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Original Research Paper

Aegle marmelos leaf juice as a complementary therapy to control type 2 diabetes – Randomised controlled trial in Gujarat, India

Vinita Nigam*, Vanisha S. Nambiar

Department of Foods and Nutrition, Faculty of Family and Community Sciences, The MS University of Baroda, Vadodara, Gujarat, India

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ABSTRACT

Objective: To compare the clinical efficacy of *Aegle marmelos* (L.) Correa (AMLC) leaf juice (supplementation (20 g/100 ml) for 60 days among type 2 diabetes mellitus subjects.
Design: Randomised-controlled trial.
Setting: Veraval. Gir-Somnath, Gujarat, India.
Intervention: Confirmed type 2 diabetes mellitus subjects ($n = 60$), were randomly divided to experimental ($n = 30$) AMLC leaf juice supplementation (20 g/100 ml) and control group ($n = 30$) for 8 weeks. Main outcome measures: body-mass-index (BMI), body-fat-percent (BF%), systolic and diastolic blood pressure (BP), fasting blood glucose, glycosylated haemoglobin and post prandial blood glucose (FBG, HbA1c and PPBG), total, high, low and very low density lipoproteins and triglycerides (TC, HDL, LDL-cholesterol and TG), C-reactive protein (CRP), liver enzyme tests serum glutamate oxaloacetate transaminase and, serum glutamate pyruvate transaminase (SGPT and SGOT), kidney function tests (creatinine), total protein (TP, albumin, globulin) and serum ferric reducing antioxidant potential (serum FRAP).
Results: At 4 weeks, significant reduction was recorded in blood pressure SBP – 6.45%; DBP – 4.6%, FBG & HbA1c-20%; PPBG-15%; total cholesterol (TC-8%), LDL-15%), triglycerides (TG-11%), liver functions SGOT-19% and, SGPT-13%, *increase* in serum (FRAP-18%) in the subjects of EG post supplementation compared to baseline. When compared to control group, it reduced BF%, FBG, HbA1c, cholesterol, TG, LDL-cholesterol, CRP and raised HDL-cholesterol as well as improved antioxidant activity.
Conclusion: AMLC leaf juice supplementation (20 g/100 ml) for 60 days showed improvement in all biochemical parameters of type 2 diabetes mellitus, with enhanced efficacy and negligible adverse-effects. This juice can therefore, be supplemented along with oral hypoglycemic drugs to keep the above parameters in control. AMLC leaf being easily available and low cost, can be used as complementary therapy in the management of diabetes possibly due to presence of active components, aegelin 2, scopoletin and sitosterol in the leaf.

1. Introduction

According to the World Health Organisation (WHO), diabetes is currently one of the world's primary health concerns. The global prevalence of diabetes in 2015 was estimated to be 387 million, with this number projected to increase to 552 million by 2030, representing 9.9% of adults [1]. Studies conducted in India in the last decade have highlighted that diabetes is increasing rapidly in the urban population [2]. As far as regional prevalence is concerned, Gujarat tops all other states with the highest number of diabetes and hypertension cases as reported in the *National Health Profile 2015*, with 161,578 diabetic persons which is 20.5 per cent of the total 787,435 population screened [3].

Type 2 diabetes is the most common form of diabetes constituting 90% of the diabetic population. Most of the patients have varying degrees of dual defects, beta cell dysfunction and insulin resistance. Genetic and acquired factors are responsible for this pathology. Sedentarism coupled with unhealthy dietary patterns are the major contributing factors for the higher prevalence of diabetes and hypertension in urban poor population

Abbreviations: A/G Ratio, albumin/globulin ratio; AIP, Atherogenic Index of Plasma; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1C, glycosylated haemoglobin; HDL, high density lipoproteins; hs-CRP, high-sensitivity C-reactive protein; LDL, low density lipoproteins; PPBS, post prandial blood sugar; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; TP, total proteins; VLDL, very low density lipoproteins.

* Corresponding author.
E-mail address: vinitanigam21@gmail.com (V. Nigam).

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[4]. High prevalence, variable pathogenesis, progressive process, and complications of diabetes all highlight the urgent need for effective treatments. Different treatments, such as insulin therapy, pharmacotherapy, and diet therapy, are now available to control or manage diabetes. There are also several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms. These mechanisms include stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing of peripheral absorption of glucose by biguanides and thiazolidinediones. In the past three decades, despite the significant progress made in the treatment of diabetes, the results of treatment in patients remains far from perfect. Currently available treatments have some disadvantages, including drug resistance (reduction of efficiency), side effects, and even toxicity [5]. Diabetes is creating significant economic burden: the total amount needed for India to treat Type 2 diabetes mellitus is estimated to be around 2.2 billion USD. [6]. However, due to unwanted side effects and their high cost, the efficacies of many currently available treatment options are debatable and there is a demand for the development of new treatment options for the treatment of diabetes [7,8].

