Combined Administration of *Rauwolfia vomitoria* extract And Vitamin C Elicits Hypolipidemid and Antioxidative Potentials in High-Fat Fed Experimental Rats.

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**Abstract:** A major risk factor in the etiology of cardiovascular disease is hyperlipidemia. Current research is focus at discovering pharmacological compounds of natural origin that can reduce the risk of hyperlipidemia. This study was set up to assess the effect of separate and combine administration of *Rauwolfia vomitoria* and vitamin C on hyperlipidemia and oxidative stress in rat fed with high fat diet. Forty adult male rats randomized into 5 groups of 8 each were used. Group 1 was the control; groups 2, 3, 4 and 5 were fed with lard supplemented diet. Leaves extract of *Rauwolfia vomitoria*was administered to groups 3 and 4 (250mg/kg) while groups 4 and 5 were treated with vitamin C (10UI/Kg). All administrations were performed orally as a single dose for 28 days after which the rats were sacrificed. Plasma and heart homogenate were then used for analysis. The study indicates that high fat diet predisposes to hyperlipidemia, increased malondialdehyde, decrease GSH and decrease catalase and superoxide dismutase activities. Combine administration of *Rauwolfia vomitoria* and vitamin C offer protective effect by reversing the metabolic alterations occasioned by the diet. The study suggests that *Rauwolfia vomitoria* contain bioactive components that may act synergistically with vitamin C to provide cardioprotective effect.

**Keywords:** Cardiovascular risk, antioxidants, atherogenic index, hyperlipidemia, lipoprotein, *Rauwolfia vomitoria*.

I. Introduction

Cardiovascular diseases refer to any disease that affects the cardiovascular system, mainly cardiac diseases, vascular diseases of the brain and kidney, and peripheral arterial disease [1]. The disease has been reported to be the principal cause of death globally accounting for 17.3 million deaths per year, a number that is expected to grow to 23.6 million by 2030[2]. Obesity, high blood pressure, insulin resistance, and aging are associated with the development of cardiovascular diseases [3]. Other important factors are diet, lifestyle, environmental, genetic, and epigenetic interactions [4].

One of the major risk factors for the development of cardiovascular disease is dyslipidemia, which may be primary or associated with hypertension, diabetes mellitus and obesity[5, 6]. Dyslipidemia usually involve elevated plasma levels of triglycerides, total, LDL and VLDL cholesterol and a low level of HDL cholesterol[6]. Therefore, any nutritional and pharmacologic intervention that improves or normalizes abnormal lipid metabolism may be useful for reducing the risk of cardiovascular diseases[6, 7].

The role of dietary fat in health has been under intensive research and debate during the past decades [1]. Many observational studies reported that the total amount of dietary fat has only a minor or no effect on the risk of lifestyle diseases such as cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), and cancer or the level of the risk factors of these diseases, or markers (abdominal adiposity, blood pressure (BP), serum lipid profile, and insulin sensitivity) [8]. However, the quality of fat has been shown to have a significant effect on serum lipid profile and BP as well as endothelial function and low-grade inflammation and these has been shown to affect the risk of CVD [8, 9, 10].

Oxidative stress has been reported to play a central role in the pathogenesis of atherosclerosis [[11]. An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis[11, 12] ROS cause damage to cellular structures within the vascular wall, and they trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenictranscriptomic profile. ROS levels increase in stress condition and, because of their high reactivity, participate in a variety of chemical reactions. They are involved in cell damage, necrosis, and apoptosis via oxidation of lipids, proteins, and DNA [13].
Herbal medicine is an important aspect of human life. As such, many medicinal plants are used for the management of various ailments in traditional settings around the globe [14, 15]. Africa as a continent is endowed with an enormous wealth of plant resources. Over 5,000 different species are known to occur in the forest regions alone, and most of them have been used for several centuries in traditional medicine for the prevention and treatment of disease [16] Rauvolfia vomitoria occurs naturally in forest but is mostly found in forest regrowth where fallow periods are prolonged. It belongs to the family of Apocynaceae, Its common names are swizzle stick (English) and Asofeyeje (Yoruba) [7]. Rauvolfia vomitoria is a shrub or small tree up to 8m. The branches are whorled and the nodes enlarged and lumpy leaves in threes. It is widely planted as ornamental plant and it is used as firewood for instance in Sierra Leone. The bark yields a good fibre, and yellow dye is obtained from the bark. The seeds are used in making decorative necklaces. The sweet scent of Rauvolfia vomitoria flowers are frequented by bees. It is also used as timber. Reported medicinal use of Rauvolfia vomitoria include the use of the bark and root powder in Gabon to kill fleas by mixing with water or palm oil. Rauvolfia vomitoria is also used to treat leprosy in the Democratic Republic of Congo. The plant is very important and useful in the treatment of lunatic patients; the root is added to gin and given to mentally ill persons. It can also be ground into powder and taken with pap, and can be taken in form of decoction for rheumatic pains. An infusion of the root bark is used to treat jaundice and gastro-intestinal disturbance.

