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Amla (*Emblica officinalis*) and Guava (*Psidium guajava*) supplementation: Impact of Low Carbon footprint local seasonal fruits on Lipemic Status of Morning Walk*er*s

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ABSTRACT

Dyslipidemia, a major risk factor of atherosclerosis is associated with an increased risk of cardiovascular diseases. Local seasonal vitamin C rich fruits Amla and Guava are purported to have hypolipidemic properties. Consumption of seasonal fruits is associated with large reductions in environmental impacts. The investigation was carried out to study the impact of supplementation of Amla and Guava on lipid metabolism of morning walkers. A total of 46 morning walkers were enrolled and divided into four groups: Amla group (N=13), Guava group (N=15), combination (Amla + Guava) group (N=10) and control group (N=8). Daily, Amla group and Guava group subjects received one Amla (approximately 35g) and one Guava (approximately 60g) for a period of 21 days respectively. The combination group received one Guava for 10 days followed by one Amla for a period of 11 days. Fasting blood glucose and lipid profile levels were monitored at baseline and at 21 days. Only the Amla group showed a favourable impact on lipid profile with a significant reduction of 6.4%, 13.0% and 11.7% in the levels of total cholesterol ($p<0.05$), low-density lipoprotein cholesterol ($p<0.01$) and non high-density lipoprotein cholesterol ($p<0.01$). The results obtained suggest that vitamin C and bioactive component rich Amla holds promise to be used in complementary therapy for the management of dyslipidemia.

Key words : *Dyslipidemia, Amla* (*Emblica officinalis*), Guava (*Psidium guajava*), *Hypolipidemic, Carbon footprint, Vitamin C*

Introduction

The prevalence of Non-Communicable Diseases (NCDs) continues to rise unabatedly across the globe. The proportion of all deaths in India due to NCDs increased from 37.9% in 1990 to 61.8% in 2016 and among NCDs, the category of cardiovascular diseases (CVDs) was the leading cause of death (Indian Council of Medical Research, Public Health Foundation of India and Institute for Health Metrics and Evaluation, 2017). The behavioral and biological risk factors, associated with the development of NCDs, are use of alcohol and tobacco, physical inactivity, overweight and obesity, increased saturated fat and salt intake, low fruit and vegetable intake, raised blood pressure, blood glucose and total cholesterol (TC) levels (World Health Organization, 2014). Dyslipidemia, including elevated levels of TC, low-density lipoprotein cholesterol (LDL-C), and triacylglycerol (TG) and low levels of high-density lipoprotein cholesterol (HDL-C) is a major risk factor of atherosclerosis and is associated with an increased risk of CVDs (Lin *et al*., 2018). A recent review of population based studies in India shows that over a 20-year period TC, LDL-C and TG levels have increased among urban populations and that high cholesterol is present in 25-30% of urban and 15-20% rural subjects (Gupta *et al*., 2017).

A recent surge in the popularity of alternative medicine and natural products in many parts of the world has revived interest in traditional medicines that have been used for the management of CVDs through centuries. Several medicinal plants, have been reported to have therapeutic benefits for the treatment of CVDs (Rastogi *et al*., 2016; Al-Snafi, 2017). The reported advantages of herbal medicines include effectiveness, safety, affordability and acceptability (Dhaliya Salam *et al*., 2013). Compounds available in food supplements and medicinal plants including dietary fibers, vitamins, flavonoids, sterols, and other antioxidants could be effective for the metabolism of lipids by influencing the metabolic reactions of different tissues (Bahmani *et al*., 2015).

Local seasonal fruits Amla (*Emblica officinalis* Gaertn.) commonly known as Indian Gooseberry and Guava *(Psidium guajava Linn*.) have been some of the most important herbs in Ayurveda, the Indian traditional system of medicine. Herbal food supplements like Guava, Amla, fenugreek, cinnamon etc. have been used for human consumption for centuries and their safety in humans has been well recognized (Deng, 2012; Joseph and Priya, 2011). Amla has been reported to be a rich source of vitamin C, minerals, amino acids and an extensive variety of bioactive phytochemicals including tannins, alkaloids, flavonoids, terpenoids, saponins and phenolic compounds and beneficial therapeutic properties like antioxidant, hypolipidemic, hypoglycemic and cardioprotection have been attributed to it (Bhandari and Kamdod, 2012; Jain *et al*., 2016). Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamin C, fibre and fatty acids (Kamath *et al*., 2008). The benefits of Guava fruit include controlling blood pressure, lowering cholesterol, battling diabetes, combating cancer and protecting the prostate (Kamala *et al*., 2011).

