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A PILOT CLINICAL STUDY TO EVALUATE THE EFFECT OF *EMBLICA OFFICINALIS* EXTRACT (AMLAMAX™) ON MARKERS OF SYSTEMIC INFLAMMATION AND DYSLIPIDEMIA

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ABSTRACT

Emblica officinalis Gaertn., commonly known as the Indian gooseberry or "Amla", has been used as health food for centuries in India and other Asian countries. The biological effects of amla have been attributed to the antioxidant properties of the low-molecular weight hydrolysable tannins present in the fruit. Amlamax™ is a purified, standardized, dried extract of amla containing about 35% galloellagi tannins along with other hydrolysable tannins. Our earlier studies on rabbits showed significant reduction in total cholesterol and triglycerides as well as increase in HDL. The present study extends these results to human volunteers. Two doses of the extract were evaluated - 500 mg and 1000 mg per day for 6 months. Blood samples were collected at the 3rd and 6th months showed reduction in total and LDL cholesterols and enhancement of beneficial HDL cholesterol. In addition, blood CRP levels, a marker for inflammation, were also significantly reduced. Since dyslipidemia and inflammation are the two major components of cardiovascular diseases, the present results must be considered encouraging and indicate the potential of Amlamax™ in the management of heart diseases.

KEY WORDS

Emblica officinalis, Tannins, Cardiovascular diseases, Inflammation, Dyslipidemia.

INTRODUCTION

Amla *(Emblica officinalis*), commonly known as Indian gooseberry is widely used in many of the indigenous medical preparations against a variety of disease conditions (1). In vitro and animal studies have indicated that amla have potent anti-oxidant effect against several test systems such as superoxide radicals, lipid peroxide formation induction by Fe^{+++}/ADP ascorbate system, hydroxyl radical scavenging action and in systemic augmentation of antioxidant enzymes in the brain of laboratory animals (2-5). In rats, the flavonoids from *E. officinalis* effectively reduced lipid levels in serum and tissues and exerted a significant inhibitory effect of hepatic HMG CoA reductase enzyme activity (6). Our earlier study showed the beneficial effects of Amlamax™ on atherosclerosis and dyslipidemia in rabbits (7). The antioxidant activity of fruits of *E. officinalis* has been traced to its tannoid principles both in vitro and in vivo (8). A study conducted in rats found that gallo ellagi tannins enriched fractions of fresh juice of Emblica fruits showed antioxidant activity in ischemia - reperfusion - induced oxidative stress in rat heart (9). The aqueous extract of E. officinalis fruit increases cardiac glycogen levels and decreases serum GOT, GPT and LDL in rats having induced myocardial necrosis (10). Elevation of HDL as first observed in our study on rabbits (7) is a significant result obtained with Amlamax™ for which a patent application is under processing (11). Amlamax™ was found to be nontoxic in both acute and subacute toxicity studies in rats up to a dose of 2g/kg for three months (12).

Coronary vascular disease has emerged as the pre-eminent disease of our times claiming more lives than those accounting for the next four causes combined (13). Drug therapy mainly revolves around cholesterol synthesis inhibitors (statins). In patients with established disease, aspirin, a beta blocker and

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an ACE inhibitor are additionally prescribed (14). Dyslipidemia (15) and inflammation (16) are two major factors contributing to the disease process. Even though statins have moderate anti-inflammatory effects, there are no drugs currently in use targeting inflammation.

C-reactive protein (CRP), an acute phase protein, has been clinically used as a sensitive marker for systemic inflammation (17). High-sensitivity CRP (hs-CRP) has been noted to add to the prediction of first myocardial infarction when combined with blood lipid measures (18) and has been suggested as potential risk factor for Cardio vascular diseases (CVD) (19,20). Investigators have begun to develop therapies to lower hs-CRP concentrations and presumably, CVD risk (19, 21, 22).

The aim of the present study was to explore the effect of Amlamax on the lipid parameters and inflammation in mildly hypercholesterolemic humans. The results presented here demonstrate the utility of Amlamax^{TM} in controlling both dyslipidemia and inflammation especially CRP.

