

Effect of *Erythrina variegata* on experimental atherosclerosis in guinea pigs

Sir,

Leaves of *Erythrina variegata* (Indian coral tree) (family: Fabaceae) eaten as a pot herb, are used as an antiobesity drug in Siddha medicine.^[1] It has folkloric reputation as antiinflammatory in India, China and South East Asia, and different parts of the plant are reported with insecticidal, hemagglutinating, curaric, skeletal muscle relaxant, feeding deterrent, antispasmodic, antimycobacterial and antiosteoporotic activities.^[2] In this study, the influence of the total alcohol extract of *E. variegata* (Ev) on experimental atherosclerosis in guinea pigs has been evaluated (IAEC approved, Ref: IAEC/SRMC and RI/41/2005). The extract was well tolerated, with no signs of toxicity up to 2 g/kg b.wt. in the acute toxicity study.

Ten month-old guinea pigs (750 g) were used for the study. They were fed high-fat diet (HFD) (Guinea pig pellet diet + 0.2% w/w cholesterol) for 30 days. Six animals were

sacrificed and evaluated for the onset of early atherosclerotic changes in the coronary artery, aorta and major organs.^[3] Animals were divided into four groups of six animals each and were treated as follows:

group I – pellet diet only,

group II – HFD fed group,

group III – HFD + 100 mg/kg Ev, and

group IV – HFD + 10 mg/kg atorvastatin calcium.

At the end of experimentation, they were fasted overnight, sacrificed under ether anesthesia and blood was collected by cardiac puncture for serum lipid estimation. Aorta was accessed through the left ventricle, slit open longitudinally, and the entire length of the aorta from the base of the aortic arch up to the diaphragmatic hiatus was resected out, washed in ice-cold saline, trimmed of adventitial fat and stored in formal calcium (10% formalin 1% CaCl₂). The area of atherosclerotic plaque in the aorta was histomorphometrically measured by Oil Red O staining^[4] using Image ProPlus Image analysis system. The entire anterior descending left coronary artery was quickly identified and dissected out for histopathological examination. Heart and liver were harvested, washed with ice-cold saline, trimmed of adventitial fat, weighed and stored at –80°C until needed for analysis. They were evaluated for measurement of thiobarbituric acid reactive substances (TBARS)^[5] reduced glutathione^[6] superoxide dismutase (SOD)^[7] and glutathione peroxidase (GPX).^[8] The experimental data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's 't' post test. $P < 0.05$ was considered significant.

Ev of 1.39% w/w yielded β -sitosterol (433 mg, 1.445 w/w), oleanolic acid (65 mg, 0.217% w/w) and β -sitosterol glycoside (108 mg, 0.36% w/w) on column chromatographic processing. Histopathological assessment of the left coronary artery from animals sacrificed after the initial HFD administration of 1 month revealed initiation of atherosclerotic changes. Serum lipid levels of the experimental animals summarized in Table 1 shows a statistically significant rise ($P < 0.001$) in total cholesterol (TC; 229%), low density lipoprotein (LDL)C (890%) and the atherosclerosis index (AI) (254%) in group II animals compared to normal controls. Lipid profile results of the two treatment groups (III and IV) are in comparison

Table 1: Serum lipid profile and tissue antioxidant status of experimental animals

Group (treatment)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AI	Significant alteration in antioxidant status in				
						Liver		Heart		
						TBARS	GSH	SOD	GPX	GSH
I (Normal control)	27.67 ± 0.79	66.07 ± 4.12	5.32 ± 1.1	7.23 ± 2.67	3.24 ± 1.02	-	-	-	-	-
II (high-fat diet)	91.1 ± 10.33*	75.59 ± 2.28	6.35 ± 0.74	71.6 ± 27.99*	14.71 ± 6.28*	↑	↓	↓	↓	↓
III (Ev 100 mg/kg)	60.71 ± 8.06	45.95 ± 8.12	5.87 ± 0.59	45.65 ± 18.24	10.26 ± 2.14	-	-	-	↑	↑
IV (Atorvastatin 10 mg/kg)	65.52 ± 6.40	31.28 ± 6.40 [#]	27.35 ± 1.21	31.75 ± 15.65 [#]	1.58 ± 0.87 [#]	↓	↑	↑	-	-

Values are mean ± SEM; n = 6 animals in each group, * $P < 0.001$ when compare to group-I. [#] $P < 0.001$ when compare to group-II

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with positive control. Ev administration reduced TC (33%), triglyceride (TGL; 39%) and LDL (36%), while high density lipoprotein (HDL) levels remained unaltered demonstrating its marginal hypolipidemic influence. Atorvastatin calcium brought about a typical hypolipidemic response: TC (28%), TGL (69%), HDL-C (+330%), LDL-C (56%) and AI (−89%). Body weight changes of the experimental animals, recorded on a month-wise basis during experimentation, showed a 12% increase in group II, 8% increase in Ev treated group III (statistically different from group II at $P<0.01$), and 11.5% increase in group IV as against 10% increase for normal control. The least increase in body weight due to Ev is noteworthy in view of the antiobesity claims for the drug in traditional medicine.

Heart and liver tissue antioxidant status in experimental animals is reflective of hyperlipidemia related pro-oxidant damage in group II which showed a decrease in GSH ($P<0.001$) and elevation in TBARS ($P<0.01$) in liver.

There has been a decrease in GPX, SOD ($P<0.05$) and GSH compared to normal control in heart. Ev showed a significant improvement in GPX and GSH in heart over positive control. Thus, its hypolipidemic effect has not been augmented by the antioxidant component in the liver.