The production and consumption of foods is known to impact the environment significantly. The modern consumers of fruits and vegetables, are more conscious of issues such as environmental sustainability and socioeconomic issues and depend on such factors to inform their choices (Tecco *et al*., 2016). Fruit and vegetables have been reported to have lower greenhouse gas emissions compared to animal products (Poore and Nemecek, 2018). According to decision recommendations from the environmental assessment of 34 fruits and vegetables of a large Swiss retailer, seasonal consumption of local foods needs to be preferred over out-of-season fruits and vegetables that are imported by plane (Stoessel *et al*., 2012).

There is a dearth of studies that have compared the hypolipidemic action of vitamin C rich fruits Amla and Guava. This study was thus planned, with an objective to assess the effect of Amla supplementation on the lipid profile of morning walkers and to compare its effects with those of Guava supplementation and a combination of Amla and Guava supplementation.

Materials and Methods

Subjects

Forty-six morning walkers who gave verbal consent were enrolled for the study from a garden in Vadodara city, Gujarat, India. The subjects were then divided into four groups i.e. Amla group (N=13), Guava group (N=15), combination (Amla + Guava) group (N=10) and control group (N=8) based on their liking and willingness to consume the supplemented fruit. The Amla group received one Amla (weighing approximately 35g) and the Guava group received one medium sized Guava (weighing approximately 60g) daily for a period of 21 days. The combination group was supplemented with one medium sized Guava for a period of 10 days followed by one Amla for 11 days. The subjects were asked to consume the supplemented fruit either with lunch or with dinner. The control group did not receive any supplement. During the study duration, no modifications in the diet or medication were made. None of the subjects took other complementary or alternative medications during the course of supplementation. Baseline data was collected on general information, anthropometry, and medical history along with fasting blood glucose (FBG) and lipid profile. Anthropometric measurements of height, weight, waist circumference and hip circumference were taken using standard methods. FBG and lipid profile levels were estimated after an overnight fast as per standard procedures using enzymatic kits. The lipid fractions LDL-C, non high-density lipoprotein cholesterol (Non HDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were estimated by calculation. All the parameters were monitored at both baseline and at the end of the 21 days period.

Statistical Analysis

Results are expressed as mean ± standard error. Baseline and post supplementation values were compared using the paired t test. A result was declared to be statistically significant only if the p-value of an analysis was less than 0.05 on two tailed testing. Statistical analysis was carried out using Microsoft® Office Excel 2003.

Results and Discussion

Of the total 46 enrolled subjects who belonged to the middle age group, 28 subjects were males and 18 were females. Based on the Asia Pacific classification (World Health Organization Expert Consultation, 2004), the subjects were overweight or obese as indicated by their mean body mass index (Table 1). In all the four groups, abdominal obesity as indicated by a high waist – hip ratio was present in the female subjects. Data on medical history revealed that none of the subjects had complications like diabetes, hypertension or coronary heart disease.

Supplementation with Amla led to a significant reduction in the levels of TC ($p<0.05$) and the atherogenic lipoprotein LDL-C ($p<0.01$) (Table 2). TC and LDL-C levels decreased by 6.4% and 13.0% respectively. At the end of the supplementation period, an improvement was observed in HDL-C levels but the change was not statistically significant. Amla supplementation also brought about a significant reduction of 11.7% in the values of Non HDL-C ($p<0.01$) which represents a mixture of atherogenic lipoproteins. The TG and FBG values remained unaltered after Amla supplementation. Such decreasing trends in lipid parameters were not observed in the control group, Guava group and combination group. In line with other data, the Amla supplemented group showed a marked improvement in the atherogenic indices also (Table 3). There were significant reductions in the TC/HDL-C ($p<0.01$), LDL-C/HDL-C ($p<0.01$) and Non HDL-C/HDL-C ($p<0.01$) ratios after Amla supplementation. Atherogenic indices remained unaltered in the other three groups.