In India, there are a number of alternative systems of medicine for the treatment of diabetes which use herbal medicines. Some of these alternative systems (e.g. Ayurveda, Siddha, Unani, Homeopathy) are formally recognized by the Indian government. The Ayurvedic system of medicine existed even before the birth of modern medicine and uses plants, herbs and minerals for treatment of diabetes [9]. Many of the botanical products and food components are not simply identifiable as nutrients but they are effective as pharmacological hypoglycaemic agents showing improvement in lipid metabolism, antioxidant status and capillary function in their clinical trials. These include fenugreek seeds (*Trigonella foenum-graecum:* powder 25–50 g twice a day), bitter melon (*Momordica charantia*: 57 g of juice/day or 15 g aqueous extract), spirulina (*Arthrospira* spp: 2 g/day), Soybean (*Glycine max*: 69 g/day), flaxseeds (*Linum usitatissimum*: 40 g ground/day) curry leaves (*Murraya koenigii*), cinnamon (*Cinnamomum cassia*: 1, 3 or 6 g/day), Tulsi (*Ocimum tenuiflorum*: 2 g powder/day), Amla (*Phyllanthus emblica*: 35 g/day), etc. which have been clinically proved [9–12] and Panchratna juice (a juice containing Amla, Ginger, Mint, Tulsi and Turmeric) [13].

A potentially promising treatment for diabetes, which has a long history of use in traditional Indian medicine, is the plant *Aegle marmelos* (L.) Correa (AMLC). AMLC (also known as Bael in Hindi or Billipatra in Gujarati) has been used in India from Vedic or prehistoric times. It has been described in the ancient medical treatise *Charak Samhita* where Charaka describes the plant as Rasayana (health giving), besides its other uses and cures [14]. AMLC has been known for its antihyperglycemic effect [15]. The leaves and the shoot of the plant are also used as green vegetable in Indonesia [16,17].

Several animal studies have indicated a positive role of AMLC leaf extract, 250–500 mg/kg BW given orally to the glucose fed hyperglycaemic rats for a period of one to two months improved their blood sugar levels [18–21]. Miyazaki (2007) reported hypoglycaemic activity after administration of the AMLC extracts at doses of 50, 70, 90 and 100 mg/kg body wt for 14 consecutive days to male and female Wistar rats. He also concluded that AMLC have a high margin of drug safety and there was no short term toxicity [22]. AMLC produces hypoglycaemic effect enhancing the peripheral utilisation of glucose, correcting the impaired hepatic glycolysis and limiting its gluconeogenic formation similar to insulin [19,23]. AMLC has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India [24], Bangladesh [25] and Sri Lanka [26]. Despite these unique properties, a history of safe use (and resultant good safety profile), and the low cost of this potential treatment, there have been no well-designed clinical trials that have tested the safety and effectiveness of AMLC for diabetes in humans. In light of the substantial and increasing burden of diabetes on the health system, and increasing concerns about the cost, safety and compliance of current oral hypoglycaemic therapy, this study was conducted to assess the impact of AMLC leaf juice supplementation on diet, anthropometric, bio-physical profile, blood glucose levels, lipid profile, liver and kidney functions of Type 2 diabetes subjects in a private hospital setup of Veraval, Gir Somnath district in Saurashtra region of Gujarat, India.

2. Materials and methods

Randomised controlled clinical trial was used for the present study.

2.1. Study area for the clinical trial

Veraval city in Gir Somnath district of Gujarat state in India was selected purposively for sampling and clinical trials to study the impact of AMLC leaf juice supplementation on blood sugar level of type 2 diabetic subjects with the hypothesis that *Aegle marmelos* leaves may exhibit high antioxidant capacity and supplementation of the leaves may help to control the blood glucose level and cholesterol level of diabetes type-2 patients (Figs. 1 and 2).

2.2. The primary outcome of the study

Beneficial effect of AM leaf juice supplementation on blood glucose and lipid profile of T2DM subjects.

2.3. Secondary outcome measure

Beneficial effect of AM leaf juice supplementation on liver and kidney functions of T2DM subjects.

2.4. Enrolment of subjects

For this study, based on a minimum sample of 30, required for clinical trial, 65 confirmed Type 2 diabetic subjects from the largest private hospital in Veraval city, dist Gir Somnath, Gujarat, India who gave their written consent were enrolled (having FBS $\leq$ 250 mg/dl; HbA1c $\geq$ 7%) were purposively selected and randomly divided into two groups, Experimental (N = 30) and Control group (N = 30). Out of 65 Subjects, 5 dropped out (1 from E Gp and 4 from C Gp), thus for the study, data of 60 Type 2 diabetic subjects was included.

2.5. Baseline information

2.5.1. Socio-economic data

General information was collected regarding age, education, family information, income level, occupation, lifestyle pattern and medical and family history using semi-structured questionnaire.

2.6. Dietary intake – 24 h dietary recall

A 24-h recall method (24-HDRM) was used to assess the dietary intake of the subjects. Information on dietary intake was taken by recall of diet of the previous day (24 h) with details of ingredients and amounts using standard cups and spoons. All data were entered in MS excel and average daily calorie intake and nutrient intake was calculated using Diet cal software and daily % RDA met were calculated for each subject.

