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Antihyperhomocysteinemic Activity of an Ethanol Extract from *Embelia ribes* in Albino Rats

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Abstract

An ethanol extract from *Embelia ribes* Burm (Myrsinaceae) fruits was investigated for its antihyperhomocysteinemic and lipid-lowering potential in methionine-induced hyperhomocysteinemia rats. Hyperhomocysteinemia was induced by methionine (1 g/kg, p.o., 30 days) through drinking water in male albino rats. A significant ($p < 0.01$) increase in homocysteine, lactate dehydrogenase, total cholesterol, triglycerides, and low-density lipoprotein levels in serum and lipid peroxides levels in heart homogenates with a concomitant decrease in serum high-density lipoprotein and myocardial glutathione levels were observed in pathogenic control rats compared with normal healthy control rats. Furthermore, ethanol *Embelia ribes* extract (100 and 200 mg/kg, p.o., 30 days) treatment in both the doses significantly reversed all the above-mentioned parameters compared with pathogenic control rats. The results of test drug were comparable with folic acid (100 mg/kg, p.o.), a standard antihyperhomocysteinemic agent. The results indicated that ethanol *Embelia ribes* extract has significant antihyperhomocysteinemic and lipid-lowering potential in hyperhomocysteinemic rats.

Keywords: *Embelia ribes*, homocysteine, lipid profiles, methionine.

Introduction

Plant infusions and decoctions have been used as popular medicine in several underdeveloped and developing countries as an alternative treatment for various pathophysiologic conditions. Local communities residing in the biodiversity-rich areas of the north eastern region of India have traditionally used and relied on herbs for treating various ailments (Kayang et al., 2005). This practice has continued even today where the low cost and availability, coupled with the poorly equipped government health facility and rising cost of drugs, has left the rural community with hardly any options but to rely on traditional health care practices. These increasing trends in the use of plants as medicines locally and globally necessitate scientific investigations especially where information regarding toxicity is lacking on such plants or their extracts.

Hyperhomocysteinemia has emerged as an independent risk factor for development of coronary, cerebrovascular, and peripheral arterial occlusive disease (Omenn et al., 1998). Although severe hyperhomocysteinemia is rare, mild elevations in homocysteine concentration have been found in nearly 7% of the general population and in 20% to 30% of patients with coronary and peripheral vascular disease (Clarke et al., 1991; Folsom et al., 1998; McCully, 1996). A mere increase of 12% over the normal level of homocysteine has been associated with a threefold increase in risk for myocardial infarction (Nygard et al., 1997). Recent epidemiologic studies support a positive association between plasma homocysteine concentration and risk for cardiovascular disorders (Arnesen et al., 1995; Graham et al., 1997). Methionine is the only dietary source of homocysteine, a potent agent that disrupts endothelial integrity (Hening et al., 1993; Ross, 1993; Toborek & Hening, 1994). Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis by increasing homocysteine levels (Toborek et al., 1995).

Embelia ribes Burm is a threatened woody shrub belongs to the family Myrsinaceae, which is sparsely distributed in India, SriLanka, Malaysia and southern China (Guhabakshi et al., 2001). The whole plant is used in the treatment of anti-inflammatory to relieve rheumatism and fever (Kapoor et al., 1983). The fruit is bitter in taste, a good appetizer, cures tumors, ascites, bronchitis,

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jaundice and mental disorders (Kirthikar & Basu, 1987). Fruits contain a quinone derivative, embelin (3-undecyl 2,5-dihydroxy, 1,4-benzoquinone), an alkaloid, christembine (Tyagi et al., 1978), and a volatile oil, vilangin; its chemical constituent is 2,5-dihydroxy-4-undecyl-3,6-benzoquinone (Rao & Venkateswaralu, 1961). It is highly esteemed in Ayurveda as a powerful anthelmintic (Hordegen et al., 2006). In a preliminary study, Tripathi has reported the antihyperglycemic activity of decoction of the *Embelia ribes* fruits in glucose-fed albino rabbits. Further, Bhandari et al. (2002) have reported diabetic dyslipidemic activity of *Embelia ribes* (200 mg/Kg, p.o.,) in streptozotocin-induced diabetes in rats. However, no antihyperhomocysteinemic activity has been carried out on ethanol extract of *Embelia ribes*. Therefore, it was thought worthwhile to determine the antihyperhomocysteinemic activity of ethanol extract of *Embelia ribes* with reference to biochemical cardiac markers. Hence, the current study was a pilot study designed to determine whether the ethanol extract of dried fruits of *Embelia ribes* could exert any protective action against methionine-induced hyperhomocysteinemia as judged by biochemical markers.

Materials and Methods

Chemicals

Methionine and folic acid were procured from CDH (Bombay, India). Other chemicals used were of analytical grade. Double-distilled water was used for all biochemical assays.

Plant material

The dried fruits of *Embelia ribes* Burm were purchased from a local market, in New Delhi, India, in October 2005, and botanical authentification was carried out by the Department of Botany, Faculty of Science, Hamdard University, New Delhi. India. A voucher specimen is kept in the herbarium of the university (voucher specimen no. UB 2).

