The butanol fraction of *Eclipta prostrata* (Linn) effectively reduces serum lipid levels and improves antioxidant activities in CD rats

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**Abstract**

*Eclipta prostrata* (Linn) has been used as a traditional medicinal plant to prevent lipidemia and atherosclerosis in Asia. However, its functional properties and the underlying mechanism of action have not been clearly defined. This study was conducted to elucidate the biological basis for hypolipidemic and antioxidant activities of *E prostrata*. Charles River Sprague-Dawley CD rats (specific pathogen-free/viral antibody-free Crj/Bgi male, 180 ± 10 g) were fed experimental diets supplemented with 0 mg (control), 25 mg (E25), 50 mg (E50), or 100 mg (E100) of a freeze-dried butanol fraction of *E prostrata* per kilogram of diet for 6 weeks. Serum triacylglycerol and total cholesterol levels were significantly lower in the E50 and E100 groups by 9.8% to 19.0% and by 10.7% to 13.4%, respectively, and low-density lipoprotein–cholesterol levels were significantly reduced in the same groups by 10.3% to 13.0% compared with the untreated control group. The E50 and E100 groups also showed significantly increased high-density lipoprotein–cholesterol levels (13.0%-19.1%) compared with the control group. Atherogenic indices were decreased by 9.8% to 30.5% in all groups fed diets supplemented with *E prostrata*. Furthermore, serum hydroxyl radical, lipid peroxide, and oxidized protein levels were significantly decreased in the E50 and E100 groups. These results clearly demonstrate the effects of *E prostrata* on serum lipid and oxidative metabolism in rats. The health-promoting effects of *E prostrata*, which were demonstrated in this study in a rat model, may have implications for atherosclerosis and hypercholesterolemia in humans.

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**Keywords:** *Eclipta prostrata*; Rat; Antiatherogenic; Hypolipidemic; Antioxidant

**Abbreviations:** EAI, atherogenic index; CAD, coronary artery disease; CD, Cesarean derived; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein

1. Introduction

*Eclipta prostrata* (Linn), a member of the Asteraceae plant family and commonly known as False Daisy [1], has been used as a traditional medicine to treat hyperlipidemia, atherosclerosis, hepatic disorders, spleen enlargement, and skin diseases in Asia [2,3]. Therapeutic effects of *E prostrata* on hepatic cirrhosis and infective hepatitis [4] and in the prevention of CCl4-induced liver damage in guinea pigs [5] were reported. HIV-1 protease and integrase inhibitory substances were found in *E prostrata* [6]. In fish, dietary feeding of crude extract of *E alba*, another name for *E prostrata*, enhanced nonspecific immune responses and disease resistance of *Oreochromis mossambicus* (Mozambique tilapia) against *Aeromonas hydrophila* [7]. In a recent report, the treatment of mildly hypertensive
male subjects aged 40 to 55 years with encapsulated *E. alba* powder (3 g/d) showed diuretic, hypotensive, and hypocholesterolemic effects and alleviated complications due to oxidative stress [8]. Feeding a crude extract of *E. prostrata* to albino rats reduced serum triacylglycerol level [9].

The functional efficacy of medicinal plant extracts generally depends on the solvents and methods used for their extraction [10-13]. For example, a butanol extract of *Panax* (ginseng) prevented the accumulation of abnormal lipids in hyperlipidemic rats [11], whereas fractions prepared using a more polar solvent (ethyl acetate/butanol) showed considerable antioxidant activity compared with a less polar solvent [14]. Thus, we speculate that the butanol fraction of *E. prostrata* may effectively reduce serum lipid levels and improve antioxidant activities. However, its functional properties in animal models have not been clearly defined. Therefore, this study was conducted to evaluate hypolipidemic and antioxidant activities of *Eclipta* in rats. The scientific elucidation of the health-promoting effect of *E. prostrata* will provide important information that can contribute to the effective management of atherosclerosis and hypercholesterolemia in humans.