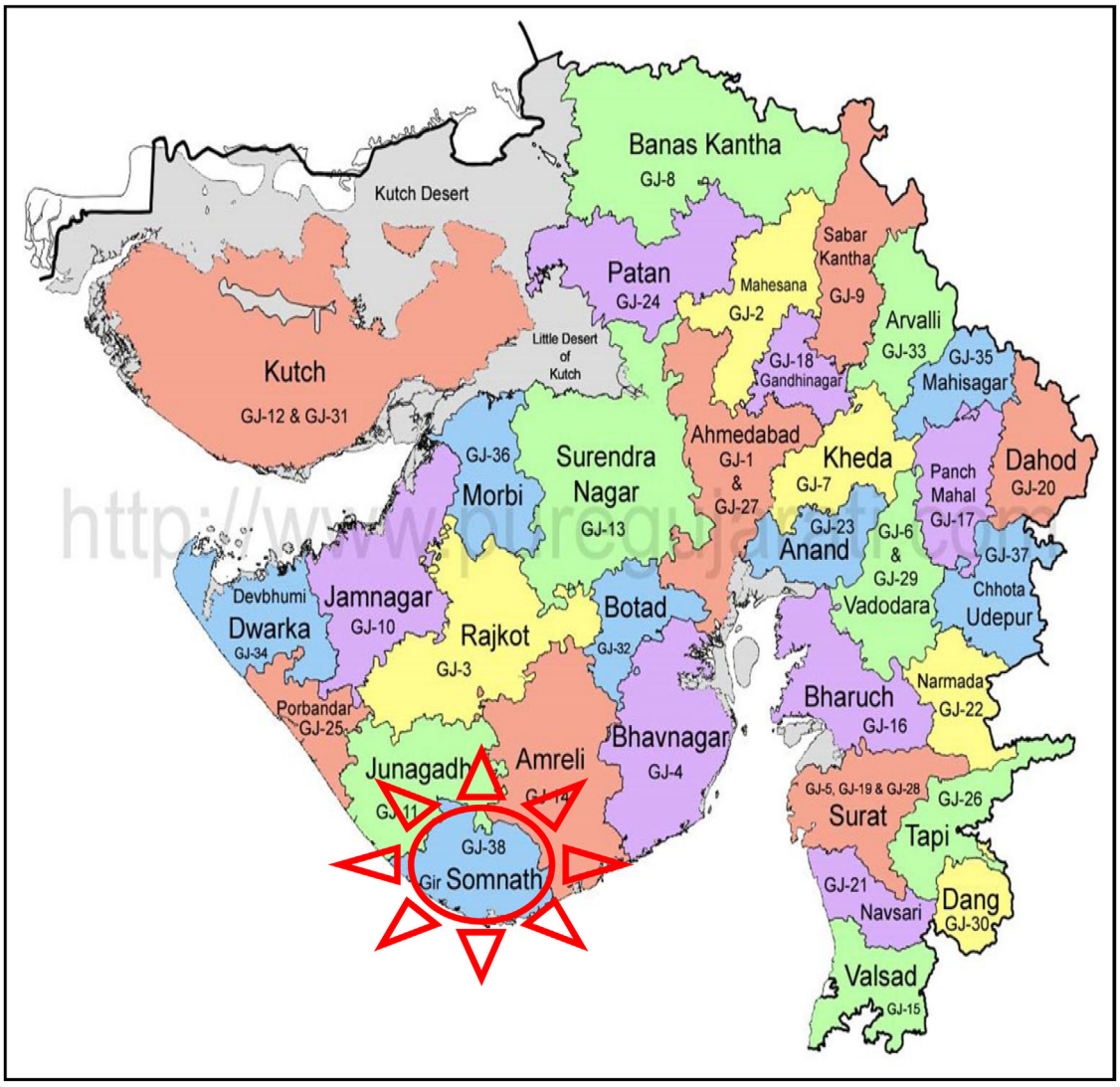

Fig. 1. Map indicating location of Gir Somnath District in Gujarat.

Fig. 2. Clinical trial-study venue: Spiritual Centre Of Veraval City, Bhalka, Dist Gir Somnath, Gujarat, India.

2.7. Anthropometric, clinical and biochemical measurements

Anthropometric measurements (weight, height and BMI) were taken using standard methods. Fasting blood sample was collected for bio-chemical investigation after 10 hours overnight fast. Cut-off normal values for individual lipid levels were taken as per the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults [27]. The term dyslipidemia is used when two or more individual lipid levels were abnormal. All diabetic patients were categorised as obese or non-obese using body mass index (BMI) criteria of $\geq$23 kg/m^2 proposed for South Asian population [28] and as hypertensive if their blood pressure is $\geq$140/90 mmHg [29]. ADA criteria for diabetes (HbA1C $\geq$ 7% or fasting blood glucose > 125 mg/dl (6.94 mmol/l) and postprandial blood glucose $\geq$ 140 mg/dl (7.77 mmol/l) were used [30]. All the observations were tabulated and results were expressed as percentage and mean SD (standard deviation).

