

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

Dae-Ik Kim^a, Sung-Hyen Lee^{b,c,*}, Jin-Ho Choi^a, Hyun Soon Lillehoj^b,
Mi-Hee Yu^d, Gun-Soon Lee^e

^aDepartment of Food Science and Biotechnology, Pukyong National University, Busan 608-737, South Korea

^bAnimal and Natural Resources Institute, Beltsville Agricultural Research Center, ARS-USDA, Beltsville, MD 20705, USA

^cNational Rural Resources Development Institute, NIAST, RDA, 88-2 Seodun Gweonson, Suwon, 441-853, South Korea

^dDepartment of Food Science and Technology, Keimyung University, Daegu 704-701, South Korea

^eKorea National Agricultural College, RDA, 11-1 Donghwa Bongdam Whasung, Gyeonggi 445-760, South Korea

Received 3 March 2008; revised 30 April 2008; accepted 8 May 2008

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.

© 2008 Elsevier Inc. All rights reserved.

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

Abbreviations: AI, 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, atherosclero-

sis, 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

* Corresponding author. Animal and Natural Resources Institute, Beltsville Agricultural Research Center, ARS-USDA, Building 1043, BARC-East, Beltsville, MD 20705, USA. Tel.: +1 301 504 8771/6170; fax: +1 301 504 5103/5306.

E-mail address: sunghyen.lee@ars.usda.gov (S.-H. Lee).

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* extract per kilogram of diet, whereas controls were fed basal diet without *E prostrata* extract. All diets contained 18% protein, 58.3% carbohydrate, 15% fat, and 0.5% cholesterol (Table 1). The butanol fraction of *E prostrata* was prepared by successive extraction steps starting from crude methanol extract using chloroform, ethanol, ethyl acetate, and butanol. The final butanol fraction was concentrated by evaporation, freeze drying, and storing at –20°C until use. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Pukyong National University.

2.2. Analytical procedures

Blood samples were collected from the rats fed experimental diets for 6 weeks after overnight fasting, and euthanasia

Table 1
Composition of the control and experimental diets (g/kg)

Composition	Control	E25	E50	E100
<i>Eclipta</i> powder ^a	–	0.025	0.050	0.100
Cornstarch	45.0	45.0	45.0	45.0
Sucrose	13.3	13.275	13.250	13.200
Casein	18.0	18.0	18.0	18.0
Lard	10.0	10.0	10.0	10.0
Corn oil	5.0	5.0	5.0	5.0
Cellulose	3.0	3.0	3.0	3.0
AIN-76 mineral mix ^b	1.0	1.0	1.0	1.0
AIN-76 vitamin mix ^b	3.5	3.5	3.5	3.5
DL-methione	0.3	0.3	0.3	0.3
Choline chloride	0.2	0.2	0.2	0.2
Cholesterol	0.5	0.5	0.5	0.5
Sodium cholate	0.2	0.2	0.2	0.2

^a 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.

^b AIN-76 mineral and vitamin mixtures were obtained from ICN (Solon, Ohio).

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*-phthaldehyde 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,

Table 2
Effects of *E prostrata* extract on serum triacylglycerol, total cholesterol, LDL-cholesterol, HDL-cholesterol, and AI in CD rats^a

	Control	E25	E50	E100
Triacylglycerol (mg/dL)	124.6 ± 4.2	120.3 ± 2.7	112.4 ± 2.2*	100.9 ± 3.9**
Total cholesterol (mg/dL)	126.5 ± 3.6	119.6 ± 1.5	112.9 ± 2.7**	109.6 ± 3.0**
LDL-cholesterol (mg/dL)	57.1 ± 1.9	54.9 ± 1.4	51.2 ± 0.9*	49.7 ± 1.5**
HDL-cholesterol (mg/dL)	24.1 ± 0.6	25.0 ± 0.6	27.2 ± 0.6**	28.6 ± 0.7**
AI ^b	4.6 ± 0.3	4.1 ± 0.1	3.6 ± 0.1**	3.2 ± 0.6**

Values are means ± SEM (n = 7). Values are significantly different at $P < .05$ as assessed by the Dunnett *t* test.

