Effects of *Hibiscus Sabdariffa* Calyces Aqueous Extract on Serum Cholesterol, Body Weight and Liver Biomarkers of *Rattus Novergicus*

Emmanuel I Nnamonu  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: nnamonuei@yahoo.com

Vincent C Ejere  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email:vincent.ejere@unn.edu.ng

Anthony O Ejim  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email:selssy@gmial.com

Paul C Echi  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: paul_echi@yahoo.com

Jude V Egbruji  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: angeluriel@yahoo.com

Tochukwu R Eze  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: nnabuikenwaw@yahoo.com

Joseph E Eyo*  
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: joseph.eyo@unn.edu.ng

**ABSTRACT**

The present study investigated the effect of *Hibiscus sabdariffa* calyx aqueous extract on the serum cholesterol, body weight and liver marker enzymes activities of normal albino rats. The aqueous extract was orally administered (100 – 800 mg/kg body weight) for 28 days to normal male albino rats. Total cholesterol, body weight, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels were measured. *Hibiscus sabdariffa* administration significantly reduced serum cholesterol and body weight in a dose and duration dependent pattern. AST, ALP and ALT levels were significantly elevated in a dose and duration dependent pattern. The significant increase in the levels of the liver enzymes tends to suggest dysfunction in the coordinating of the liver activity. The extract ability to lower the total cholesterol level and body weight suggests its usefulness as a hypocholesterolemic and anti-obesity agent.

**Keyword-** *Hibiscus sabdariffa*, aqueous extract, liver marker enzymes, serum cholesterol, body weight, albino rats

**1. INTRODUCTION**

*Hibiscus sabdariffa* is an herb belonging to the family Malvaceae. It is grown in all parts of the world and usually cultivated for its leaf, fleshy calyx, seed or fibre [1]. Various studies on extracts of different parts of *H. sabdariffa* using animal models suggest manifold beneficial effects to human welfare. *H. sabdariffa* sepal (calyx and epicalyx) are the most important economic parts of the plant which is used in food (jam and jelly) and cosmetic industries as a source of natural colouring agent [2]. Physiologically, obesity is a disarray of energy balance primarily considered as a disordor of lipid metabolism [3]. Cholesterol is the main lipid found in blood, bile and brain tissues. It is the main lipid associated with arteriosclerotic vascular diseases. Cholesterol is required for the formation of steroids and cellular membranes. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephritic syndrome and cirrhosis. On the other hand, decreased levels are found in malabsorption, malnutrition, hypothyroidism, anaemias and liver diseases [4].

The aqueous extract of red and green *H. sabdariffa* petals has been reported to cause significant decreases in the LDL–cholesterol levels, while no significant effect was observed on HDL–cholesterol and triglycerides levels [5]. Carvaja-Zarrabal et al. [6] reported that the aqueous extract of *H. sabdariffa* calyces could be considered a possible anti-obesity agent. Chen et al. [7] reported of its anti-atherosclerotic property, while Mckay et al. [8] noted its ability to lower the blood pressure of pre-hypertensive and mildly hypertensive adults. In the same vein, Adegunloye et al. [9] had earlier reported that the dried red
calyces of *Hibiscus sabdariffa* was used as traditional medicine for diuretic, hypcholesterolemic, antihypertensive and mucolytic effects. Kalt et al. [10] reported that the pigments (anthocyanin) which are responsible primarily for red colour were delphinidino-3-glucoside and cyanidin-3-glucoside. Anthocyanin was also found to have many times more antioxidant activity than ascorbate [11]. The ethanolic extract of *H. sabdariffa* seed has been reported to increase serum prolactin levels in a dose dependent manner, while the aqueous seed extract was found to produce a significant reduction in cat blood pressure [12]. A group of phenolic natural pigments (anthocyanin) present in the dried flower of *H. sabdariffa* and *Hibiscus rosasinensis* have been reported severally to have cardioprotective [13-15], hypcholesterolemic [7,14,15], anti-oxidative and hepatoprotective [11,16] effects in animals. It has also been reported that delphinidin-3-sambubioside, a *Hibiscus* anthocyanin induced apoptosis in human leukemia cells through reactive oxygen species–mediated activity [17], and polysaccharides extracted from *H. sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes [18]. However, repeated exposure to aqueous extract of *H. sabdariffa* may potentially pose risk. The oral administration of the dried calyx of *H. sabdariffa* at high doses has been reported to be toxic to the hepatic system, and cause muscular dystrophy, increased serum creatinine, while extract obtained with alcohol (absolute 50%) had more damaging effects on the liver enzymes function in addition to an increase in plasma creatinine levels [19]. Furthermore, sub-chronic administration of calyx aqueous extract of *H. sabdariffa* increased significantly levels of AST, ALT, ALP and bilirubin [20]. Major toxic effects of *H. sabdariffa* extracts have been reported in the kidneys and reproductive organs of male rats [21]. In line with the above literatures, there is need for further studies on the effects of *H. sabdariffa* extracts on the activities of liver marker enzymes. The aim of this study therefore, was to evaluate the effect of a 28-day oral administration of the aqueous calyx extract of *H. sabdariffa* on the serum cholesterol, body weight and liver marker enzymes of normal male albino rats.