Biochemical analysis included the following parameters such as glycemic profile (FBG, HbA1c, PPBG), lipid profile (TC, TG, LDL, HDL, VLDL), Serum antioxidant status (serum FRAP), Hs-CRP (Anti-Inflammatory Status), SGOT, SGPT (Liver function), serum creatinine and total protein (kidney function). After an overnight fast of 13 h, fasting venous blood samples (5 ml) were drawn by trained technician using disposable syringes and needles and blood was dispensed into respective vaccutainers. Serum was then separated for further biochemical analysis. The bio-chemical analysis was done using enzymatic kits which was out-sourced to largest laboratory of Veraval. Fasting blood glucose estimations were done by the enzymatic kit supplied by Glaxo India Limited by GOD/POD method. The triglycerides in the serum were estimated by the GPO/POD method using the enzymatic kit. Total cholesterol was estimated using diagnostic kit supplied by Glaxo India Ltd., and serum HDL-C was also estimated. VLDL was calculated by dividing triglyceride values by five (TG/5). The LDL was calculated by dividing the difference LDL-C = TC − (HDL-C + VLDL). Serum creatinine, total proteins, Glycosylated haemoglobin (HbA1c), highly sensitive-C reactive protein (HsCRP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT). Total antioxidant capacity of the subjects was measured using Ferric Reducing Antioxidant Potential (FRAP) assay (Benzie and Strain, 1996). This method is based on the reduction of a ferric tripyridal triazine (TPTZ) complex to its ferrous, coloured form in the presence of antioxidant. This test was carried out at Central Marine Fisheries Research Institute CFMRI, Veraval, Dist Gir Somnath under the guidance of senior scientist.

2.8. Supplementation of Aegle marmelos (L.) Correa leaf juice (AMLC Juice)

Fresh leaves were plucked from the identified zone of Gir Somnath district (21°04′49.7″ N 70°35′10.6″ E) in the evening, cleaned and kept for the preparation of juice to be supplemented to the Experimental group (E Grp) next morning. For this group (n = 30), 3000 ml (100 ml/subject) fresh AMLC juice was prepared by the research team under the supervision of investigator daily using 600 g AMLC leaves (20 g/18–20 leaves/per subject) using a kitchen mixer grinder, strained using strainer with single fine mesh, 7-Inch diameter. The control group (C Grp) was not given any intervention. During study period of two months (60days), none of the subjects took other complementary or alternative medicine.

2.9. Administration of juice

AMLC juice (100 ml volume using measuring cup) was served to the subjects empty stomach in disposable glasses in the morning daily between 7:45 am and 8:15 am in a large spiritual centre located in Bhalka area on the main road of Veraval city as this place was centrally located.

2.10. Compliance

The AMLC juice was given to all subjects in the presence of the investigator every day. It was a close monitored supervised trial. In case of absence of a subject, an extension was made in the number of days, so that the total number of juice supplementation amounted to 60 days for all subjects (n = 30) for the Experimental group. For biochemical analysis blood sample was drawn by the technician of the respective laboratory and other data were collected by team members in presence of investigator.

2.11. Impact analysis (post data)

Post data were collected after 60 days for alterations if any on the anthropometric measures, biophysical parameters, dietary information and bio-chemical parameters with respect to same parameters as pre data. Biochemical analysis (Pre and Post analysis) was performed twice by private local laboratory in Veraval city. Thus, all the parameters were analysed twice (pre-measurement-before supplementation and post-measurement – at the end of supplementation).

2.12. Ethical approval

The study was approved by Ethics Committee of the department.

2.13. Data management and statistical analysis

The data was entered in an excel spreadsheet and checked for its validity and then subjected to appropriate statistical analysis. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS 17.0 version, SPSS Inc., Chicago, IL, USA). Descriptive statistics was used: mean comparison, standard deviation, percentages, paired t-test was performed to compare the mean values between pre and post data of the same group. The significance levels were set at $p < 0.05$.

3. Results

3.1. Socio-economic background of the subjects

Out of 60 diabetic patients enrolled in the study, 25 were male and 35 were female patients. The mean age of study population was 51.3 ± 9.13 years (male: 50.8 ± 11.14 years and female: 51.7 ± 7.52 years). The mean age of the females (n = 35) was 51.7years, with a range of 25–69 years with a median value of 52 years indicating that these women were mainly in their post menopausal age, wherein, they are at a greater risk for CVDs and do tend to have an abnormal lipid profile (Table 1).

Ninety four percent subjects were Hindus, 4% Muslims (females) and 1% (female) was Christian. Eighty eight percent male and 83% females subjects were married. Of all the subjects, 56.6% lived in joint families. 45.41% females and 8% males were illiterate. 51.6% females subjects were house wives. Ten percent of the subjects were retired. The average family size of all the subjects was found to be 5–6 members. Majority of the subjects (81.67%) were having average monthly family income between 3000 and 25,000 Rupees (41.91-349.28 USD; 32.69-27.42 GBP) . Sixty six percent subjects had per capita income less than 5000 Rupees (69.87 USD; 54.32 GBP). The mean duration from the first diagnosis of diabetes for the study patients was 6.6 ± 5.31 years. In the first

Table 1
Baseline information of type 2 diabetic subjects.