The reduction in lipemic parameters upon Amla supplementation could be attributed to the bioactive components present in Amla. In a study where 28 type 2 diabetic subjects were supplemented with a medium sized Amla (35g) berry for a period of sixty days, a significant decrease in the levels of TC (5.7%), Non HDL-C (8.3%) and LDL-C (9.4%), and an increase in HDL-C levels (5.5%) were reported (Iyer *et al.*, 2009). The authors attributed the favourable impact on lipid profile to the nutrient and phytochemical composition of Amla. Another study that evaluated the lipid-lowering properties of Amla fruit powder in normal and diabetic human subjects reported significant decreases in TC, TG

Table 1. Clinical profile of the morning walkers (Mean ± SE)

Variable		Control group N=8	Guava group N=15	Amla group N = 13	Amla + Guava group N=10
Sex	Male	4	12	6	6
	Female	4	3	7	4
Age (y)	Male	48 ± 6.50	44.5 ± 4.23	51.6 ± 1.43	51.5 ± 2.50
	Female	48 ± 1.03	36.6 ± 7.54	49.2 ± 2.74	48.7 ± 1.10
Height (m)	Male	1.75 ± 0.01	1.73 ± 0.02	1.72 ± 0.03	1.73 ± 0.03
	Female	1.62 ± 0.03	1.62 ± 0.01	1.54 ± 0.01	1.60 ± 0.04
Weight (Kg)	Male	77 ± 1.08	73 ± 3.21	71 ± 4.86	70 ± 3.46
	Female	71 ± 4.90	68 ± 0.88	70 ± 3.43	65 ± 6.76
BMI (Kg/m^2)	Male	25.13 ± 0.61	24.18 ± 0.75	24.33 ± 1.45	23.35 ± 0.76
	Female	27 ± 1.26	25.9 ± 0.48	28.81 ± 1.64	26.22 ± 3.98
WHR	Male	0.91 ± 0.04	0.92 ± 0.01	1.02 ± 0.02	0.85 ± 0.04
	Female	0.86 ± 0.01	0.85 ± 0.01	0.85 ± 0.32	0.87 ± 0.02

BMI= Body Mass Index; WHR= Waist Hip Ratio and LDL-C levels and significant improvement in HDL-C levels on day 21 in subjects given 2 or 3 g Amla powder per day (Akhtar *et al*., 2011). Potent antihyperlipidemic activity of aqueous extracts of Amla has been demonstrated in type 2 diabetic patients wherein treatment with 250 mg or 500 mg twice daily for 12 weeks led to a significant reduction in the levels of TC, LDL-C, and TG, and increase in HDL-C levels compared with baseline and placebo (Usharani *et al*., 2013). In another study where 500mg of Amla extract was administered twice daily for 12 weeks to patients with dyslipidemia, levels of TC, TG, LDL-C and VLDL-C were significantly lower in the Amla group in comparison to the placebo group. A significant reduction in the ratio of Apolipoprotein B to Apolipoprotein A1 and a 39% reduction in atherogenic index of plasma was also noted in the Amla group (Upadya *et al*., 2019).

Animal studies have revealed the central role of gallic acid in Amla mediated anti-hyperlipidemic action through upregulation of peroxisome proliferator-activated receptors-α, increased activity of lipid oxidation and decreased activity of hepatic lipogenic enzymes along with increased cholesterol uptake through increased LDL-receptor expression on hepatocytes and decreased LDL-receptor degradation due to decreased proprotein convertase subtilisin/kexin type 9 expression (Variya *et al*., 2018). Flavonoids from Amla have been shown to be able to effectively lower lipid levels in serum and tissues by significantly inhibiting hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and enhancing degradation and elimination of cholesterol in animal studies (Anila and Vijayalakshmi, 2002).

Table 2. Impact of vitamin C rich fruit supplementation on the lipid profile of morning walkers (Mean ± SE)

Variable		Control group N=8	Guava group N=15	Amla group N=13	Amla + Guava group N=10
FBG (mg/dl)	Baseline	85 ± 10	80 ± 4	86 ± 7	86 ± 3
	Post data	85 ± 8	82 ± 4	86 ± 6	81 ± 3
TC (mg/dl)	Baseline	199 ± 7	217 ± 9	217 ± 6	224 ± 15
	Post data	208 ± 10	218 ± 12	203 ± 4 *	223 ± 19
HDL-C (mg/dl)	Baseline	46 ± 3	43 ± 1	46 ± 2	50 ± 6
	Post data	51 ± 2	44 ± 2	52 ± 2	52 ± 4
LDL-C (mg/dl)	Baseline	117 ± 10	139 ± 8	131 ± 5	141 ± 8
	Post data	112 ± 7	137 ± 8	114 ± 6 **	132 ± 16
VLDL-C (mg/dl)	Baseline	45 ± 11	35 ± 3	36 ± 4	32 ± 3
	Post data	45 ± 11	39 ± 5	36 ± 5	38 ± 6
TG (mg/dl)	Baseline	226 ± 58	175 ± 14	182 ± 18	163 ± 17
	Post data	226 ± 56	195 ± 23	187 ± 23	162 ± 16
Non HDL-C (mg/dl)	Baseline	150 ± 8	174 ± 9	171 ± 6	174 ± 16
	Post data	156 ± 10	173 ± 11	151 ± 5 **	170 ± 16