Preparation of an ethanol extract of *Embelia ribes*

The dried and coarsely powdered drug (100 g) was packed in a Soxhlet apparatus and subjected to extraction with ethanol over 72 h. The filtrate was evaporated under vacuum, and a brown mass residue obtained was stored at 4°C for further use. The average yield of the ethanol *Embelia ribes* extract was approximately 7.9%. For experimental studies, the weighed amount of ethanol *Embelia ribes* extract (100 and 200 mg/kg) was dissolved in 1% Tween 80 in normal saline and administered to adult male Wistar albino rats by the oral route.

Standardization of extract

Preliminary phytochemical screening of ethanol extract of dried fruits was carried out for the detection of phytoconstituents, using standard chemical tests. Alkaloids, carbohydrates, and saponins were detected in the extract. High Performance Thin Layer Chromatography (HPTLC) fingerprints of ethanol extract was established using CAMAG HPTLC (WinCAT software, version 2.2) and benzene:petroleum ether:acetic acid (8:2:0.5) as solvent system, which showed the presence of four spots (R_f values: 0.01, 0.04, 0.15, and 0.94) at 254 nm.

Animals

Healthy, male, adult, albino Wistar rats (200–250g) procured from the Central Animal House Facility, Hamdard University, New Delhi, and acclimatized under standard laboratory conditions at 25 ± 2°C, relative humidity (50 ± 15%), and normal photoperiod (12-h light-dark cycle) for 7 days were used for the experiment. Commercial rat pellet diet (Nav Maharastra chakan Oil Mills Ltd, Delhi, India) and water were provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (registration no. 173/CPCSEA, dated 28 January, 2000).

Experimental procedures

After acclimatization, all the animals were randomly divided into five groups of 10 animals each and treated as follows:

Group I, normal healthy control: rats received only 1% Tween 80 in normal saline.
Group II, pathogenic control: rats received only methionine (1 g/kg, p.o.) for 30 days.
Group III: rats received ethanol *Embelia ribes* extract (100 mg/kg, p.o.) coadministered with methionine (1 g/kg, p.o.) for 30 days
Group IV: rats received ethanol *Embelia ribes* extract (200 mg/kg, p.o.) coadministered with methionine (1 g/kg, p.o.) for 30 days.
Group V: rats received folic acid (100 mg/kg, p.o.) coadministered with methionine (1 g/kg, p.o.) for 30 days.

At the end of the experiment, blood samples were withdrawn from the retroorbital plexus using microcapillary technique (Sorg & Buckner, 1964) from all the groups of overnight-fasted rats, and serum was separated for biochemical estimation of homocysteine (Primus et al., 1988), lactate dehydrogenase (Lum & Gambino, 1974), total cholesterol (Demacher & Hijamaus, 1980),

Table 1. Effect of ethanol *Embelia ribes* extract administration on homocysteine and lactate dehydrogenase levels in serum.

Treatment	Homocysteine (μg/mL)	LDH (IU/L)
Normal healthy control	8.517 ± 0.191	28.301 ± 0.805
Pathogenic control	22.652 ± 0.034*	59.361 ± 0.694*
Ethanol *Embelia ribes* extract (100 mg/kg) treated	15.582 ± 0.071#	39.026 ± 0.448#
Ethanol *Embelia ribes* extract (200 mg/kg) treated	15.065 ± 0.055#	36.393 ± 0.695#
Folic acid (100 mg/kg) treated	14.715 ± 0.032#	34.502 ± 0.361#

*$p < 0.01$ as compared with group I, #$p < 0.01$, compared with group II.

triglycerides (Foster & Dunn, 1973), and high-density lipoproteins (Burstein et al., 1970) in serum.

After blood collection, all animals were sacrificed by cervical dislocation and hearts were dissected out for biochemical estimation in heart homogenates. Lipid peroxides were measured in heart by using the TBA method of Ohkawa et al. (1979). Glutathione activity was assayed by the method of Sedlak and Lindsay (1968) based on the reaction with 5,5′-dithiobistrinitro benzene (DTNB).

Statistical analysis

All data were expressed as mean ± SEM. All the groups of data were analyzed by one-way analysis of variance followed by Dunnett *t*-test using GraphPad Prism 3.0 (GraphPad Software; San Diego, CA, USA). $p < 0.01$ values were considered as statistically significant.

Results

Methionine administration in the pathogenic control group resulted in significant ($p < 0.01$) elevation of homocysteine, lactate dehydrogenase (LDH), total cholesterol, triglycerides (TG), and low-density lipoprotein (LDL-C) levels in serum along with the significant ($p < 0.01$) decrease in high-density lipoprotein (HDL-C) levels in serum compared with normal healthy control group. Ethanol *Embelia ribes* extract and folic acid treatment in hyperhomocysteinemic rat significantly ($p < 0.01$) decreased the homocysteine, LDH, total cholesterol, TG, and LDL-C levels and increased the HDL-C levels in serum compared with pathogenic control group (Table 1 and 2). Further, methionine treatment significantly ($p < 0.01$) increased the lipid peroxides (LPO) levels and decreased glutathione (GSH) levels in heart homogenates in pathogenic control group compared with normal healthy control group, and ethanol *Embelia ribes* extract and folic acid treatment in hyperhomocysteinemic rats significantly ($p < 0.01$) decreased the myocardial LPO levels and increased the GSH levels compared with pathogenic control group (Table 3).