Variable	Total sample *N* (%)					
	Male *N* (%)		Female *N* (%)		Total *N* (%)	
Frequency, (%)	25 (41.67)		35 (58.33)		60 (100)	
Age (Mean ± SD) (years)	50.8 ± 11.14		51.74 ± 7.52		51.35 ± 9.13	
	N	(%)	*N*	(%)	*N*	(%)
Marital status						
Single	2	3.33	2	3.33	4	6.6667
Married	22	88	29	82.85	51	85
Separated/divorcee)	1	1.67	4	6.67	5	8.34
Religion						
Hindu	25	41.67	31	51.67	56	93.34
Muslim	0	0	3	5	3	5
Christian	0	0	1	1.67	1	1.67
Educational qualification						
Illiterate	2	3.33	16	26.67	18	30
Primary education	9	15	9	15	18	30
Secondary	6	10	6	10	12	20
Higher secondary	3	5	0	0	3	5
Graduate	1	1.67	3	5	4	6.67
Post graduate	3	5	1	1.67	4	6.67
Technical degree	1	1.67	0	0	1	1.67
Occupation						
Service	9	15	2	3.33	11	18.34
Home maker	0	0	31	51.67	31	51.67
Retired	5	8.33	1	1.67	6	10
Business	10	16.67	1	1.67	11	18.34
Unemployed	1	1.67	0	0	1	1.66
Family type						
Single	1	1.67	2	3.33	3	5
Nuclear	8	13.33	15	25	23	38.33
Joint/extended	16	26.67	18	30	34	56.66
Total family income (Rupees)						
3000–25,000	20	80	29	82.85	49	81.67
26,001–48,000	1	4	5	14.28	6	10
>48,001	4	16	1	2.85	5	8.34

five years, 53.34% were diagnosed as diabetics. Around, 30% were diabetics since 6–10 years and about 16.67% of patients had diabetes since >10 years (Table 1).

3.2. Impact on dietary intake (24 h dietary recall method)

The 24 h dietary recall of the E Gp and C Gp diabetic subjects is given in Table 2. Dietary information obtained from 24 h dietary recall of pre and post supplementation diet of Experimental subjects revealed that *macronutrients such as* the mean energy, protein, fat, carbohydrate and fibre intake was less compared to the pre-diet intake. However there was no significant statistical difference ($p > 0.05$) in their pre and post supplementation (Table 2). As far as micronutrients is concerned, mean mineral intake such as iron, calcium, zinc, copper, phosphorus etc. was less compared to the pre-diet intake. Mean Vitamin intake for vitamin A, C and B-carotene also showed no statistical difference ($p > 0.05$) between the pre and post diet intake (Table 2).

In C Gp there was significant difference ($p < 0.05$) in the mean intake of macronutrients such as energy, proteins, fat and carbohydrate of the male and female subjects post supplementation of 2 months. However there was no statistical difference ($p > 0.05$) in the mean vitamin and mineral content post supplementation.

3.3. Impact of AMLC leaf juice supplementation on the anthropometric profile of E Gp And C Gp

The mean basal mass index (BMI) of the subjects decreased from 26.57 kg/m^2 to 25.66 kg/m^2 post supplementation. There was significant decrease in weight ($p < 0.05$) and BMI ($p < 0.05$) of the subjects in E Gp. Whereas no such trend was recorded in C Gp (Table 3). There was improvement in nutritional status of E Gp. Post supplementation, obese subjects decreased from 20% to 16% whereas normal subjects increased from 13% before supplementation to 20% after 2 months in E Gp (Table 4).

3.4. Impact of AMLC leaf juice supplementation on bio-physical profile of the E Gp and C Gp

3.4.1. Impact of juice on body fat

A significant decrease was recorded in the body fat percent among females ($p < 0.01$) and ($p < 0.001$) total subjects in the E Gp whereas no such alteration was recorded in C Gp (Table 5).

3.4.2. Impact of juice on blood-pressure (BP)

Overall AMLC leaf juice supplementation was effective in altering the BP measurements. Around 5–6% significant decrease was seen in SBP in both the groups (Pre-post Expt./C Gp SBP values: 138.8 ± 19.1 mmHg – 129.8 ± 19.0 mmHg/134.3 ± 20.2–127.1 ± 18.0 mmHg).

There was significant ($p < 0.05$) drop (5.3%) in DBP values in E Gp post supplementation for all the subjects (Pre-post DBP mean ± SD values: 86.6 ± 10.0 mmHg/82.0 ± 11.6 mmHg). However in C Gp, there was no significant difference in DBP values in the total subjects (pre-post DBP values – 82.4 ± 9.3 mmHg/81.5 ± 10.1 mmHg) (Table 6).

3.5. Impact of AMLC leaf juice supplementation on the glycemic and lipemic profile of the type 2 diabetic subjects

AMLC leaf juice supplementation caused highly significant decrease in mean FBG, HbA1c and PPBG values in males, females and total subjects of E Gp ($p < 0.0001$) in comparison to C Gp post supplementation.

The impact of the AMLC leaf juice supplementation on HbA1c revealed high significant reduction for males, females and total group ($p < 0.0001$) in E Gp post supplementation. It was also observed that percent fall in the initial HbA1c and FBG was more pronounced in the female subjects (Table 7). But the percent fall in the initial PPBG was more pronounced in the male subjects in comparison to female subjects. These results indicate the beneficial role of AMLC leaf juice supplementation in E Gp.

