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Serum lipid profile of hyperlipidemic rabbits (*Lepus townsendii*) treated with leaf extracts of *Hibiscus rose-sinesis, Emilia coccinea, Acanthus montanus* and *Asystasia gangetica*

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There are efforts directed towards the search for medicinal plants capable of ameliorating hyperlipidemia. The present study sought to investigate the capacity of ethanolic leaf extracts of *Hibiscus rose-sinesis, Emilia coccinea, Acanthus montanus* and *Asystasia gangetica* to ameliorate hyperlipidemia in animal model. Hyperlipidemia was induced by placing experimental animals on lipogenic diet containing 2.5% cholesterol, 20% sunflower oil and 0.5% sodium cholate. Separate leaf extracts of the four plants (dose = 400 mg/kg) were administered to various groups of the experimental animals by intra peritoneal injection at regular time intervals of 12 h for 14 days. At the end of treatment, blood samples of the rabbits were measured for total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations. Generally, treatment of hyperlipidemic rabbits (HyL-Rs) with the four plant extracts caused different levels of lowered serum lipid components with concomitant elevation of HDL-C concentration. Specifically, the capacities of the four plant extracts to reduce serum levels of TC in the experimental animals were in the order: *H. rose-sinesis > A. montanus > A. gangetica > E. coccinea*. In addition, serum lipid profile (SLP) of HyL-Rs treated with extract of *H. rose-sinesis* was comparable with the control groups. The present study showed that the tendency but varied capacities of the four experimental plant extracts to alter SLP of HyL-Rs were associated with their phytochemical peculiarities, which suggested that certain phytochemicals exhibit hypolipidemic activity *in vivo*.

**Key words:** Serum lipid profile, *Hibiscus rose-sinesis, Emilia coccinea, Acanthus montanus* and *Asystasia gangetica*.

INTRODUCTION

*Hibiscus rosa-sinensis* L. commonly referred to as Chinese hibiscus, China rose or shoe flower (*Jasvand*) belongs to the family Malvaceae (*Kirtikar and Basu, 1987*). It is an ornamental plant widely grown in the tropical and subtropical regions of East Asia. *Emilia coccinea* is an annual weed widely distributed in tropical Asia and some part of Africa (Ghana and Nigeria) (*Chillendon*, 1956). The plant is edible, commonly used as salad in South East Asia and herbal medicine in South Eastern Nigeria (*Edeoga et al., 2005; Jimoh et al., 2010*).
Acanthus montanus (Nees) is of the family Acanthaceae, also known as Bear’s breeches, Mountain thistle or Alligator plant. The plant is a striking small shrub with sparse branches and soft stem. A. montanus is commonly found in South Eastern Nigeria and Congo DRC (Igoli et al., 2004; Okoli et al., 2008). It is also found growing in the wild fields of grasslands, woods, scrub and rocky hills of the Balkans, Romania, Greece and Eastern Mediterranean (Huxley, 1992). Asystasia gangetica (L.), commonly known as Chinese violet is an ornamental plant and source of forage for cattle, goats and sheep in south East Asia. The plant could also be used as cover crops and prevention of growth of noxious weeds. The phytochemical compositions, pharmacological and therapeutic potentials of H. rose-sinesis, E. coccinea, A. montanus and A. gangetica have previously been reported elsewhere (Edeoga et al., 2005; Okoli et al., 2008; Kensa, 2011; Soni et al., 2011; Bhaskar et al., 2011; Kumar et al., 2012; Gopal et al., 2013).

Elevation of plasma lipid components describes a metabolic disorder referred to as hyperlipidemia. The multifaceted etiology of hyperlipidemia could be classified into primary genetic defect or secondary causes associated with diet, drugs, or underlying pathophysiologic disorders (Raasch, 1988; Eliot and Jamali, 1999; El-Demerdash et al., 2005). Hyperlipidemia is associated with increased levels of atherogenic lipoproteins, which is a contributing factor and primary indicators of atherosclerosis, susceptibility to coronary heart disease (CHD) and cerebrovascular accidents as well as enhanced oxidative stress in hepatocytes, renal tissue and inflammatory reactions (Ilan et al., 2005; Suanarunsawat et al., 2011; Gao, 2012; Adekunle et al., 2013).