MATERIALS AND METHODS

Fresh fruits of amla were collected from the southern parts of India during January 2006 and pharmacognostically identified by comparing with voucher specimen number AE-HBRS-011. The chemicals used were of AR grade purchased from Merck.

Preparation of Extract : Fresh fruits of amla *(Emblica officinalis)* (250 kg) were cleaned, crushed, deseeded and refluxed with water for 2 hours. Then it was cooled and filtered. The filtrate was collected and the residue was extracted with water. The combined filtrates were pooled and concentrated at 90°C under vacuum. The dried powdered extract was collected (yield – 4.6%). This crude extract was analyzed for total polyphenols, proteins and galloellagi tannins. This standardized extract (Amlamax^{TM}) was made into 500mg tablets which were used for the study.

Study Design: The trial and formulation were approved by the local ethics committee. This study was a two centre two dosage study initiated by the Research and Development Laboratory of Arjuna Natural Extracts Ltd., Binanipuram – 683502, Ernakulam. The two centres selected are : (1) Kanjiramattom - 40 Kms towards the south of Ernakulam city – Group I; (2) Kokkunnu – 40 Kms towards the north of Ernakulam city – Group II.

Written informed consent was obtained from each patient before enrollment. A total of 26 subjects both male and female under the age group of 26 – 72 years were screened for Group I and a total of 25 subjects both male and female under the age group of 32 – 60 years were screened for Group II. Their lipid profile (total cholesterol, triglyceride, HDL, LDL and VLDL), blood routine analysis (Hb, neutrophil, lymphocytes, eosinophil, total count), Fasting blood glucose, CRP and TSH (thyroid stimulating hormone) were analyzed. Physical parameters like blood pressure and body weight were also recorded. Serum total cholesterol and HDL were estimated by enzymatic method (23,24). Triglyceride was estimated by GPO – PAP method (25). LDL and VLDL were calculated using Friedwald formula (26).

Exclusion Criteria: The patients suffering from chronic disorders (vascular heart disease, congestive cardiac failure, diabetes, renal and liver disorders) and those consuming regular drugs were excluded from the study.

Inclusion Criteria: The subjects having total cholesterol level 190 – 310 mg/dL and the CRP level in the range of 1.5 – 5 mg/L were selected for the study.

Treatment Plan: The subjects were briefed about the objectives of the study and were asked to sign a letter of informed consent. They were advised to follow their usual food habits and take Amlamax^{TM} 500 mg one tablet at bedtime along with a glass of water for Group I and Amlamax^{TM} 500 mg two tablets at bedtime along with a glass of water for Group II for a period of 6 months. The two groups were arbitrarily selected to test the efficacy of the two doses. They were advised to report after 3 months. After 3 months their fasting blood samples were collected and analyzed for lipid parameters and CRP. There were no dropouts and drug compliance was very good as judged by the number of unused samples returned after 3 months. They were further provided with tablets for the next 3 more months. At the end of study, fasting blood samples were collected as before and analyzed. Data analysis was carried out by multi factor analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Our earlier results had indicated that amla could correct dyslipidemia in animals including an enhancement of HDL cholesterol (7). The present study extends these results to mildly hypercholesterolemic humans. We further sought to study the effect of Amlamax^{TM} the inflammatory component of atherosclerosis. Accordingly, the present study was done on volunteers recruited from two centres. Two dosage levels were selected. One group was administered 500 mg/day of

Table 1 : Lipid profile, CRP and Blood glucose values of Group I volunteers (Mean ± SD)