### 2. MATERIALS AND METHODS

#### 2.1 Collection and Preparation of *Hibiscus Sabdariffa* Calyx Extract

Fresh calyces of *H. sabdariffa* were collected from Kalaah farm at Mubi, Adamawa State, Nigeria. The calyces were identified [22] and voucher specimen store in the herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria. The *H. sabdariffa* calyces were air-dried for three weeks under room temperature after which the dried plant materials were ground into powder using a laboratory milling machine (Thomas Willey model 4, USA). 135g of the powdered plant material was introduced into 2000 ml flat bottom flask and 500ml of distilled water was added. The content was mixed thoroughly and left for 24 hours with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with Whatman filter paper (grade 1: 11 µm) and the resulting filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK). The solid extract was weighed and re-dissolved in normal saline according to the body weights of the animals for oral administration.

#### 2.2 Procurement and Management of Experimental Animals

Sixty adult male albino rats weighing between 120 - 295g were obtained from the Genetics and Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats had no history of drug consumption (i.e. they have not been used for any investigation). They were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats had unhindered access to feed (chick growers mash, 18 % crude protein, Vital Feeds, Nigeria) and clean drinking water. All the animals were maintained under standard laboratory conditions for temperature, humidity and light throughout the experiment. The fecal droppings in the tray were removed daily.

#### 2.3 Experimental Design

A completely randomized block design (CRBD) was used for the experiment with 60 adult male albino rats divided into five (5) blocks (groups). Each block had three replicates with 4 rats per replicate. Group I (control) were fed commercial growers chick mash and water. Group II – Group V represented the experimental groups. They were fed commercial growers chick mash (18 % crude protein), water and the extract daily. Group II – V rats were orally administered 100, 200, 400 and 800 mg/kg body weight of rat, respectively for 28 days. All dosages were re-suspended in 2 ml of saline and administer orally using 5ml hypodermal syringe.

#### 2.4 Collection of Blood Samples and Determination of Liver Maker Enzymes

5 ml of blood was collected from each replicate of anaesthetized rats using the ocular puncture method [23]. The sampled bloods were allowed to stand for 30 minutes to clot before being centrifuged at 2000rpm for 10 minutes. The sera obtained were used to estimate the levels of AST, ALT [24], ALP [25] and total cholesterol [26]. Blood collection was done before the start of the experiment (day 0) and at weekly intervals during treatment (day 7, 14, 21 and 28).

#### 2.5 Determination of Body Weight

The rats were weighed using a Mettler electronic balance PC 2000 before treatment (Day 0) which served as the initial weight and during treatment before blood samples were collected at days 7, 14, 21 and 28.