Furthermore, hyperlipidemia causes increase cardiac peroxynitrite formation and low bioavailability of nitrous oxide engendering deterioration of cardiac performance and possibly associated cardiac pathologies (O’nody et al., 2003). According to Mackay and Mensah (2003), cardiovascular disease was the leading cause of death worldwide in 2002 with estimate put at 16.7 million deaths. This challenge to public health is further compounded by associated 85 million disability-adjusted life-years lost worldwide due to CHD and stroke, with a projection of over 143 million in 2020 (Mackay and Mensah, 2003; Fon Tacer et al., 2007).

There are drugs available for the management and treatment of hyperlipidemias (Segal et al., 1972; Mayes, 1983; Ochani and D’mello, 2009). However, their desirability and safety, especially on long-term usage, coupled with cost and simplicity of administration has been a drawback to fulfill conditions of full compliance by patients. For these reasons, there are efforts directed towards the search for edible medicinal plants capable of ameliorating hyperlipidemia. Although, the medicinal values of these plants are applied for the treatment/management of several pathologic challenges (Edeoga et al., 2005; Okoli et al., 2008; Kensa, 2011; Soni et al., 2011; Bhaskar et al., 2011; Kumar et al., 2012; Gopal et al., 2013), their application as agents of lipidemic control are yet to be investigated and exploited. The present study sought to investigate the capacity of ethanolic leaf extracts of H. rose-sinesis, E. coccinea, A. montanus and A. gangetica to ameliorate hyperlipidemia elicited by lipogenic diet using animal model.

**MATERIALS AND METHODS**

**Collection of plant samples and preparation of extracts**

Fresh leaf samples of H. rose-sinesis, E. coccinea, A. montanus and A. gangetica were harvested in July, 2012 within the environment of Federal University of Science and Technology, Owerri, Nigeria. The plant specimens were identified and authenticated by Dr. A. Ibe of the Department of Crop Science and Biotechnology, Federal University of Science and Technology, Owerri, Nigeria. Voucher specimens were deposited at the herbarium for reference purposes. Ethanol/water extracts (1:2 v/v) of the various samples were prepared by methods of Ibegbulem and Chikezie (2012). The leaves were washed under a continuous stream of distilled water for 15 min and air-dried at room temperature (25±5°C) for 5 h. The samples were chopped and further dried at 60°C for 5 h in an oven and subsequently ground with Thomas-Willey milling machine. Twenty-five grams of the pulverized specimens were suspended in separate 250 ml of ethanol/water mixture (1:2 v/v) in Stoppered flasks and allowed to stand in a thermostatically controlled water bath at 40°C for 24 h. The suspensions were filtered with Whatman No. 24 filter paper, concentrated in a rotary evaporator at 50°C and dried in vacuum desiccator. The yields (w/w) were calculated to be as follows: H. rose-sinesis = 4.9%, E. coccinea = 2.6%, A. montanus = 4.1% and A. gangetica = 3.6%. The extracts were re-dissolved in 20 ml of PBS (pH = 7.4) and incubated at 37°C for 30 min with thorough shaking. The extracts were administered by intraperitoneal (i.p.) injection to the rabbits at a dose of 400 mg/kg at regular intervals of 12 h for 14 days.

**Experimental animals**

Male rabbits (Lepus townsendii) (8 to 10 weeks old) weighing 1.1 to 1.5 kg were obtained from a commercial breeding farm in Ezibodo, Owerri West local Government Area, Nigeria. The rabbits were maintained at 25±5°C, 30 to 55% of relative humidity on a 12-h light/12-h dark cycle, with access to distilled water (DW) and standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) ad libitum for 2 weeks acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

**Study design**

The animals were deprived of food and water for 12 h before commencement of treatments (control and test experiments) as previously described (Ibegbulem and Chikezie, 2012). Lipogenic diet (LpD-SCF) consisted of 2.5% cholesterol, 20% sunflower oil and 0.5% sodium cholate (LpD) (Bolkent et al., 2005) compounded with SCF was fed to the rabbits for 30 days to induced hyperlipidemia before commencement of study. A total of twenty four (24) normal and hyperlipidemic rabbits (HyL-Rs) were categorized into six groups of four (n = 4) each as follows: Group C1, Control/Normal rabbits received SCF + PBS (Vehicle; 1.0 ml/kg/12 h, i.p.) for 14 days; Group C2, Control/HyL-Rs received LpD-SCF + PBS (Vehicle; 1.0 ml/kg/12 h, i.p.) for 14 days; Group T1, HyL-Rs received LpD-SCF + H. rose-sinesis (400
mg/kg /12 h, i.p.) for 14 days; Group T2, HyL-Rs received LpD-SCF + E. coccinea (400 mg/kg /12 h, i.p.) for 14 days; Group T3, HyL-Rs received LpD-SCF + A. montanus (400 mg/kg /12 h, i.p.) for 14 days; Group T4, HyL-Rs received LpD-SCF + A. gangetica (400 mg/kg /12 h, i.p.) for 14 days.