2. Methods and materials

2.1. Animals and feeding studies

Forty Charles River Sprague-Dawley CD rats (specific pathogen-free/viral antibody-free Crj/Bgi male, 180 ± 10 g) purchased from the Laboratory Animal Center (Biogenomics, Seongnam, Korea) were housed in stainless-steel wire cages and were maintained on a 12-hour light/dark cycle in a temperature-controlled environment (22°C) with free access to standard rat diet (Harlan Teklad, Madison, Wis) and water ad libitum for 2 weeks. Twenty-eight rats, which were individually housed in stainless-steel wire cages in the same environment, were randomly divided into 4 groups (7 rats per group) and were fed 4 different experimental diets that consist of a standard rodent diet [15] supplemented with high fat and high cholesterol [16,17] for 6 weeks. Experimental diets were prepared by mixing 25 mg (E25), 50 mg (E50), or 100 mg (E100) of freeze-dried *E. prostrata* butanol extract per kilogram of diet. There was no mortality in the rats fed diets containing *E. prostrata* butanol extract during the experimental period of 6 weeks and no observable changes in animal autonomic or behavioral patterns.

Table 1

<table>
<thead>
<tr>
<th>Composition</th>
<th>Control</th>
<th>E25</th>
<th>E50</th>
<th>E100</th>
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</thead>
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<tr>
<td><em>Eclipta</em> powder*</td>
<td>–</td>
<td>0.025</td>
<td>0.050</td>
<td>0.100</td>
</tr>
<tr>
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<td>13.250</td>
<td>13.200</td>
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<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
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<tr>
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<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
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<tr>
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<td>1.0</td>
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<tr>
<td>AIN-76 vitamin mix*</td>
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<td>3.5</td>
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<tr>
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<tr>
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<td>0.2</td>
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<tr>
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<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Sodium cholate</td>
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<td>0.2</td>
<td>0.2</td>
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</tr>
</tbody>
</table>

* Experimental diets contained 0 mg (control), 25 mg (E25), 50 mg (E50) and 100 mg (E100) of freeze-dried *E. prostrata* butanol extract per kilogram of diet.

2.2. Analytical procedures

Blood samples were collected from the rats fed experimental diets for 6 weeks after overnight fasting, and euthanasia was carried out by cervical displacement. Serum was prepared by centrifugation at 3000 rpm for 10 minutes. Serum triacylglycerol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol were determined by enzymatic colorimetric methods using commercial assay kits (Sigma, St. Louis, Mo, or Eiken, Tokyo, Japan). Serum total cholesterol level was determined by the method of o-phthalaldehyde described by Rudel and Morris [18], and the atherogenic index (AI) was calculated using the following formula: (total cholesterol – HDL-cholesterol) / HDL-cholesterol [19]. Serum hydroxyl radical, lipid peroxide, and carbonyl content were measured using methods previously described [20-22]. Lipid peroxide content was analyzed by the thiobarbituric acid reactive substance assay [23-25]. Optical densities were measured using a UV/visible spectrophotometer (Amersham Biosciences, Buckinghamshire, UK).

2.3. Statistical analysis

Data analyses were performed using the GraphPad Instat software (GraphPad, San Diego, Calif), and all data were expressed as means ± SEM. Statistical differences between experimental and control groups were determined by the Dunnett *t* test at the level of *P* < .05 [26,27].

3. Results

There was no mortality in the rats fed diets containing *E. prostrata* butanol extract during the experimental period of 6 weeks and no observable changes in animal autonomic or behavioral patterns.

3.1. Serum triacylglycerol, total cholesterol, LDL-cholesterol, and HDL-cholesterol

Table 2 shows the serum lipid profiles of the 4 rat experimental groups fed diets supplemented with 0, 25, 50,
or 100 mg/kg of *E prostrata* extract. Serum triacylglycerol levels (in mg/dL) were significantly lower in the E50 (*P* < .05) and E100 (*P* < .01) groups compared with the untreated control group. Serum concentrations of total cholesterol (in mg/dL) were significantly lower in the E50 and E100 groups compared with the control group (*P* < .01). Serum LDL-cholesterol levels (in mg/dL) were generally lower in the groups treated with *E prostrata*-supplemented diets. The E50 (*P* < .05) and E100 (*P* < .01) groups showed significant differences in LDL-cholesterol levels compared with the untreated control group, and there was a general trend toward decreased LDL-cholesterol levels with increased *E prostrata* concentrations in the diet. In contrast, the serum HDL-cholesterol levels (in mg/dL) were generally lower in the groups treated with *E prostrata* at 50 and 100 mg/kg diet than in the control group (*P* < .01). Atherogenic indices were significantly decreased in E50 and E100 groups compared with control (*P* < .01).