#### 2.6 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), while the differences among groups means
were separated using Duncan New Multiple Range Test. All analysis was done using statistical package for social sciences (SPSS) version 20.0 (IBM Statistics UK). All data were expressed as mean ± standard error, while level of significance was set at p<0.05.

3. RESULTS

The effects of the aqueous extract of H. sabdariffa on ALT, AST, ALP and total cholesterol are presented in Table 1. At days 0 and 7, results obtained on ALT, AST and ALP showed no significant difference (Table 1) when compared with the control. A significant dose and time dependent increase (p>0.05) was observed on ALT, AST and ALP levels at higher doses (200 mg/kg and 800 mg/kg body weight) at days 14, 21 and 28. Serum cholesterol levels showed a significant increase (p<0.05) on day 0, no significant difference at day 7 but decreased significantly at days 14, 21 and 28 when compared with the control. A mixed trend was observed on the effect of the extract on body weight (Table 2). A significant decrease (p<0.05) was observed at most doses as the administration of the extract continued with time as compared between initial and final body weight and also with the body weight of the control group (Table 2).

Table 1 Opposite

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentrations (mg/kg)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT(u/l)</td>
<td>CRL</td>
<td>4.0±0.00</td>
<td>9.3±0.33</td>
<td>8.0±2.31</td>
<td>11.3±1.77</td>
<td>14.6±1.45</td>
</tr>
<tr>
<td></td>
<td>AQ100</td>
<td>4.0±0.00</td>
<td>9.7±0.33</td>
<td>11.3±1.76</td>
<td>15.7±0.88</td>
<td>13.6±0.88</td>
</tr>
<tr>
<td></td>
<td>AQ200</td>
<td>4.0±0.00</td>
<td>9.3±0.33</td>
<td>13.6±1.67</td>
<td>13.7±3.84</td>
<td>32.00±2.51</td>
</tr>
<tr>
<td></td>
<td>AQ400</td>
<td>4.0±0.00</td>
<td>9.7±0.33</td>
<td>21.0±2.31</td>
<td>26.7±2.85</td>
<td>31.6±1.67</td>
</tr>
<tr>
<td></td>
<td>AQ800</td>
<td>4.0±0.00</td>
<td>9.3±0.33</td>
<td>22.3±3.53</td>
<td>28.00±3.61</td>
<td>35.3±1.20</td>
</tr>
<tr>
<td>AST(u/l)</td>
<td>CRL</td>
<td>5.0±0.00</td>
<td>7.6±0.33</td>
<td>9.0±0.58</td>
<td>10.0±1.73</td>
<td>12.0±1.16</td>
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<tr>
<td></td>
<td>AQ100</td>
<td>5.0±0.00</td>
<td>7.3±0.33</td>
<td>11.7±0.88</td>
<td>15.7±0.67</td>
<td>19.6±0.88</td>
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<tr>
<td></td>
<td>AQ200</td>
<td>5.0±0.00</td>
<td>7.6±0.33</td>
<td>12.3±0.88</td>
<td>17.3±0.33</td>
<td>24.3±0.88</td>
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<tr>
<td></td>
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<td>7.3±0.33</td>
<td>16.0±1.53</td>
<td>18.6±1.20</td>
<td>31.3±2.40</td>
</tr>
<tr>
<td></td>
<td>AQ800</td>
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<td>7.6±0.33</td>
<td>16.3±0.84</td>
<td>26.3±0.67</td>
<td>31.00±2.31</td>
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<tr>
<td>ALP(u/l)</td>
<td>CRL</td>
<td>20.01±0.11</td>
<td>47.6±0.33</td>
<td>26.7±0.33</td>
<td>23.7±2.03</td>
<td>26.7±3.33</td>
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<tr>
<td></td>
<td>AQ100</td>
<td>20.01±0.11</td>
<td>47.3±0.33</td>
<td>47.00±2.00</td>
<td>50.67±2.91</td>
<td>59.67±5.04</td>
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<td>47.6±0.33</td>
<td>54.6±2.60</td>
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<td>47.00±0.33</td>
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<td>65.3±1.45</td>
<td>75.00±3.06</td>
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<tr>
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<td>47.6±0.33</td>
<td>59.6±0.67</td>
<td>67.6±3.84</td>
<td>75.6±2.33</td>
</tr>
<tr>
<td>CHOL(mg/dl)</td>
<td>CRL</td>
<td>2.36±0.81</td>
<td>1.58±0.00</td>
<td>2.53±0.10</td>
<td>2.86±0.09</td>
<td>2.57±0.06</td>
</tr>
<tr>
<td></td>
<td>AQ100</td>
<td>2.12±0.10</td>
<td>1.58±0.00</td>
<td>1.59±0.15</td>
<td>1.44±0.09</td>
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<td>AQ200</td>
<td>2.27±0.24</td>
<td>1.58±0.00</td>
<td>1.50±0.09</td>
<td>1.22±0.00</td>
<td>1.20±0.01</td>
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<tr>
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<td>AQ400</td>
<td>2.66±0.13</td>
<td>1.57±0.00</td>
<td>1.27±0.03</td>
<td>1.22±0.00</td>
<td>1.23±0.03</td>
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<tr>
<td></td>
<td>AQ800</td>
<td>2.93±0.03</td>
<td>1.58±0.00</td>
<td>1.25±0.01</td>
<td>1.21±0.01</td>
<td>1.73±0.03</td>
</tr>
</tbody>
</table>

