Effects of Oral Administration of Cnidoscolus aconitifolius Leaf Extract (Chaya Tea) on Biomarkers of Cardiovascular System of Wistar Albino Rats

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Abstract: This present study is designed to evaluate the effects of Oral Administration of Cnidoscolus aconitifolius (CA) leaves extract (Chaya tea) on biomarkers of cardiovascular system of Wistar albino rats. A total of 30 albino rats grouped into six groups (A, B, C, D, E and F); Group A (Control male) five rats, Group B (Boiled extract of C. aconitifolius) five male rats, Group C (raw extract of C. aconitifolius) five male rats, Group D (Control female), Group E (Boiled extract of C. aconitifolius) five female rats and Group F (Raw extract of C. aconitifolius) five female rats. Systolic and diastolic blood pressure, pulse rate, creatinine kinase, lactate dehydrogenase, body weight of the animal and weight of the organ (heart) were estimated on each sample. Results from this study showed that albino rats fed with boiled and raw extract of C. aconitifolius have changes in biomarker of cardiovascular system increased activity of creatine kinase, Significant increase in the lactate dehydrogenase activity following the administration of the raw extract of C. aconitifolius may be due to hydrocyanic glycosides probably leading to tissue breakdown. The body weight of the albino rat in the groups showed a significant increase (P<0.05) it could be due to the fact that young chaya leaves contain nutritional components, which may increase the appetite and resulted in increased food intake or consumption which ultimately may lead to increase in the weights observed. The weights of the organ (heart) showed no significant difference (P>0.05). No marked variation was seen in the weights as compared to controls. In spite of this, we suggest that the aqueous extract of C. aconitifolius may have a positive effect on the growth of laboratory animals. The blood pressure (systolic and diastolic) and pulse rate showed no significant difference statistically when compared with the controls. There was no significant alteration in boiled and raw extract of C. aconitifolius when compared with the controls. The heart obtained from the controls group showed no difference in their normal gross anatomical features, the cardiac chambers contains blood cells, the ventricular wall myocytes appear unremarkable, this is size, colour and consistency etc.

Key words: Cnidoscolus aconitifolius • Biomarkers • Body Weight and Albino Rats

INTRODUCTION

Cnidoscolus aconitifolius, commonly known as chaya or tree spinach, is a large, fast growing leafy perennial shrub belonging to the Euphorbiaceae family. It is believed to have originated in the Yucatan Peninsula of Mexico, cultivated in Central American and United State. It is commonly found in the tropic and sub tropical regions worldwide, including Africa, South of Sahara, North and South America, India, etc. It is commonly eaten as vegetable in soup [1] in South Western Nigeria where it is called Iyana Ipaja. Young chaya leaves and the thick, tender steam tips cut and boiled as spinach. The nutritional composition of Chaya leaves revealed that per 100g, it contained protein (5.7%), crude fiber (1.9%), calcium (199.4mg/100g), potassium (217.2mg/100), iron (11.4mg/100g), vitamin C (164.7mg/100mg), kind carotene (0.085mg/100g) [2-4].

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Chaya traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids and eye problems [5]. Chaya shoots and leaves have been taken as a laxative, diuretic, circulation stimulant, to improve digestion, to stimulate lactation and to harden the fingernails [6].

Like most food plants such as lima beans, cassava and many leafy vegetables, the leaves contain hydrocyanic glycosides, a toxic compound easily destroyed by cooking. Even though some people tend to eat raw chaya leaves it is unwise to do so [2]. Chaya leaf furnishes appreciable quantities of several of the essential mineral macronutrients necessary for human health maintenance. For example, potassium has been shown to be an important mineral nutrient in the control of hypertension and in the reduction of risks of stroke [7]. Calcium is important for ossification and iron necessary for normal haematopoiesis [8]. The nutritional value and composition of raw and cooked chaya leaves have been demonstrated [9, 10].

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the world [11]. Primary prevention and secondary prevention of CVD are public health priorities [12]. Substantial data indicate that CVD is a life course disease that begins with the evolution of risk factors that in turn contribute to the development of subclinical atherosclerosis [13, 14]. Subclinical disease culminates in overt CVD [15, 16]. The onset of CVD itself portends an adverse prognosis with greater risk of recurrent events, morbidity and mortality [17, 18]. It is also increasingly clear that although clinical assessment is the keystone of patient management; such evaluation has its limitations [19-22]. Clinicians have used additional tools to aid clinical assessment and to enhance their ability to identify the “vulnerable” patient at risk for CVD, as suggested by National Institutes of Health (NIH) panel [23]. Biomarkers are one such tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately and to effectively prognosticate and treat patients with disease.