### 3.2. Serum hydroxyl radical, lipid peroxide, and carbonyl content

The antioxidant effects of *Eclipta prostrata* are shown in Fig. 1. Serum hydroxyl radical (nmol/mg protein per minute) and serum lipid peroxide (nmol/mg protein) levels were significantly lower in the E50 and E100 groups compared with the untreated group (*P* < .01). Carbonyl content of oxidatively modified proteins was significantly decreased in the E100 group compared with the untreated control group (*P* < .01).

4. Discussion

When a physiologic imbalance such as hyperlipidemia exists, the result is the progressive development of atherosclerosis and cardiovascular diseases [28]. Serum triacylglycerol and total cholesterol levels in rats fed *E prostrata* at 50 and 100 mg/kg diet were lower by 9.8% to 19.0% and 10.7% to 13.4%, respectively, as compared with the untreated control group. Hypercholesterolemia is a risk factor for coronary artery disease (CAD), and numerous clinical studies have shown that LDL-cholesterol plays a major role in the pathogenesis of CAD [29,30]. *E prostrata* significantly decreased serum LDL-cholesterol levels by 10.3% to 13.0% in the E50 and E100 groups. In contrast, HDL-cholesterol levels were significantly increased (13.0%-19.1%) in these groups compared with the control group.

![Fig. 1. Effect of *E prostrata* extract on serum hydroxyl radical, lipid peroxide, and carbonyl contents in CD rats. Experimental groups were fed diets supplemented with 0 mg (control), 25 mg (E25), 50 mg (E50) or 100 mg (E100) of freeze-dried *E prostrata* extract per kilogram of diet. Values are means ± SEM (n = 7). Values are significantly different at *P* < .05 as assessed by the Dunnett *t* test. *P* < .05; ** *P* < .01 compared with the control group.](image-url)
Atherogenic indices were also decreased by 9.8% to 30.5% in all groups supplemented with E prostrata. Kumari et al [9] reported that a crude E prostrata extract reduced serum triacylglycerol at 100 mg/kg diet, and our results confirm that the butanol extract of E prostrata significantly reduced serum triacylglycerol level in the E50 group compared with the control group. This result suggests that the active phytochemical(s) in E prostrata may be soluble in polar solvents. Furthermore, the serum cholesterol modulating effect of the butanol extract of E prostrata was evident by the decreased serum LDL-cholesterol and increased HDL-cholesterol levels observed in this study. To the best of our knowledge, this is the first report demonstrating the effects of E prostrata on LDL-cholesterol and HDL-cholesterol levels.

Oxidized lipids have undesirable effects on human health, and reactive oxygen species are suggested to be causative agents of various diseases such as arthritis, asthma, dementia, Down syndrome, cancer, and Parkinson disease [31]. E prostrata extract clearly decreased serum hydroxyl radical, lipid peroxide, and oxidized protein levels significantly in this study. The hypolipidemic and antioxidant effects of E prostrata may rely on the presence of polypeptides, steroids, and flavonoids [32].

Finally, the results of this study indicate that the butanol fraction of E prostrata effectively enhanced serum lipid and oxidant metabolism in rats and corroborates the use of this medicinal plant in traditional medicine for treatment of hyperlipidemia and atherosclerosis. The use of E prostrata may contribute to the prevention of atherosclerosis and hypercholesterolemia in humans and to the improvement of the health of patients with CADs. However, additional studies on the biochemical and functional characterizations of the active components of E prostrata, which influence serum lipid and oxidant metabolism and its components in clinical medicine, are needed for better understanding of this plant and its benefits.

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References