*Values with different alphabetic (lower case) superscripts differ significantly (p<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (p<0.05) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.

Table 2

Effect of aqueous H. sabdariffa extract on the body weight of albino rats

<table>
<thead>
<tr>
<th>Concentration (mg/kg)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>224.47±14.23</td>
<td>225.00±14.43</td>
<td>232.53±7.86</td>
<td>233.70±8.21</td>
<td>252.70±5.30</td>
</tr>
<tr>
<td>100</td>
<td>175.13±2.92</td>
<td>174.98±2.93</td>
<td>177.87±1.73</td>
<td>177.65±1.76</td>
<td>176.63±1.48</td>
</tr>
<tr>
<td>200</td>
<td>130.47±2.98</td>
<td>130.27±3.01</td>
<td>208.10±8.15</td>
<td>207.86±8.21</td>
<td>200.13±14.87</td>
</tr>
<tr>
<td>400</td>
<td>120.27±0.07</td>
<td>119.27±0.22</td>
<td>150.37±0.24</td>
<td>149.94±0.23</td>
<td>200.28±0.10</td>
</tr>
<tr>
<td>800</td>
<td>130.25±2.96</td>
<td>129.63±2.89</td>
<td>150.18±1.44</td>
<td>149.63±1.44</td>
<td>168.40±9.3</td>
</tr>
</tbody>
</table>

*Values with different alphabetic (lower case) superscripts differ significantly (p<0.05) between different concentrations within the same exposure duration.
4. DISCUSSION
This study evaluated the effect of a 28-day oral administration of the aqueous calyx extract of H. sabdariffa on the serum cholesterol, body weight and liver marker enzymes activities of normal albino rats.