The term biomarker (Biological marker) was introduced in 1989 as a Medical Subject Heading (MeSH) term: “Measurable and quantifiable biological parameters (Example, specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies, etc.” In 2001, an NIH working group standardized the definition of a biomarker as “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” and defined types of biomarkers [24].

These cardiovascular biomarkers can be classified into the following groups: Telemetry; Electrocardiogram, heart rate, blood pressure, temperature [25, 26]. Serum Biomarkers: Troponins T & I, Natriuretic protein, Myeloperoxidase, Creatine kinase, Lactate dehydrogenase, Ischemia-modified albumin, Glycogen phosphorylase 2, Matrix metalloproteinase, Inflammatory cytokines (e.g., TNFa, IL-6, CRP), Choline, Myoglobin [27-32]. Tissue Biomarkers: G protein-coupled receptor kinase-2 (Heart & lymphocytes), beta-Adrenergic receptor (Heart), Super oxide dismutase (Heart), DNA damage (Heart), Aconitase (Heart) [33-36]. Proteomic Profiles: Heart, Serum [37, 38]. Gene Analysis (Heart): Energy production (e.g. ATP synthase), Lipid metabolism, Mitochondria (Dehydrogenases), Heart structural proteins, Adhesion/cell connection proteins, Growth factors, Cytokines/chemokines of inflammation, Temperature regulation, G protein signals/kinases, Ion transport, Protein degradation [39-42]. Tissue pathology: Electron microscopy, Apoptosis stain, Morphometry, Histochemistry, Special stains (e.g., trichrome & oil red O) [31, 36, 43-47]. Imaging: (Magnetic resonance microscopy & computed tomography) [48, 49].

Therefore, this present study is designed to evaluate the effects of Oral Administration of Cnidoscolus aconitifolius leaves extract (Chaya tea) on some biomarkers of the cardiovascular system of Wistar albino rats.

MATERIALS AND METHOD

Experimental Animals: Thirty (30) albino rats (Fifteen (15) each for male and female) weighing 150-180g were obtained from the Animal House of Department of Human Physiology, University of Port Harcourt. The animals were housed under a standard laboratory condition with 12 hours dark/light cycle and with access to standard diet (Guinea feed, Benin-Auchi, Road, Edo State) and water ad libitum.

Preparation of Extracts: Cnidoscolus aconitifolius leaves were obtained freshly from a farm in Choba, Obio/Akpor LGA, Rivers State, Nigeria and authenticated by a
Resource Staff at the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria. Cnidoscolus aconitifolius leaves were subjected to some conventional food processing techniques as reported [50]. About 10g of the leaves were extracted with boiling water (1000ml) for 30 minutes until the volume of the water were reduced to 90% of the original.

The resulting decoction were filtered using a filtration sieve (pore size 0.5mm diameter) the extract were stored in a refrigerator at 4°C. The raw extracts were obtained by using the method described elsewhere [51-54] and were modified in our laboratory. Briefly, 10g of the leaves were soaked in 1000ml of distilled water in a beaker, stirred for about 6 minutes and left overnight. Thereafter, the solution were filtered using a filtration sieve (pore size 0.5mm diameter) the extract were stored in a refrigerator at 4°C.

**Experimental Design:** The experiment animals were divided randomly into six (6) groups A-F of five animals each. There were two control groups (A & B) (five animals each for male and female) received tap water. Two experimental groups (C-D) (five animals each for male and female) received boiled C. aconitifolius and last two experimental groups (E-F) (five animals each for male and female) received raw C. aconitifolius. The extracts of boiled, raw C. aconitifolius and water were administered via oral route for 14 days.

**Measurement of Blood Pressure:** Before and after the administration of the extract in all the groups, a 58500 blood pressure recorder known a UGO BASILE BIOLOGICAL RESEARCH APPARATUS were used to measure the blood pressure for the experimental animals (Diastolic BP, Systolic BP, Pulse rate, Gender).

**Sample Collection:** At the end of experimental period, rats were weighed and anaesthetized with chloroform. Blood samples were collected by cardiac puncture in non heparinized tubes, centrifuged at 2000 rpm for 20 min and blood sera were then collected and stored at 4°C prior immediate determination of Lactate dehydrogenase and Creatine kinase.

**Statistical Analysis:** The data of heart, biomarker assay, body weights and blood pressure analysis were analyzed and expressed as mean ± standard of error (Mean ± S.E.M.). The data obtained, were analyzed using two way analysis of variance (ANOVA).