Although the use of the plant in treating diabetes and some other cardiovascular related disorder has been noted in Nigeria, as at the time of our study, no report has been cited in the literature to support this medicinal use. This is the goal of this study. Furthermore, the study attempt to compare the effect of the plant on oxidative stress associated with CVD with that of vitamin c.

II. Materials And Methods

2.1. Plant extract preparation

Fresh leaves of Rauvolfia vomitoria were collected from a local garden in Ikenne, Nigeria in July, 2013. The plant materials were authenticated at the Department of Plant Science, Faculty of Science, Olabisi Onabanjo University, Nigeria. A voucher number Ars 013 NF was assigned and voucher specimen was thereafter deposited at the Herbarium. Samples of the leaves of Rauvolfia vomitoria were then air dried for seven days, pulverized into fine powder and thereafter stored in sealed plastic containers. Twenty (20) grams of the powdered sample was then extracted in 100 ml of 70% methanol by maceration. The extract was then filtered, concentrated using rotary evaporator (Yamato Scientific RE301A, W, Tokyo) and lyophilized with Hull brand (SP Scientific Series, USA) freeze-drier. Stock solution was prepared by dissolving the dried extracts in distilled water and was stored at -20°C until use.

2.2 Animal handling

Forty (40) male Wistar strain rats, weighing between 150 and 220 g self-reed at the Animal holding, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne, Nigeria were used in the study. The animals were kept under ambient condition and were allowed to acclimatize for a week while being fed with standard rat chow (obtained from Animal Care Nig. Ltd) and water ad libitum. Experimental animals were handled appropriately as outlined by the guidelines of Experimental Animals Ethics Code of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Nigeria. The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use [18] and in accordance with the principles of good laboratory procedure. The rats were randomly assigned in to 5 groups of 8 rats each. The rats were then treated as follow

**Group 1:** Normal Control (standard rat chow)
**Group 2:** Test Control (high fat diet)
**Group 3:** Test I (high fat diet and administered with Rauvolfia vomitoria, extract)
**Group 4:** Test II (high fat diet diet and administered with Rauvolfia vomitoria extract + vitamin C)
**Group 5:** Test III (high fat diet and administered with vitamin C)

High fat diet was prepared by supplementing the normal rat chow with 15% lard (19). All the rats were fed with their respective diet ad libitum. The extract and vitamin c were both administered orally using oral intubator at a dose of 250mgKg⁻¹ and 10 UIKg⁻¹ respectively. All administrations were carried out once daily for 28 consecutive days.

2.3 Preparation of organ homogenate and blood sample

After the last dose administration, the rats were fasted for 12 hours and then anesthetized in a closed jar of cotton-soaked diethyl ether. Blood was withdrawn from the rats by cardiac puncture after which they were sacrificed by cervical dislocation. The blood samples were collected in heparinized bottles and the heart
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excised, weighed and stored in buffered petri-dishes before being homogenized in phosphate buffer (pH 7.3). Afterwards, blood samples and heart homogenates were centrifuged and supernatant collected for biochemical analysis.

2.4 Biochemical Assay

Assay for catalase and superoxide dismutase activities was according to the previously described methods of Sinha [20] and Marklund and Marklund[21] respectively. Lipid peroxidation was assessed by determining the malondialdehyde as described by Varshney and Kale [22]. Reduced Glutathione was measured by the method of Beutler et al.[23]. Heart lipid was extracted using the method of Folch et al.[24]. Randox™ diagnostic kits (Randox Laboratories, U.K.) was then used for estimation of the lipid content. Cholesterol and triglyceride were determined enzymatic [25]. HDL-cholesterol was estimated by the HDL precipitant method [26]. VLDL and LDL-cholesterol concentration were calculated using Friedewald’s formula [27]. Atherogenic index of plasma (AIP) was calculated using the formula of Abotet al.[28] and Coronary Risk Ratio (CRR) was obtained by the method of Alladiet al. [29].