Representative photographs of histopathological sections of the coronary artery are presented in Figure 1. Normal coronary artery shows an intact intima [Figure 1a]. Myocardial tissue appears normal. HFD treated group II [Figure 1b] shows discontinuous endothelium with fatty changes in the surrounding cardiac tissue. Apart from intracellular lipid, extensive aggregates of foam cells are seen in the media [Figure 1c]. These have completely replaced its muscular pattern, typical of primary medial destruction in early atherosclerosis. Ev treatment appears to have reversed these changes. Sections from these groups [Figure 1d and e] show a normal continuous endothelium. Also, the cardiac tissue is devoid of fatty degeneration. Sections of coronary artery from

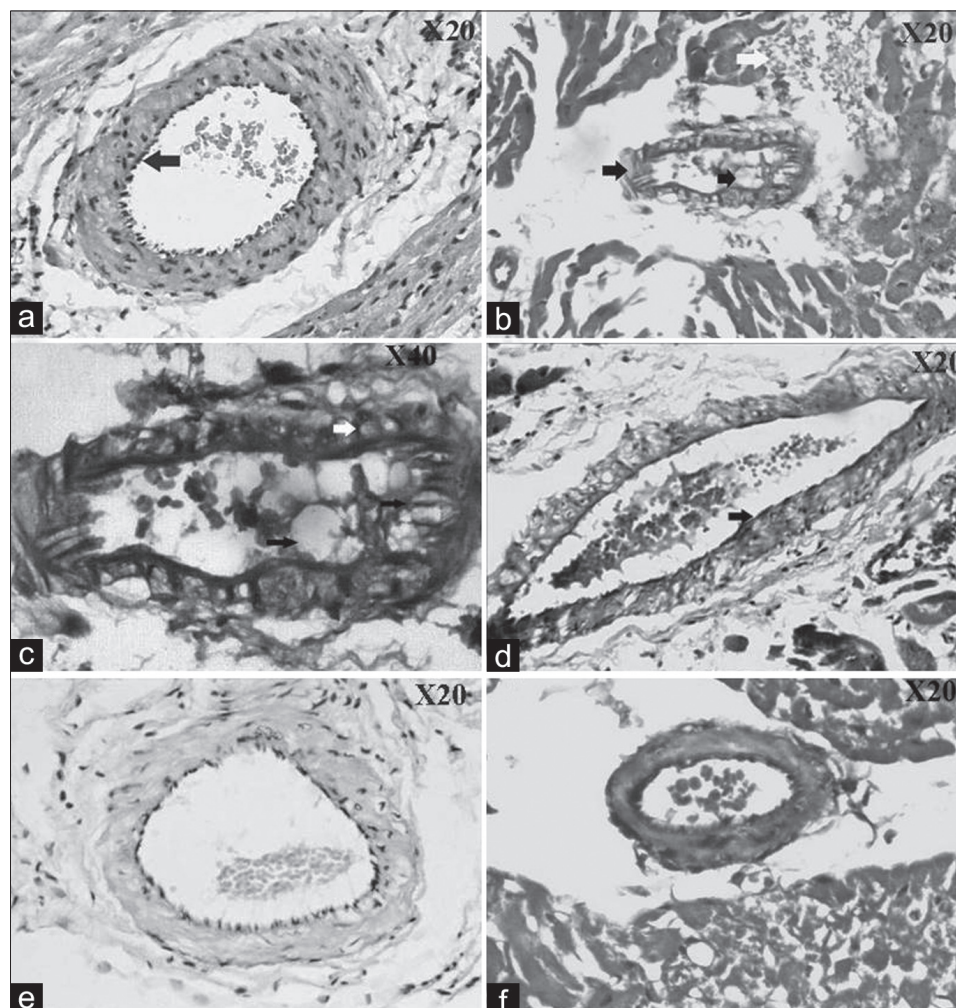


Figure 1: Histopathological examination of coronary artery sections from experimental animals (H and E). (a) Vehicle treated normal control group on pellet diet. (b) Positive control; black arrows show coronary artery with damaged intima with large foam cells. Note the foam cells in the media [white arrow]. (d and e) Ev treated and (f) atorvastatin calcium treated groups show normal coronary artery. Foam cells are less evident in both intima and media in these sections

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atorvastatin calcium treated animals exhibit normal coronary histopathology.

While Oil Red O stained intimal surface of aorta from untreated control was devoid of plaque, brick red lipid deposits measuring 15, 8 and 11% of total intimal surface were seen in HFD, Ev and atorvastatin treated groups, respectively.

Antioxidant status of the tested tissues in HFD fed group II suggests hyperlipidemia associated oxidative stress that triggers lipid peroxidation. Resulting cellular damage evidenced by coronary intimal, cardiac tissue damage due to proinflammatory changes has triggered atherosclerotic changes as evidenced by lipid-laden lesion areas in the aorta.

In Ev treated group III, these changes have been beneficially altered. Hypolipidemic activity of the leaf extract reported by us earlier in HFD fed rats,^[9] present evidence of healing in coronary artery over high-fat control group II and 47% reduction in the extent of aortic lipophilic lesion areas, and minimal increase in body weight relative to other groups strongly suggest its atheroprotective and anti-obesity influence. There has also been an antioxidant effect in heart tissue. In view of the antioxidant, anti-inflammatory, antihyperlipidemic and DNA protective activities^[10] of β -sitosterol, oleanolic acid and β sitosterol glycoside isolated in appreciable quantity from Ev, it may be suggested that the observed anti-atherosclerotic activity of the extract could be consequent to hypolipidemic and anti-inflammatory influence of the isolated phytoconstituents.

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