Collection of blood and measurement of serum lipid profile

Collection of blood samples from the various experimental groups commenced 12 h after the administration of the last dose of the leaf extracts. Blood samples were obtained from the various experimental groups by carotid artery puncture for measurement of serum lipid profile (SLP). Total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) were determined using commercial kits (Randox Laboratory Ltd., UK). Low-density lipoprotein cholesterol (LDL-C) concentration was determined by difference according to the formula described by Friedewald et al. (1972): LDL-C = TC – (HDL-C) – (TAG/5), as reported by Shaker et al. (2010). Atherogenic index (AI) was calculated thus: [TC–(HDL-C)]/(HDL-C) (Suanarunsawat et al., 2011).

Statistical analyses

The data were analyzed by the use of student’s t distribution test of significance as described by Pearson and Hartley (1966).

RESULTS

The concentration ratio of serum lipid components (TC, LDL-C, TAG and HDL-C) of group C1 was approximately 3:1:2:1.6. The rabbits placed on LpD-SCF but denied extract treatment (group C2) exhibited wide-ranging elevation in SLP patterns. Specifically, increase in C2TC represented 69.23% compared to C1TC. Likewise, increase in C2LDL-C was 3.69 folds > C1LDL-C, whereas C2TAG was elevated by 57.91% compared to C1TAG; p < 0.05. Conversely, C2HDL-C was 2.29 folds < C1HDL-C (p < 0.05).

Generally, HyL-Rs treated with the four plant extracts (groups T1, T2, T3 and T4) showed reduced levels of serum lipid components (TC, LDL-C and TAG) with concomitant elevation of HDL-C in relation to the group C2 (Figure 1). However, a cursory look at Figure 1 showed that SLP of group T1 was not significantly different (p > 0.05) from group C1. Likewise, T2TAG was not significantly different (p > 0.05) compared to C1. Also, it is important to note that T4HDL-C was not significantly different (p > 0.05) from the other experimental groups except in comparison with C2HDL-C. T3LDL-C, T3TAG and T3HDL-C were not significantly different (p > 0.05) from their corresponding T2 parameters. Serum levels of HDL-C of the various experimental groups were in the order: C1 = 71.34 mg/dl > T1 = 62.42 mg/dl > T4 = 60.23 mg/dl > T3 = 56.88 mg/dl > T2 = 55.78 mg/dl > C2 = 31.22 mg/dl.

Table 1 showed that the four plant extracts exhibited varied capacities to reduce the levels of serum TC after 14 days treatment of the various experimental groups, which was in the order: H. rose-sinesis > A. montanus > A. gangetica > E. coccinea relative to group C2. Also, rabbits treated with leaf extract of H. rose-sinesis (T1H. rose-sinesis = 400 mg/kg) caused 60.64% reduction on serum LDL-C. From the same perspective, T4E. coccinea = 400 mg/kg
Table 1. Changes in Serum lipid profile of hyperlipidemic rabbits treated with ethanolic leaf extracts of *H. rose-sinesis*, *E. coccinea*, *A. montanus* and *A. gangetica*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative serum lipid concentrations (%)</th>
<th>TC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LDL-C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TAG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HDL-C&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td></td>
<td>43.18</td>
<td>60.64</td>
<td>48.15</td>
<td>99.94</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>20.46</td>
<td>37.72</td>
<td>25.93</td>
<td>78.67</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>12.95</td>
<td>28.79</td>
<td>22.22</td>
<td>82.19</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>4.50</td>
<td>19.95</td>
<td>18.52</td>
<td>92.92</td>
</tr>
</tbody>
</table>

Superscript a indicated decreased %serum lipid components, whereas superscript b indicated increased %serum HDL-C.

Table 2. Atherogenic index of normal and hyperlipidemic rabbits treated with *H. rose-sinesis*, *E. coccinea*, *A. montanus*, *A. gangetica* and *E. coccinea* is exemplified by the characteristic relative improvement in AI of Hyl-Rs treated with the four leaf extracts compared to the untreated group C2 (Table 2).