\[
\text{VLDL} = \frac{\text{Serum triglyceride}}{2.2}
\]

\[
\text{LDL} = \frac{\text{Total serum cholesterol} - \text{Total serum triglycerides}}{5} - \text{Total serum HDL}
\]

\[
\text{AIP} = \frac{\text{Total serum triglycerides}}{\text{Total serum HDL}}
\]

\[
\text{Coronary Risk Ratio} = \frac{\text{Total cholesterol}}{\text{HDL}}
\]

2.5 Statistical Analysis

All results were expressed as mean ± SEM of 7 determinations. SPSS v20.0 software package was used for data processing. Data analyses were done with one-way ANOVA and level of significance tested at p≤0.05 with Duncan Multiple Range Test (DMRT).

III. Results

High fat diet significantly raised the plasma total cholesterol and triglyceride level from 133.94±0.95 and 58.96±0.36 mmol/L respectively observed in the normal control to 224.56±9.03 and 102.30±6.68 mmol/L respectively (Table 1). Separate administration of Rauwolfia vomitoria and vitamin c into rats placed on high fat diet reduced the plasma total cholesterol and triglyceride level. The observed plasma total cholesterol of 166.19±1.22 and 168.18±4.08 observed in rats treated with Rauwolfia vomitoria and vitamin C respectively were not different from each other, they were however higher than that of the normal control but lower than that of the rats fed with high fat diet without corresponding treatment. When co-administered into rats placed on high fat diet, Rauwolfia vomitoria and vitamin C significantly reduced plasma total cholesterol. The observed concentration of 136.88±2.00 mmol/L was not different from that of the normal control. Similarly, LDL cholesterol and VLDL cholesterol were both significantly (p<0.05) raised when rats were fed with high fat diet. Administration of R. vomitoria did not significantly reverse the observed elevation in LDL and VLDL cholesterol. When R. vomitoria and vitamin c were however co-administered into rats placed on high fat diet, the LDL and VLDL level were significantly (p<0.05) lowered. The observed LDL cholesterol and VLDL cholesterol level of 33.14±4.21 and 16.59±0.66 mmol/L observed in this group of rats were not significantly (p<0.05) different from the normal control value. Plasma HDL cholesterol was reduced with high fat diet from the observed value of 79.19±0.77 in the normal control to 30.87±0.04 mmol/L. Separate treatment with R. vomitoria and vitamin C and their combined administration into rats placed on high fat diet raised the plasma HDL cholesterol concentration to the pretreatment level. The observed concentrations in all the treatment groups were not different from each other.

Table 2 is the result of the effect of treatment on the heart cholesterol and triglyceride level. Increased fat diet was not observed in the study to have significantly altered the cholesterol or the triglyceride level of the heart. Separate and combined treatment with R. vomitoria and vitamin C was also not observed in the study to have significantly (p>0.05) altered the heart cholesterol and triglyceride level.

The result of the treatment effect on Plasma Artherogenic Index (PAI) and Coronary Risk Ratio (CRR) indicates that both parameters were raised with high fat diet. Separate and combine administration of R. vomitoria and vitamin c however reduced both the PAI and CRR in rats fed with high fat diet. The API value of 1.29±0.03 and 1.26±0.06 obtained when rats were co-administered with R. vomitoria and vitamin c and when treated with vitamin c alone (respectively) were not different from each other but higher than 1.51±0.21 obtained when treated with R. vomitoria alone. Again, the CRR value of 2.16±0.21 obtained in rats co administered with R. vomitoria and vitamin C was significantly lower than 2.59±0.12 and 2.30±0.15 obtained in rats separately treated with R. vomitoria and vitamin C respectively. The observed values in all these treated groups were however found to be lowered that that of 1.68±0.01 obtained in the normal control rats.