Analysis of enzymological and biochemical profile of blood are widely used as indicators to access the functional status of the animal health and the internal environment of the organism [27,28]. Enzymes like ALP, ALT, and AST have serve as good bio-indicators [29]. The observed dose dependent significant increases in the activities of such liver enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) tend to suggest liver dysfunction in the experimental animals [30]. Usually an elevation in the liver enzymes may indicate inflammation or damage to the cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes into the blood stream which can result in elevated liver enzymes on blood tests. This observation is somehow consistent with some earlier reports which showed that a prolonged usage of the aqueous and methanolic extracts of the calyx of H. sabdariffa in albino rats could cause liver injury even at dose levels as low as 150 mg/kg – 180 mg/kg [31-33]. In actual sense, Ojokoh [31] discovered visible signs of degeneration of the hepatocytes during physical examination of the liver of albino rats fed with H. sabdariffa calyx extracts. This view may not be surprising as H. sabdariffa calyx had been analyzed to contain phytic acid, tannin and glycosides such as delphinidin-3-monoglucoside and delphinidin which are toxic to animal and human tissues at high doses [22]. Nevertheless, the present observation is at variance with some other earlier reports. Prommetta et al. [34] observed that doses ranging from 250 – 1000 mg/kg/day, did not elicit any adverse effect on several important organs such as liver, kidney and the blood system. Similarly, reports abound on the hepato-protective effects of H. sabdariffa extracts [35,36] as well as chemo-protective and anti-oxidative effects [37,38]. In an attempt to explain the observed increases in the activities of these marker enzymes following the administration of Hibiscus sabdariffa extracts, Yakubu et al. [39] opined that such may be a unique adaptation by the liver to the assault from the plant extract or as a result of fresh synthesis of the enzyme molecules following extract administration. It seems also plausible that the effect of the extracts on the activities of Aspartate and Alanine Aminotransferase may be the case of organ chain reactions. This is more so as the two enzymes have been found to be localized normally within the cells of the liver, heart, kidney, gill, muscle and other organs [21]. Therefore, the need to fully resolve the present apathy in the actual bioactivity of Hibiscus sabdariffa extracts within these vital organs is essential. Despite the fact that these enzymes have been reported as important markers in assessing and monitoring liver damage [40], there is the need to correlate the exact mechanisms and constituents of the extract on the levels of these enzymes in the different tissues. This is more so as major toxic effects of H. sabdariffa extracts have been reported in the kidneys and reproductive organs of male rats [21].

An adequate resolution of this will further ensure that the efforts currently being put in place globally catering for the well being of the citizens especially Third World countries are not jeopardized.

In the present study, the result of the effects of the extracts of H. sabdariffa on serum cholesterol showed that the extracts of H. sabdariffa are capable of reducing the serum cholesterol levels of treated rats on a dose dependent fashion. This observation on the hypocholesterolemic ability of H. sabdariffa is consistent with several past reports. Tzu-Li et al., [41] observed that the consumption of Hibiscus sabdariffa extracts can significantly decrease serum cholesterol levels in human beings. This view was corroborated by the findings of [5] and [42] who observed a significant decrease in the serum cholesterol level of treated men and women. Similarly, Chen et al. [7] reported of its anti-atherosclerotic property, while [8] noted its ability to lower the blood pressure of pre-hypertensive and mildly hypertensive adults. In the same vein, Adegunloye et al., [9] had previously stated that the dried red calyces of Hibiscus sabdariffa are used as traditional medicine for diuretic, hypocholesterolemic, antihypertensive and mucolytic effects. This hypocholesterolemic effect has been attributed to its abundant antioxidant composition [43].

The decrease in the body weight of the treated rats in comparison with the control further attests to the anti-obesity property of H. sabdariffa. This result agrees with those of [19] and [6] who observed a drastic loss of weight among animals treated with various concentrations of H. sabdariffa extracts. In contrast however, [5] observed no significant decrease in the body weight of rats that were chronically treated with 25mg/kg and 50mg/kg body weight of H. sabdariffa extracts. Carvajal-Zarrabal et al. [6] were of the opinion that such weight decreases might have been as a result of dietary palatability problem when H. sabdariffa concentration was increased. The loss of appetite in treated animal models due to daily administration of H. sabdariffa extracts had earlier been reported [21]. Hence, there is every reason to believe that the earlier observation of [5] must have been occasioned by the low concentration of H. sabdariffa extract they used.

5. CONCLUSION
The significant increase in the levels of the liver enzymes (ALP, AST and ALT) tends to suggest a dysfunction in the coordinating activity of the liver in the physiology of the animal. The extract ability to lower the total cholesterol level and body weight suggests its usefulness as a hypocholesterolemic and anti-obesity agent.

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