Parameters tested	Duration of the study		
	0 month	3 months	6 months
Total Cholesterol (mg/dL)	242 ± 26.36622	211.71 ± 32.2541	210.82 ± 31.2038
LDL- Cholesterol (mg/dL)	161 ± 28.7545	137.82 ± 32.6897	139.88 ± 30.0937
VLDL (mg/dL)	40.71 ± 14.4335	32.29 ± 11.6607	26.94 ± 9.8606
Triglycerides (mg/dL)	203.53 ± 72.1674	161.47 ± 58.3036	134.71 ± 49.3031
HDL-Cholesterol (mg/dL)	40.29 ± 6.7193	41.59 ± 3.7894	44 ± 5.4015
C-Reactive Protein (mg/L)	3.2 ± 0.9062	2.08 ± 0.8773	1.94 ± 0.4827
Blood Glucose (mg/dL)	103.71 ± 32.3215	95.53 ± 23.0807	89.41 ± 21.8472

Amlamax^{TM} while the other consumed 1000 mg/day. Of the total number of participants initially screened, 22 were selected for Group 1 and 17 for Group 2 as per the inclusion criteria. All the participants completed the study. They were advised to record and report any adverse effects of the drug during the treatment period. There were none.

The serum levels of total cholesterol, LDL, VLDL, HDL, triglycerides and CRP were determined at the start, at 3 months and at 6 months of treatment. There was a significant reduction in total cholesterol, LDL, VLDL and triglycerides whereas there was a significant elevation in the HDL level (Table 1 and 2). The fall in CRP levels was quite significant at around 40% (Table 1). Statistical analysis by ANOVA of the results confirmed the significance of the above observation with the reduction in LDL, VLDL and triglycerides ($p<0.01$) and HDL elevation ($p< 0.05$). In addition, the haemogram showed improved levels of hemoglobin and RBC and other cells. There was a significant fall in blood glucose showing better glucose metabolism (Table 1 and 2). The vital functions showed improvement establishing the rejuvenating properties of amla. No significant differences were noted in results obtained with the two dosage levels studied.

The results show that treatment with Amlamax at doses of 500 mg/day and 1000 mg/day brought about significant reduction in the level of risk factors of CVD arising from dyslipidemia and inflammation. Considering that presently no therapeutic options are available that specifically targets inflammation in atherosclerosis, the results of Amlamax^{TM} should be considered significant. Safety of the product is an added feature. A well-controlled, randomized study is warranted.

CVD is a multicomponent, multifactorial disease. The third component, after inflammation and dyslipidemia, is immunity (27). Considering that the traditional uses of amla have all centered on the vitalizing and immune boosting properties of the product, Amlamax^{TM} can potentially modulate all the three components of the disease, the first agent to do so. Large trials are an urgent need to substantiate these potential benefits.

The mechanisms by which Amlamax^{TM} exerted the beneficial effects is presently not clear. Amla, like statins, is credited with HMG CoA reductase inhibitory activity (6). Ellagitannins and the ellagic acid obtained on hydrolysis of these tannins (by lipases and/or esterases) are inhibitors of squalene epoxidase, a rate-limiting enzyme of cholesterol biosynthesis (28). These inhibitory activities may explain the beneficial effects of Amlamax on lipid parameters. Inflammation/infection is known to reduce HDL levels (29,30) and the enhancement

Table 2 : Lipid profile, CRP and Blood glucose values of Group II volunteers (Mean ± SD)

Parameters tested	Duration of the study		
	0 month	3 months	6 months
Total Cholesterol (mg/dL)	216.35 ± 19.5807	210.41 ± 24.4590	197.94 ± 28.8352
LDL- Cholesterol (mg/dL)	146.71 ± 18.6035	141.59 ± 24.8881	130.18 ± 26.8465
VLDL (mg/dL)	32 ± 8.9047	26 ± 11.1672	24.06 ± 9.3963
Triglycerides (mg/dL)	160 ± 44.5236	130 ± 55.8359	120.29 ± 46.9816
HDL-Cholesterol (mg/dL)	37.65 ± 7.4592	42.82 ± 6.6530	43.71 ± 6.4786
C-Reactive Protein (mg/L)	2.6 ± 1.0160	2.5 ± 0.8791	1.9 ± 0.4547
Blood Glucose (mg/dL)	96.53 ± 8.7388	87.24± 20.3832	84.94± 18.4246

of HDL observed in the present study may arise from the control of inflammation by Amlamax™.

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