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When compared with the normal control, the result of the lipid peroxidation and antioxidant status (Table 4) showed that increase dietary fat significantly (p<0.05) raised the level of malondialdehyde in the rat and lowered significantly the level of GSH and catalase activity. Separate administration of R. vomitoria and vitamin c and when co-administered reversed the alterations in malondialdehyde level and GSH level in rats placed on high fat diet. The observed MDA level of 4.89±0.14nmol MDA/g tissue and 4.56±0.04nmol MDA/g tissue in rats separately treated with R. vomitoria and vitamin C respectively were not different significantly (p<0.05) from each other, they were however higher than the normal control value of 4.21±0.19nmol MDA/g tissue and the value of 4.36±0.17nmol MDA/g tissue observed in the rats co administered with R. vomitoria and vitamin C. The observed GSH level of 27.07±0.18 x103 µg/g tissue in rats’ co treated with R. vomitoria and vitamin C was not different from the normal control level of 28.13±1.16x103µg/g tissue. Although separate administration of R. vomitoria and vitamin C increased the GSH level above that of the group fed with high fat diet, the observed value of 24.76±1.48x103µg/g tissue and 25.97±0.85x103µg/g tissue respectively were lower than the normal control value. No significant (p<0.05) difference was observed in the SOD activity of the rats placed on high fat diet (3.33±0.02x103 ng/mg protein) when compared with the normal control value of 3.30±0.01x103 ng/mg protein. The observed SOD activities in the rats separately and co administered with R. vomitoria and vitamin C were also not significantly (p>0.05) different from the normal control value.

IV. Discussion

Dyslipidemia refers to a condition of elevated plasma levels of triglycerides, total cholesterol, LDL and VLDL cholesterol and a low level of HDL cholesterol [30, 31, 32]. One of the major risk factors for the development of cardiovascular disease is dyslipidemia [6, 32]. Data from the present study indicates that lard is a dietary fat capable of predisposing to cardiovascular disease. We observed in this study that there was increased plasma cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol and an associated reduce HDL cholesterol when rats were placed on lard supplemented diet. Lard is an animal fat which is known to contain saturated fatty acid[33]. Report from previous studies suggests an association between intake of dietary saturated fatty acids (SFA) and serum cholesterol levels [34, 35]. Data from our study agrees with this report. Any nutritional and pharmacologic intervention that improves or normalizes abnormal lipid metabolism may be useful for reducing the risk of cardiovascular diseases[1, 36]. Presently, several drugs are available for the management of dyslipidemia. However, there is renewed interest in the use of herbal products [37, 38]. This is partly due to the perceived safety of herbal medicine and the cost of getting access to good medical care among the poor rural populace. Report from the present study indicates that R. vomitoria leaves contain phytochemicals capable of eliciting positive effect over hyperlipidemia. However, we also observed in this study that the efficacy of the plant is enhanced when co administered with vitamin c than when administered alone. Our data indicates that co-administration of the extract with vitamin c, reduced the level of plasma cholesterol, triglyceride, LDL and VLDL cholesterol. These are lipid constituents that are known risk factors in cardiovascular diseases. A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases [39, 40] and is often associated with hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus [40, 41].

Our study demonstrated a reduction in triglyceride level occasioned by R. vomitoria in rats fed with high fat diet. We also observed that apart from triglyceride and total cholesterol, R. vomitoria also caused significant reduction in plasma LDL and VLDL cholesterol and increases HDL cholesterol, suggesting its efficacy in reducing cardiovascular disease risk. This is in agreement with report from previous studies which indicated that an elevated LDL cholesterol concentration in plasma is atherogenic [42, 43, 44], whereas, a high HDL level is cardioprotective[37, 45]. Increases in plasma HDL cholesterol have been reported to reduce risk in coronary heart disease [42, 46]. High HDL exerts a protective effect by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues, which it esterifies with oborating the fact that the aid of lecithin: cholesterol acyltransferase, and delivers to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins, and eventual elimination from the body [46, 47] and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant [47] and anti-inflammatory property [46].

Atherogenic indices are powerful indicators of the risk of heart disease and have been successfully used as an additional index when assessing cardiovascular (CV) risk factors: the higher the value, the higher the risk of developing cardiovascular disease and vice versa [48, 49, 50, 51]. The atherogenic index of plasma (AIP) is based on the ratio of the values of triglycerides to high-density lipoprotein (HDL) levels. AIP values of ~0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk [49]. Data from the present study indicates that high fat diet increases atherogenic indices (AIP and CRR) corroborating the fact that high fat diet predisposes to cardiovascular diseases. Though R. vomitoria was observed in the study to significantly reduced atherogenic indices (CRR and AIP), better efficacy was observed when the extract was co-administered with vitamin C.
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We decided to assess the oxidative status of the heart in this study in order to investigate the role of oxidative stress in the development of cardiovascular diseases and the possible mechanism by which R. vomitoria ameliorate the disorder. Result from the study indicate that rats fed with high fat diet showed increased malondialdehyde level, decreased catalase activity and decrease reduced glutathione concentration. Administration of R. vomitoria during treatment however prevented these metabolic alterations suggesting that the extract is capable of reducing oxidative stress induce by high dietary fat. The imbalance between ROS production and the cellular antioxidant defense system resulting from oxidative stress has been implicated in cell damage, necrosis, and apoptosis via oxidation of lipids, proteins, and DNA [13] and provoke also endothelial dysfunction and infiltration. Studies indicated that an increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis[11, 12]. ROS production can rise when the breakdown of metabolites in the tricarboxylic acid (TCA) cycle exceeds the capacity of the electron transport chain (ETC) to assimilate the resulting electrons [52]. ROS cause damage to cellular structures within the vascular wall, and they trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile. In this study, we observed increased malondialdehyde level with increased fat diet suggesting that high fat diet increased metabolic activity in the TCA cycle.