^a 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.

^b (total cholesterol – HDL-cholesterol) / HDL-cholesterol.

* $P < .05$.

** $P < .01$ as compared with the control group.

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 were significantly higher 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 ($P < 0.01$).

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 E 100 groups. In contrast, HDL-cholesterol levels were significantly increased (13.0%–19.1%) in these groups compared with the control group.

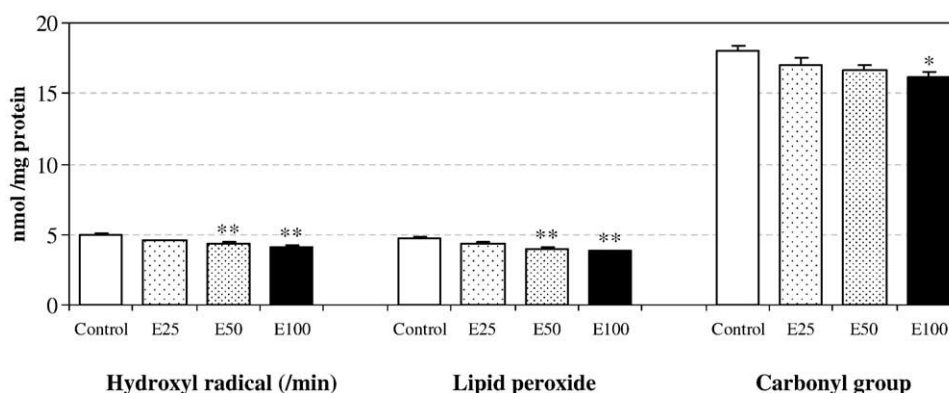


Fig. 1. Effect of *E prostrata* extract on serum hydroxyl radical, lipid peroxide, and carbonyl contents in CD rats. Experimental groups were fed diets supplemented with 0 mg (control), 25 mg (E25), 50 mg (E50), or 100 mg (E100) of freeze-dried *E prostrata* extract per kilogram of diet. Values are means ± 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.

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.

Acknowledgment

This work was partially supported by the fund from the Pukyong National University. The authors thank Hyo-Gwon Im for his contribution to this research and Erik P. Lillehoj for his editorial critique.