Discussion

The current study examined the antihyperhomocysteinemic and lipid-lowering potential of ethanol *Embelia ribes* extract (100 and 200 mg/kg, p.o.) in methionine-induced hyperhomocysteinemia in rats. The mechanisms associated with homocysteine-induced endothelial dysfunction are mediated by increased oxidative stress (Kanani et al., 1999), leading to increased levels of oxidized LDL (Ventura et al., 2000). Hyperhomocysteinemia may promote the generation of reactive oxygen species (ROS) such as H_2O_2 and hydroxyl radicals via the autooxidation of sulfhydryl (-SH) group (Heinecke et al., 1987) or by decreasing the intracellular levels of GSH, which is involved in the elimination of free radicals.

Homocysteine, a thiol containing amino acid derived from demethylation of dietary methionine, may generate partially reduced ROS that are able to stimulate the lipid peroxidation involved in atherosclerotic process. Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis by increasing homocysteine levels (Toborek et al., 1995).

The data in our current study showed that methionine (1 g/kg, p.o.) treatment in pathogenic control group rats significantly ($p < 0.01$) elevated the levels of homocysteine,

Table 2. Effect of ethanol *Embelia ribes* extract on lipid profile levels.

Treatment	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Normal healthy control	100.595 ± 0.868	87.149 ± 1.311	43.589 ± 0.560	39.576 ± 0.571
Pathogenic control	194.212 ± 1.646*	179.516 ± 2.148*	144.076 ± 1.713*	14.232 ± 0.438*
Ethanol *Embelia ribes* extract (100 mg/kg) treated	160.850 ± 2.299#	133.912 ± 1.994#	112.714 ± 1.400#	22.063 ± 0.506#
Ethanol *Embelia ribes* extract (200 mg/kg) treated	154.893 ± 1.805#	126.956 ± 1.994#	101.725 ± 1.004#	27.777 ± 0.418#
Folic acid (100 mg/kg) treated	147.574 ± 1.513#	119.226 ± 1.718#	93.465 ± 0.606#	30.464 ± 1.104#

*$p < 0.01$ compared with group I, #$p < 0.01$ compared with group II.

Table 3. Effect of ethanol *Embelia ribes* extract administration on lipid peroxides and glutathione levels in heart homogenates.

Treatment	Lipid peroxides (nmol MDA/ mg protein)	Glutathione (μmol phosphorous liberated min^{-1} mg $protein^{-1}$)
Normal healthy control	1.466 ± 0.005	11.728 ± 0.176
Pathogenic control	$4.89 \pm 0.041^{*}$	$6.030 \pm 0.325^{*}$
Ethanol *Embelia ribes* extract (100 mg/kg) treated	$3.139 \pm 0.016^{\#}$	$8.293 \pm 0.184^{\#}$
Ethanol *Embelia ribes* extract (200 mg/kg) treated	$2.906 \pm 0.012^{\#}$	$9.803 \pm 0.170^{\#}$
Folic acid (100 mg/kg) treated	$2.753 \pm 0.015^{\#}$	$10.261 \pm 0.226^{\#}$

$^{*}p < 0.01$ compared with group I, $^{\#}p < 0.01$ compared with group II.

LDH, total cholesterol, LDL-C, triglycerides in serum, and LPO in heart homogenates with a concomitant decrease in serum HDL-C and myocardial GSH levels.

An increase in the levels of serum LDH indicates cardiac muscular damage, and it could be due to the leakage of enzymes from the heart (Sheela & Shyamaladevi, 2000). Free radicals generated by hyperhomocysteinemia initiate lipid peroxidation of the membrane-bound polyunsaturated fatty acids, leading to impairment of the membrane structural and functional integrity (Ajitha & Rajnarayana, 2001). This concurs with the current findings wherein the levels of LPO were found to be significantly ($p < 0.01$) increased in animals subjected to methionine treatment. Because of this increased lipid peroxidation, GSH levels are lowered (Flohe, 1989).

In the current study, elevated levels of homocysteine, LDH, total cholesterol, LDL-C, and TG in serum and LPO in heart homogenates were reduced significantly ($p < 0.01$) by treatment with ethanol *Embelia ribes* extract, suggesting cardioprotective and lipid-lowering potential of *Embelia ribes*. Further, the levels of HDL-C in serum and GSH in heart homogenates were increased significantly ($p < 0.01$), thereby enhancing the endogenous myocardial antioxidant levels.Furthermore, the results of test drug were comparable with folic acid, a standard positive control.

Biochemical assay of various parameters in serum and heart tissues of the animals revealed that ethanol *Embelia ribes* extract in both the doses favorably modified various biochemical markers in methionine-induced hyperhomocysteinemic rats significantly ($p < 0.01$) in a dosedependent manner compared with pathogenic hyperhomocysteinemic rats.

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