**RESULTS**

Results of systolic and diastolic blood pressure, pulse rate, creatinine kinase, lactate dehydrogenase, body weight of the animal and weight of the organ (heart) treated with aqueous extract of boiled and raw C. aconitifolius are presented in Tables (1-6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Boiled CA</th>
<th>Raw CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>128.8±1.94</td>
<td>126.8±2.08</td>
<td>126.2±1.37</td>
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<tr>
<td>Diastolic</td>
<td>77.4±4.49</td>
<td>80.0±0.00</td>
<td>79.6±1.20</td>
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<tr>
<td>Pulse rate</td>
<td>373.0±6.67</td>
<td>335.40±1.92</td>
<td>397.60±5.41</td>
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<tr>
<td>Systolic</td>
<td>118.8±2.50</td>
<td>108.0±2.3</td>
<td>117.8±3.06</td>
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<tr>
<td>Diastolic</td>
<td>78.8±1.02</td>
<td>77.2±2.86</td>
<td>87.0±2.64</td>
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<tr>
<td>Pulse rate</td>
<td>382.0±4.10</td>
<td>413.8±2.28</td>
<td>390.0±1.27</td>
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<th>Parameters</th>
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<tr>
<td>Male</td>
<td>160.4±1.12</td>
<td>204.0±1.57$^a$</td>
<td>166.2±1.23</td>
</tr>
<tr>
<td>Female</td>
<td>157.6±1.12</td>
<td>203.8±1.12$^a$</td>
<td>161.4±1.23</td>
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</tbody>
</table>

$^a$ Significant difference compared to control.
Table 4: Mean organ weight (heart) (kg) of controls, boiled and raw extract of *C. aconitifolius* after administration

<table>
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<th>Raw CA</th>
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<tr>
<td>Male</td>
<td>0.18±0.19</td>
<td>0.12±0.05</td>
<td>0.14±0.06</td>
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<tr>
<td>Female</td>
<td>0.09±0.18</td>
<td>0.14±0.04</td>
<td>0.16±0.05</td>
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</table>

Table 5: Mean creatinine kinase of controls, boiled and raw extract of *C. aconitifolius* after administration

<table>
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<th>Raw CA</th>
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<tr>
<td>Male</td>
<td>892.8±2.30</td>
<td>11994.0±1.59</td>
<td>4449.2±1.85a</td>
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<tr>
<td>Female</td>
<td>2019.6±1.12</td>
<td>1424.0±2.10</td>
<td>812.6±1.20ab</td>
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Table 6: Mean lactate dehydrogenase of controls, boiled and raw extract of *C. aconitifolius* after administration

<table>
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<th>Raw CA</th>
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<tr>
<td>Male</td>
<td>2343.6±1.57</td>
<td>2360.8±1.08</td>
<td>2451.2±1.13a</td>
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<tr>
<td>Female</td>
<td>2099.2±1.99</td>
<td>2049.0±1.03</td>
<td>1693.4±1.20b</td>
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</tbody>
</table>

**DISCUSSION**

Results from this study showed that albino rats fed with boiled and raw extract of *C. aconitifolius* have changes in biomarker of cardiovascular system increased activity of creatine kinase, a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, the auto immune myositis and in acute renal failure [44] was observed when compared with the controls. Elevation of CK is an indication of damage to muscle.

Significant increase in the lactate dehydrogenase activity following the administration of the raw extract of *C. aconitifolius* may be due to hydrocyanic glycosides probably leading to tissue breakdown. The enzyme LDH is in many body tissues, especially the heart, liver, kidney, skeletal muscle, brain, blood cells and lungs [55]. Groups fed with boiled extract of *C. aconitifolius* showed no significant difference ($P>0.05$) when compared with the controls.

The body weight of the albino rat in the groups showed a significant increase ($P<0.05$) it could be due to the fact that young chaya leaves contain nutritional components [2-4] which may increase the appetite and resulted in increased food intake or consumption which ultimately may lead to increase in the weights observed [56, 57].

The weights of the organ (heart) showed no significant difference ($P>0.05$). No marked variation was seen in the weights as compared to controls. In spite of this, we suggest that the aqueous extract of *C. aconitifolius* may have a positive effect on the growth of laboratory animals. The blood pressure (systolic and diastolic) and pulse rate showed no significant difference statistically when compared with the controls.

**CONCLUSION**

Our study suggests that oral administration of *Cnidoscolus aconitifolius* leaves extract (Chaya) on cardiovascular parameters of Wistar albino might be harmful to the cardiovascular system. The investigation also shows that the *Cnidoscolus aconitifolius* causes increases in biomarker enzymes and no changes on the histology, which suggest toxicity to the heart. It is therefore recommended that further studies be conducted to determine the safe dose of this CA in Humans.

**REFERENCES**