The phytochemical peculiarities of the leaf extracts. The hypolipidemic property of *H. rose-sinesis*, *A. montanus*, *A. gangetica* and *E. coccinea* is exemplified by the characteristic relative improvement in AI of Hyl-Rs treated with the four leaf extracts compared to the untreated group C2 (Table 2).

The phytochemicals, specifically, β-sitosterol have been previously demonstrated to possess blood cholesterol-lowering activity by impeding intestinal cholesterol absorption routes and inhibitory action on hepatic cholesterol biosynthetic pathways (Ikeda and Sugano, 1983; Duester, 2001; Mayes and Botham, 2003). Expectedly, the intake of plant materials rich in β-sitosterol caused reduction in total serum cholesterol levels in the experimental animals (Weihrauch and Gardner, 1978; Moghadasian and Frohlich, 1999; Al-Dosari, 2011). Quantitative investigations had revealed the presence of substantial levels of β-sitosterol and related sterols in *H. rosa-sinesis* (Chauhan and Kumari, 1984; Mishra et al., 2011), which could be adduced to be one among other factors responsible for its hypolipidemic attributes, demonstrated by comparable AI of Hyl-Rs treated with leaf extract of *H. rosa-sinesis* (T1) with the normal/control group C1 (Table 2).

The quantitation of saponins in *E. coccinea* had been previously reported by Edeoga et al. (2005) and Jimoh et al. (2010). According to Oakenfull and Sidhu (1990), saponins have been demonstrated to possess hypocholesterolemic property and effectiveness for the control of high blood pressure associated with hyperlipidemia. The present findings showed the relatively capacity of leaf extract of *E. coccinea* to elicit reduced levels of blood lipid components in HyL-Rs, which corroborated previous reports on hypolipidemic attribute and medicinal value of plant extracts. Also, previous studies have shown that aqueous leaf and stem extracts of *A. montanus* restored hepatic functional integrity in rats occasioned by CCl<sub>4</sub> induced hepatic damage (Patrick-Iwuanyanwu and Wegwu, 2008) with envisaged concomitant return of SLP to physiologic levels in experimental animals. In a related study by Ukwe and Ubaka (2011), methanolic extract of *A. montanus* was shown to possess hypoglycemic effect in alloxanized rats. Researchers have also reported that extract *A. montanus* displayed copious levels of alkaloids, terpenoids, steroidal, glycosides and flavonoids (Odoh et al., 2010; Ukwe and Ubaka, 2011); phytochemicals earlier noted for their hypolipidemic activity.

Studies have shown that dietary fiber and polyphenolic compounds exhibited hypocholesterolaemic effect...
(Lairon, 1996; Ochani and D'mello, 2009; Yin et al., 2011) by moderating lipid absorption and metabolism in animal models. Investigations by Suanarunsawat et al. (2011) reported that phenolic compounds of O. sanctum L. leaf extract might be responsible for both lipid-lowering and antioxidant protective activity to hepatic and cardiac tissues. Phytochemical and pharmacological studies by Gopal et al. (2013) revealed the presence of flavonoids, alkaloids, glycosides, coumarins, tannins, steroids, terpenoids phenols, tannin and saponins in whole plant of A. Gangetica. Notably, these phytochemicals have been proposed to possessing blood cholesterol level lowering effect and might be useful in the treatment of cardiovascular diseases caused by hyperlipidemia (Kwiterovich, 2000; Subramani and Casimir, 2002; Oakenfull and Sidhu, 1990; Oywelo and Akingbala, 2011). The hypolipidemic activity of Citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia (Kuworska and Manthey, 2004) and hyperlipidemic metabolic syndrome (Evans et al., 2012) confirmed the role of flavonoids in ameliorating hyperlipidemia. In addition, Adeneye (2008) had proposed that alcohol decoction of Citrus paradisi Macf. (Rutaceae) ‘grapefruit’ seed caused reduction in blood lipid and glucose levels in alloxan-induced diabetic Wistar rats, which was considered as evidence for the combinatorial hypolipidemic effect of phytochemicals. Thus, the phytoterapeutic potentials, and in this regard, hypolipidemic activity of the plant extracts are the outcome of the additive effect of phytochemicals present in the plant materials.

The present study showed that the tendency but varied capacities of the four experimental plant extracts to alter SLP of Hyl-Rs were in connection with their phytochemical peculiarities, which suggested that certain phytochemicals exhibit hypolipidemic activity in vivo.

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