Hypertriglyceridemia is a common finding in T2DM subjects and is responsible for vascular complications. In present study, AMLC leaf juice supplementation for two months to E Gp observed significant decrease in serum cholesterol in all the subjects ($p < 0.01$) and females ($p < 0.001$); TG levels in all the subjects ($p < 0.01$) and in females ($p < 0.05$); mean serum LDL values in all the subjects ($p < 0.01$); significant drop in VLDL in total subjects ($p < 0.05$) in E Gp.

In C Gp, there was significant rise in serum TC levels in total group ($p < 0.0001$) and males and females ($p < 0.01$); TG levels in all the subjects ($p < 0.000$), females ($p < 0.001$) and males ($p < 0.01$); serum LDL values ($p < 0.05$) in total subjects; VLDL levels in total subjects ($p < 0.0001$) in C Gp.

3.5.1. Atherogenic Index of Plasma (AIP)

The Atherogenic Index of Plasma (AIP) defined as log (TG/HDL-C), has been proposed as marker of plasma atherogenicity because it is increased in people at a high risk for coronary heart disease and is inversely correlated with LDL-C particle size. Mean ± SD AIP values for Experiment and C Gp were 0.56 ± 0.13 and 0.55 ± 0.15. Almost all the subjects fell in to the high risk category (>0.21). Thus a high prevalence of dyslipidemia was observed among the subjects and the subjects were at greater risk for CHD as indicated

Table 2
Mean nutrient intake of experimental group and control group.