* Significantly different from baseline value at $p<0.05$
** Significantly different from baseline value at $p<0.01$

Table 3. Impact of vitamin C rich fruit supplementation on the atherogenic indices of morning walkers (Mean ± SE)

Variable		Control group N=8	Guava group N=15	Amla group N=13	Amla + Guava group N=10
TC/HDL-C	Baseline	4.43 ± 0.28	5.15 ± 0.26	4.80 ± 0.20	4.88 ± 0.6
	Post data	4.25 ± 0.36	4.88 ± 0.26	4.02 ± 0.16 **	4.47 ± 0.5
LDL-C/HDL-C	Baseline	2.34 ± 0.27	3.32 ± 0.24	2.92 ± 0.17	3.17 ± 0.52
	Post data	2.29 ± 0.17	3.08 ± 0.15	2.25 ± 0.15 **	2.7 ± 0.46
Non HDL-C/HDL-C	Baseline	3.43 ± 0.28	4.2 ± 0.28	3.80 ± 0.22	3.88 ± 0.59
	Post data	3.25 ± 0.36	3.9 ± 0.25	3.02 ± 0.18 **	3.46 ± 0.49

** Significantly different from baseline value at $p<0.01$

A*mla* is the second richest natural source of vitamin C after Barbados cherry having approximately 600 to 700 mg per fruit (Goraya and Bajwa, 2015). Vitamin C appears to inhibit the activity of HMG-CoA reductase which controls the rate-limiting step in cholesterol biosynthesis (Greene *et al*., 1985) and could lead to a reduction in hepatic cholesterol synthesis. Vitamin C also has been shown to inhibit peroxidative modification of LDL-C which could retard the progression of atherosclerosis and has been found to preserve the ability of LDL-C to be recognized by LDL-C receptors in peripheral blood lymphocytes (Sakuma *et al*., 2001). Supplementation with vitamin C has been found to significantly increase apolipoprotein A-I concentrations and thus could help preserve the reverse cholesterol transport process (Jacques *et al*., 1995).

In the present study, Guava supplementation did not bring about any alteration in the lipid levels. In previous studies that have shown hypolipidemic effects with Guava fruit supplementation, the duration of supplementation was longer than that used in the present study (Singh *et al*., 1992; Singh *et al*., 1993). A study on healthy human subjects who were supplemented with Guava fruit with peel for 6 weeks, reported a significant increase in TC, TG and LDL-C which was attributed to the low concentration of Mg^{++} in the ripe Guava peel which by affecting activities of Mg^{++} dependent lipid metabolism enzymes could increase serum TC levels (Kumari *et al*., 2016). Lipid profile levels remained unaltered in the combination group also. A possible reason could be the low duration for which each fruit was supplemented.

A difference in the content of vitamin C and other antioxidant components between Amla and Guava could be the reason for the favourable impact of Amla and not Guava on the lipid profile. In a study that explored the effect of extraction solvents on phenolic content and antioxidant activities of Amla and Guava, with the best extracting solvent, Amla obtained higher values for total phenolics, ferric reducing antioxidant power values and percent free radical scavenging activity as compared to Guava (Verma *et al*., 2018). In another study that evaluated 20 fruits including Amla and Guava for their antioxidant activity using 1,1-diphenyl-2-picrylhydrozyl radical–scavenging, ferric reducing antioxidant potential and total phenolic content assay, the highest antioxidant activity was observed in Amla which was attributed to its high content of vitamin C and other antioxidant compounds (Ali *et al*., 2011).

Conclusions

Fruits are an important part of a healthy and sustainable diet. Of the two fruits Amla and Guava, which are popularly advocated as rich sources of vitamin C, Amla showed a favourable impact on the lipid profile of the subjects. The impact of Amla supplementation on the lipid profile is noteworthy. The observation that vitamin C rich Amla with its bioactive compounds can act as a hypolipidemic agent thereby reducing the risk of cardiovascular endpoints is encouraging. Thus, along with a lower environmental footprint contribution, consumption of local seasonal vitamin C rich fruit Amla may find use in complementary therapy for the management of dyslipidemia. Long term studies with vitamin C rich local fruits need to be conducted for improving the cardiometabolic profile of the population.

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