Our study also suggests that R. vomitoria contain bioactive component which act synergistically with vitamin C as antioxidative agent to mop up ROS and thus prevent subsequent damage to the cellular macromolecules. This is indicated by the increased activity of catalase and the level GSH that we observed in rats that were co-treated with R. vomitoria and vitamin C. Antioxidants are agents that at low concentrations prevent or inhibit oxidation of oxidizable biomolecules, such as DNA, lipids, and proteins [3]. Superoxide dismutase (SOD), catalase, glutathione peroxidase, thioredoxin, and peroxiredoxin represent enzymatic antioxidants [53], while nonenzymatic antioxidants are vitamin E, vitamin C, and glutathione. Other molecules, such as uric acid and bilirubin, are also antioxidants able to protect against CVDs [54]. In this study, we assessed the activities of GSH, catalase and SOD activities. Our result indicates that R. vomitoria antioxidative effect affect only heart GSH and catalase activity but has no effect on heart SOD. A similar effect was also observed with lard supplement. Superoxide dismutase catalyzes the dismutation of superoxide to less toxic hydrogen peroxide and oxygen. The hydrogen peroxide is further detoxify by catalase and or glutathione peroxidase. Superoxide dismutase without glutathione peroxidase or catalase to remove the hydrogen peroxide is of little value [55].

V. Conclusion

We have shown by this study that on its own R. vomitoria may show some benefit in cardiovascular disease, but this activity is highly enhanced when the leaves extract is administered with vitamin C. The study thus indicates that the leaves contain some bioactive compound capable of acting synergistically with vitamin C to reduce the risk of cardiovascular disease. Work is presently going on in our laboratory to identify, isolate and investigate the pharmacological activity of this bioactive compound.

References


[2]. C. S. Sydney, C. Amy, F. Roberto, R. David, S. Holmes, L. Susanne, V. M. Diana, R. Johanna, L. Ralph, H. Sacco, T. Kathryn, A.W. David and A. Z. William, Our Time: A Call to Save Preventable Death From Cardiovascular Disease (Heart Disease and Stroke) JACC, 60 (22), 2012, 1231-1239.


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Table 1: Effect of Treatment on antioxidants, lipid peroxidation, triglyceride, cholesterol and protein in the heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>MDA (μmol MDA/g tissue)</th>
<th>Catalase (μg/mg protein)</th>
<th>SOD ([ng/mg protein] x 10^4)</th>
<th>Reduced GSH ([μg/g])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Normal control)</td>
<td>SRF</td>
<td>4.93 ± 0.11a</td>
<td>54.69 ± 4.43b</td>
<td>3.33 ± 0.11b</td>
<td>26.13 ± 0.21a</td>
</tr>
<tr>
<td>2 (Test Control)</td>
<td>HF</td>
<td>6.23 ± 0.11b</td>
<td>23.23± 2.90b</td>
<td>3.03 ± 0.15a</td>
<td>23.13 ± 0.24b</td>
</tr>
<tr>
<td>3 (Test 1)</td>
<td>HF + RF</td>
<td>5.89 ± 0.14b</td>
<td>37.59 ± 1.48b</td>
<td>3.31 ± 0.01b</td>
<td>25.76 ± 1.48b</td>
</tr>
<tr>
<td>4 (Test 2)</td>
<td>HF + RV + VC</td>
<td>5.26 ± 0.12b</td>
<td>35.94± 2.17b</td>
<td>3.54 ± 0.23b</td>
<td>26.89 ± 0.13b</td>
</tr>
<tr>
<td>5 (Test 3)</td>
<td>HF + VC</td>
<td>5.56 ± 0.04b</td>
<td>21.59 ± 2.09b</td>
<td>3.32 ± 0.01b</td>
<td>25.97 ± 0.85b</td>
</tr>
</tbody>
</table>