References

- [1] <http://plants.usda.gov/java/charProfile?symbol=ECPR>.
- [2] <http://jdm0777.com.ne.kr/a-yakchoxt/hanryuncho.htm>.
- [3] Anon. The wealth of India, raw materials, vol. 3. New Delhi: Council of Scientific and Industrial Research; 1952.
- [4] Dixit SP, Achar MP. Study of Bhingaraja (*Eclipta alba*) therapy in jaundice in children. *J Sci Res Plant Med* 1981;2:96-100.
- [5] Ma-Ma K, Nyunt N, Tin KM. The protective effect of *Eclipta alba* on carbon tetra-chloride induced acute liver damage. *Toxicol Appl Pharmacol* 1978;45:723-8.
- [6] Tewtrakul S, Subhadhirasakul S, Cheenpracha S, Karalai C. HIV-1 protease and HIV-1 integrase inhibitory substances from *Eclipta prostrata*. *Phytother Res* 2007;21:1092-5.
- [7] Christyapapita D, Divyagnaneswari M, Michael RD. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. *Fish Shellfish Immunol* 2007;23:840-52.
- [8] Rangineni V, Sharada D, Saxena S. Diuretic, hypotensive, and hypocholesterolemic effects of *Eclipta alba* in mild hypertensive subjects: a pilot study. *J Med Food* 2007;10:143-8.
- [9] Kumari CS, Govindasamy S, Sukumar E. Lipid lowering activity of *Eclipta prostrata* in experimental hyperlipidemia. *J Ethnopharmacol* 2006;105:332-5.
- [10] Jeon HJ, Kang HJ, Jung HJ, Kang YS, Lim CJ, Kim YM, et al. Anti-inflammatory activity of *Taraxacum officinale*. *J Ethnopharmacol* 2008;115:82-8.
- [11] Ji W, Gong BQ. Hypolipidemic effects and mechanisms of *Panax notoginseng* on lipid profile in hyperlipidemic rats. *J Ethnopharmacol* 2007;113:318-24.
- [12] Puri A, Khaliq T, Rajendran SM, Bhatia G, Chandra R, Narender T. Antidyslipidemic activity of *Indigofera tinctoria*. *J Herb Pharmacother* 2007;7:59-64.
- [13] Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour Technol* 2008;99:2717-23.
- [14] Aderogba MA, McGaw LJ, Ogundaini AO, Eloff JN. Antioxidant activity and cytotoxicity study of the flavonol glycosides from *Bauhinia galpinii*. *Nat Prod Res* 2007;21:591-9.
- [15] Anonymous. Report on American institute of Nutrition, Ad-hoc committee on standards for nutritional studies. *J Nutr* 1977;107:1340-8.
- [16] Lee SH, Park HJ, Chun HK, Cho SY, Jung HJ, Cho SM, et al. Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet. *Nutr Res* 2007;27:505-10.
- [17] Lee SH, Park HJ, Cho SY, Jung HJ, Cho SM, Cho YS, et al. Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice. *Nutr Res* 2005;25:869-76.
- [18] Rudel LL, Morris MD. Determination of cholesterol using o-phthalaldehyde. *J Lipid Res* 1973;14:364-6.
- [19] Haglund O, Luostarinen R, Wallin R, Wibell L, Saldeen T. The effects of fish oil on triglycerides, cholesterol, fibrinogen and malondialdehyde in humans supplemented with vitamin E. *J Nutr* 1991;121:165-9.
- [20] Halliwell B, Gutteridge JMC. Formation of a thiobarbituric acid-reactive substance from deoxyribose in the presence of iron salts. *FEBS Lett* 1981;128:347-50.
- [21] Lemon DW. An improved TBA test for rancidity. Environment Canada. New series circular No. 51. Halifax, Nova Scotia: Fishery and marine service; 1975.
- [22] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;186:464-78.
- [23] Yagi K. Simple procedure for specific assay of lipid hydroperoxides in serum or plasma. *Free Radic Antioxid Protoc* 1998;108:101-6.
- [24] Armstrong D, Browne R. The analysis of free radicals, lipid peroxidases, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Free Radic Diagn Med* 1994;366:43-58.
- [25] Lefevre G. Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. *Annals de Biologie Clinique (Paris)* 1998;56:305-19.
- [26] Dunnett CW. A multiple comparisons procedure for comparing several treatments with a control. *J Am Statistic Assoc* 1955;50:1096-121.
- [27] Sokal RR, Rohlf FJ. The principles and practice of statistics in biological research. San Francisco: Freeman and Co; 1969.
- [28] Verbeuren TJ, Coene MC, Jordaens FH, Van Hove CE, Zonnekeyn LL, Herman AG. Effect of hypercholesterolemia on vascular reactivity in the rabbit. Influence of treatment with dipyrindamole on endothelium-dependent and endothelium-independent responses in isolated aortas of control and hypercholesterolemic rabbits. *Circ Res* 1986;59:496-504.

- [29] LaRosa JC, Hunninghake D, Bush D, Criqui MH, Getz GS, Gotto Jr AM, et al. The cholesterol facts: a summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. *Circulation* 1990;81:1721-33.
- [30] Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit. *Circulation* 1998;97:946-52.
- [31] Perry G, Raina AK, Nunomura A, Wataya T, Sayre LM, Smith MA. How important is oxidative damage? Lessons from Alzheimer's disease. *Free Radic Biol Med* 2000;28:831-4.
- [32] Sarg TM, Salam NAA, El-Domiaty M, Khafagy SM. The steroid, triterpenoid and flavonoid constituents of *Eclipta alba* (L.) Hassk. (Compositae) grown in Egypt. *Sci Pharm* 1981;49:262-4.