Variable	Experiment group			Control group		
	Male	Female	Total	Male	Female	Total
Energy (k cal)						
Pre diet	1803 ± 216	1760 ± 269	1781 ± 242	1806 ± 298	1872 ± 452	1881 ± 401
Post diet	1545 ± 408	1766 ± 218	1655 ± 313	2065 ± 342	2055 ± 243	2040 ± 500
T value (*p* value)	2.75(0.02)*	0.94(0.92)NS	1.58(0.12)NS	2.73(0.01*)	2.99(0.01*)	1.98(0.05)*
Protein (g)						
Pre diet	55.78 ± 12.48	54.47 ± 9.40	54.95 ± 10.44	57.56 ± 13.76	60.28 ± 14.27	58.96 ± 13.86
Post diet	50.84 ± 12.75	56.30 ± 9.03	52.72 ± 8.47	65.52 ± 12.28	69.12 ± 11.88	67.44 ± 11.98
T value (*p* value)	1.14(0.28)NS	0.29(0.77)NS	1.05(0.30)NS	0.69(0.4)	2.99(0.01*)	4.47(0.01)*
Fats (g)						
Pre diet	66.11 ± 15.91	63.39 ± 12.20	64.39 ± 13.47	67.83 ± 9.71	74.6 ± 6.97	71.44 ± 8.90
Post diet	53.86 ± 10.60	65.49 ± 13.07	61.23 ± 13.31	73.33 ± 11.88	78.5 ± 7.97	76.09 ± 11.04
T value (*p* value)	2.22(0.05)*	0.55(0.6)NS	0.98(0.33)NS	2.94(0.01*)	2.92(0.01*)	2.17(0.01)*
Carbohydrates (g)						
Pre diet	236.9 ± 58.87	226.95 ± 46.83	230.60 ± 50.80	204.8 ± 41.1	200.1 ± 46.25	208.1 ± 47.59
Post diet	201.6 ± 80.99	218.46 ± 41.45	212.13±	245.2 ± 32.05	263.9 ± 50.03	262.0 ± 53.21
T value (*p* value)	1.80(0.10)NS	0.88(0.39)NS	1.92(0.06)NS	5.04(0.02**)	2.78(0.01*)	3.52(0.002)**
Iron (mg)						
Pre diet	14.05 ± 5.06	14.69 ±	14.38 ± 3.29	16.47 ± 4.10	17.23 ± 2.47	16.87 ± 3.29
Post diet	15.06 ± 5.50	15.56 ± 6.17	15.31 ± 5.50	17.21 ± 2.82	17.77 ± 3.36	17.51 ± 3.08
T value (*p* value)	1.78(0.20)NS	1.23(0.45)NS	1.28(0.20)NS	1.02(0.32)NS	0.70(0.48)NS	1.21(0.23)NS
Calcium (mg)						
Pre diet	853.51 ± 202.8	955.34 ± 276.61	904.42 ± 295	731.1 ± 91.06	743.8 ± 113	737.9 ± 86.34
Post diet	873.5 ± 134.1	956.29 ± 276.59	914.89 ± 272.15	749.3 ± 79.78	780.2 ± 176.2	765.8 ± 99.06
T value (*p* value)	0.54(0.77)NS	0.81(0.42)NS	1.93(0.06)NS	5.04(0.02**)	2.11(0.058)NS	1.93(0.06)NS
Phosphorus (mg)						
Pre diet	1470 ± 301	1488.46 ± 354.70	1479.23 ± 327.5	1536 ± 420.8	1538.46 ± 417.6	1537 ± 411.8
Post diet	1381.6 ± 370	1495.14 ± 350.42	1430.37 ± 360.21	1610 ± 436.9	1610 ± 379.4	1610 ± 400.1
T value (*p* value)	1.35(0.20)NS	0.82(0.22)NS	0.94(0.69)NS	0.61(0.54)NS	0.69(0.49)NS	0.94(0.35)NS
Zinc (mg)						
Pre diet	8.07 ± 2.31	8.88 ± 2.54	8.47 ± 2.42	9.81 ± 2.48	8.88 ± 3.07	9.33 ± 2.80
Post diet	7.84 ± 2.08	8.84 ± 2.54	8.34 ± 2.31	9.16 ± 2.68	9.51 ± 2.75	9.33 ± 2.80
T value (*p* value)	1.33(0.21)NS	0.50(0.31)NS	0.03(0.97)NS	0.87(0.39)NS	0.48(0.72)NS	0.03(0.97)NS
Copper (mg)						
Pre Diet	2.14 ± 0.58	2.25 ± 0.63	2.19 ± 0.60	2.50 ± 0.73	2.27 ± 0.70	2.38 ± 0.71
Post diet	1.92 ± 0.48	2.25 ± 0.63	2.08 ± 0.55	2.53 ± 0.84	2.43 ± 0.87	2.48 ± 0.84
T value (*p* value)	1.89(0.22)NS	1.06(0.28)NS	0.31(0.95)NS	0.09(0.92)NS	0.54(0.61)NS	0.55(0.58)NS
Chronium (mg)						
Pre diet	0.06 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.09 ± 0.02	0.06 ± 0.02	0.08 ± 0.03
Post diet	0.05 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.07 ± 0.02
T value (*p* value)	0.74(0.34)NS	1.10(0.0)NS	0.27(0.78)NS	0.48(0.63)NS	0.92(0.09)NS	0.80(0.24)NS
Vitamin A (μg)						
Pre diet	101.56 ± 38.32	99.50 ± 55.36	100.53 ± 46.84	158 ± 58.5	133.5 ± 97.52	144.9 ± 27.8
Post diet	80.17 ± 37.33	112.05 ± 45.65	96.11 ± 41.49	168.4 ± 88.9	118.56 ± 76.96	140.0 ± 26.3
T value (*p* value)	1.66(0.13)NS	0.78(0.44)NS	0.59(0.53)NS	0.08(0.93)NS	1.50(0.15)NS	0.53(0.59)NS
Vitamin C (mg)						
Pre diet	62.07 ± 23.58	60.36 ± 24.53	61.21 ± 24.05	50.8 ± 17.88	49.36 ± 18.71	1530 ± 400.8
Post diet	56.03 ± 23.42	59.21 ± 23.5	57.62 ± 23.21	52.35 ± 11.59	61.08 ± 20.52	1757 ± 415.3
T value (*p* value)	1.02(0.16)NS	0.02(0.09)	1.60(0.11)NS	0.30(0.76)NS	1.74(0.10)NS	0.39(0.69)NS
B Carotene (μg)						
Pre diet	1549.55 ± 400.8	1459.97 ± 1165.16	1504.76 ± 412.08	1407.55 ± 391.6	1637 ± 379.4	1530 ± 400.8
Post diet	1434.80 ± 415.3	1470.16 ± 426.02	1452.48 ± 420.65	1616 ± 420.8	1796 ± 391.3	1757 ± 415.3
T value (p value)	1.20(0.33)NS	0.81(0.42)NS	0.39(0.69)NS	0.58(0.57)NS	0.10(0.91)NS	0.39(0.69)NS
Total dietary fiber (g)						
Pre diet	14.14 ± 2.84	15.50 ± 5.90	14.82 ± 4.35	18.03 ± 3.39	17.79 ± 2.76	16.90 ± 3.02
Post diet	12.46 ± 4.96	15.85 ± 8.90	14.15 ± 6.93	19.34 ± 3.24	19.34 ± 3.24	17.68 ± 2.90
T value (*p* value)	0.92(0.37)NS		1.12(0.26)NS	2.11(0.058)NS	2.11(0.058)NS	2.12(0.06)

Data are mean ± SD unless otherwise indicated.
*significant difference at $p<0.05$; **Significant difference at $p<0.01$.

Table 3
Results of the anthropometric profile of experimental and control group.

	Experimental group			Control group		
	Male	Female	Total	Male	Female	Total
Weight (kg)						
Basal	67.89 ± 8.01	67.43 ± 9.89	67.60 ± 9.11	70.63 ± 16.14	68.94 ± 13.09	69.73 ± 14.35
Final	67.09 ± 7.75	66.72 ± 10.44	66.85 ± 9.41	70.29 ± 15.51	69.47 ± 12.64	69.85 ± 13.81
'*t*' value	1.92 NS	1.72 NS	2.49*	1.02 NS	1.47 NS	0.37 NS
Body mass index (BMI)						
Basal	24.39 ± 2.63	28.76 ± 4.79	26.57 ± 4.61	25.29 ± 5.19	28.95 ± 5.35	27.12 ± 5.51
Final	24.10 ± 2.56	27.73 ± 4.97	25.66 ± 4.71	25.19 ± 5.07	29.18 ± 5.15	27.32 ± 5.41
'*t*' value	2.17	1.81	2.57*	0.90	1.51	0.30

Data are mean ± SD unless otherwise indicated BMI body mass index.
*significant difference at $p<0.05$

Table 4
Nutritional status of experimental and control group.