Note:
- Results presented are mean ± SEM (n=8).
- Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan’s multiple range test (DMRT).
- Value in the same column with similar superscript are not significantly different (p> 0.05) from each other.
SRF = Standard Rat Feed
HF = High fat diet
RV = Rauwolfia vomitoria extract
VC = Vitamin C

Table 2: Effect of treatment on the heart cholesterol and protein level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
<th>Total protein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Normal control)</td>
<td>SRF</td>
<td>89.70 ±3.47a</td>
<td>44.50±0.93b</td>
<td>5.88±0.33a</td>
</tr>
<tr>
<td>2 (Test control)</td>
<td>HF</td>
<td>83.64±3.72b</td>
<td>56.17±2.55b</td>
<td>5.46±0.09b</td>
</tr>
<tr>
<td>3 (Test 1)</td>
<td>HF + RV</td>
<td>86.90±3.05b</td>
<td>48.41±2.79b</td>
<td>5.43±0.41b</td>
</tr>
<tr>
<td>4 (Test 2)</td>
<td>HF + RV + VC</td>
<td>80.00±4.64b</td>
<td>45.46±3.27b</td>
<td>5.24±0.14b</td>
</tr>
<tr>
<td>5 (Test 2)</td>
<td>HF + VC</td>
<td>83.60±6.99b</td>
<td>57.17±1.08b</td>
<td>5.22±0.12b</td>
</tr>
</tbody>
</table>

Note:
- Results presented are mean ± SEM (n=8).
- Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan’s multiple range test (DMRT).
- Value in the same column with similar superscript are not significantly different (p> 0.05) from each other.
SRF = Standard Rat Feed
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RV = Rauwolfia vomitoria  
VC = Vitamin C

Table 3: Effect of Treatment on Plasma lipid profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
<th>LDL-Chol (mmol/L)</th>
<th>HDL-Chol (mmol/L)</th>
<th>VLDL-Chol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Normal control)</td>
<td>SRF</td>
<td>133.94±0.95</td>
<td>58.96±0.36</td>
<td>31.95±1.48</td>
<td>79.19±0.77</td>
<td>13.10±0.07</td>
</tr>
<tr>
<td>2 (Test control)</td>
<td>HF</td>
<td>224.56±9.03</td>
<td>102.30±6.68</td>
<td>42.43±1.21</td>
<td>30.87±0.04</td>
<td>64.92±0.09</td>
</tr>
<tr>
<td>3 (Test 1)</td>
<td>HF + RV</td>
<td>166.81±2.63</td>
<td>99.41±2.79</td>
<td>42.01±3.11</td>
<td>63.15±3.13</td>
<td>64.92±0.09</td>
</tr>
<tr>
<td>4 (Test 2)</td>
<td>HF + VC</td>
<td>136.88±2.06</td>
<td>87.98±0.71</td>
<td>33.14±4.21</td>
<td>63.15±3.13</td>
<td>64.92±0.09</td>
</tr>
<tr>
<td>5 (Test 2)</td>
<td>HF + VC</td>
<td>168.18±4.08</td>
<td>92.16±3.70</td>
<td>38.46±4.23</td>
<td>73.29±0.56</td>
<td>73.29±0.56</td>
</tr>
</tbody>
</table>

Note:  
- Results presented are mean ± SEM (n=8).  
- Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan’s multiple range test (DMRT).  
- Value in the same column with similar superscript are not significantly different (p > 0.05) from each other.  

SRF = Standard Rat Feed  
HF = High fat diet  
RV = Rauwolfia vomitoria  
VC = Vitamin C

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Table 4: Effect of Treatment on Atherogenic and Coronary Risk Indeces

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AI</th>
<th>CRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Normal control)</td>
<td>SRF</td>
<td>0.77±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.68±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (Test control)</td>
<td>HF</td>
<td>3.45±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.43±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 (Test 1)</td>
<td>HF + RV</td>
<td>1.51±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.69±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 (Test 2)</td>
<td>HF + RV + VC</td>
<td>1.29±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.74±0.21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 (Test 2)</td>
<td>HF + VC</td>
<td>1.26±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.16±0.05&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note:
- Results presented are mean ± SEM (n=8).
- Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan’s multiple range test (DMRT).
- Value in the same column with similar superscript are not significantly different (p > 0.05) from each other.
- SRF = Standard Rat Feed
- HF = High fat diet
- RV = *Rauwolfia vomitoria*
- VC = Vitamin C