rats, which were consistent with earlier reports as indication of compromised membrane permeability and leakage of this soluble enzyme (28). The restoration of myocardial CK activities in animals pre-treated with the extracts indicated protection of the myocardium against isoproterenol-induced exogenous stress. Therefore, it may be suggested that these vegetables’ extracts (singly and combined) possessed cardio-protective activities due to their antioxidant and anti-inflammatory properties, in consonance with other reports (8, 25).

Aspartate aminotransferase was also evaluated in this study as a cytosolic diagnostic marker from the damaged tissue. The amount of this cellular enzyme in the plasma reflects the alterations in plasma membrane architecture (29). In this present study, rats administered isoproterenol showed significant increases in the levels of AST marker in plasma, in line with the results from previous reports. This increase is indicative of isoproterenol-induced myocardial damage, pointing to its ability to impair liver function (27, 28). Administration of the extracts decreased the harmful effect of isoproterenol, a pointer to their beneficial effect on the liver.

This result corroborates literature that vegetables contain vital components that can modulate and may prevent degenerative diseases such as heart disease (30–32).

CONCLUSION

Conclusively, the results from this study showed that the aqueous extracts of selected wild edible underutilized leafy vegetables (Basella alba, Crassocephalum crepidioides, Launaea taraxacifolia, Senecio biafrae and Solanum nigrum) possesses cardioprotective activities, probably through reduction of C-reactive protein, lactate dehydrogenase, creatine kinase and aspartate aminotransferase in the blood. Hence, the findings provides scientific basis for the usefulness of these selected wild edible vegetables for cardio-protective purposes, ultimately justifying the need to create awareness about diets rich in vegetables and specifically, less-used indigenous green leafy vegetables in promoting health and preventing diseases in the populace.

CONFLICT OF INTEREST

The authors declare no conflict of interest.
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