(*N* = 30)	Experiment group (*N*, %)		Control group (*N*, %)	
BMI (kg/m^2)/nutritional grade	Pre data	Post data	Pre data	Post data
Underweight < 18.5	–	–	1 (3.33)	1 (3.33)
Normal 18.5–22.9	4 (13.33)	6 (20)	5 (16.67)	5 (16.67)
Overweight 23–24.9	5 (16.67)	6 (20)	5 (16.67)	5 (16.67)
Pre-obese 25–29.9	15 (50)	13 (43.3)	9 (20)	10 (3.33)
Obese ≥ 30	6 (20)	5 (16.67)	10 (3.33)	9 (20)

(WHO, 2010).

Table 5
Body fat composition of experiment and control group.

	Experimental group			Control group		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
Basal	22.84 ± 4.66	39.58 ± 9.71	31.21 ± 11.55	24.67 ± 7.67	39.65 ± 8.81	32.16 ± 11.87
Final	21.50 ± 4.91	38.43 ± 9.16	29.96 ± 11.37	24.09 ± 8.25	39.30 ± 8.40	31.69 ± 11.97
'*t*' value	1.69	3.06**	3.48**	1.26	0.37	0.20

* Significant difference at $p < 0.05$.
** Significant difference at $p < 0.01$.

Table 6
Impact Of AMLC leaf juice supplementation on mean blood pressure of experimental and control group (Mean ± SD).

	Experimental group		Control group	
BP (mmHg)	Basal	Final	Basal	Final
Male (*N* = 11)			**Male (*N* = 14)**	
Male SBP	139.23 ± 18.11	136.21 ± 21.99	127.3 ± 18.76	121.9 ± 18.04
T value	0.64		2.07	
DBP	88.65 ± 8.81	85.52 ± 13.65	79.23 ± 8.30	79.5 ± 9.21
T value	0.69		1.60	
Female (*N* = 19)			**Female (*N* = 16)**	
Female SBP	138.39 ± 20.24	123.50 ± 16.20	138.9 ± 21.38	132.9 ± 16.21
T value	4.48***		1.28	
DBP	84.62 ± 11.20	79.56 ± 9.68	84.3 ± 6.57	84.3 ± 11.5
T value	1.90		0.22	
Total (*N* = 30)			**Total (*N* = 30)**	
Total SBP	138.81 ± 19.17	129.85 ± 19.09	133.13 ± 20.96	127.10 ± 18.02
T value	2.68*		2.36*	
DBP	86.63 ± 10.00	82.54 ± 11.66	81.76 ± 9.32	81.91 ± 10.11
	2.01*		0.63	

* Significantly different at $p < 0.05$.
*** Highly significant at $p < 0.001$.SBP, systolic blood; DBP, diastolic blood pressure.

by baseline AIP levels. After juice supplementation, AIP decreased significantly among the total subjects ($p < 0.01$) (0.56 ± 0.13; 0.49 ± 0.13), and moderately among male subjects ($p < 0.05$) (0.59 ± 0.11; 0.48 ± 0.13) in the E Gp. There was increase in AIP in the C Gp subjects ($p < 0.01$) (Table 8).

3.6. Impact of AMLC leaf juice supplementation on other bio-chemical parameters of E Gp and C Gp

AMLC leaf juice supplementation caused significant decrease in mean SGPT values ($p < 0.001$) (25.02 ± 7.8; 21.79 ± 5.7 IU/L) and mean SGOT values ($p < 0.01$) of all the subjects (29.03 ± 9.9; 23.32 ± 8.6 IU/L) in the E Gp after supplementation of 2 months (Table 9). However in C Gp there was significant rise in SGPT values ($p < 0.0001$) and SGOT values ($p < 0.0001$) in total subjects. Overall there was 13% fall in SGPT and 19% fall in SGOT in E Gp and 15% increase in these parameters in C Gp. As can be seen from Table 9, there was no alteration in serum creatinine values ($p > 0.05$) in E Gp post intervention. However there was significant rise in mean serum creatinine values in males ($p < 0.01$) of C Gp. [Pre values 1.34 ± 0.37 mg/dl (0.074 mmol/l); Post values 1.47 ± 0.36 mg/dl (0.081 mmol/l)]. These values were found to be in normal range of 0.7–1.4 mg/dl (0.03–0.07 mmol/l). There was no significant

Table 7
Impact of AMLC leaf juice on the aip of experimental and control group.

	Experimental group			Control group		
	Male	Female	Total	Male	Female	Total
Atherogenic Index of Plasma (AIP)						
Basal	0.59 ± 0.11	0.55 ± 0.14	0.56 ± 0.13	0.54 ± 0.15	0.56 ± 0.15	0.55 ± 0.15
Final	0.48 ± 0.13	0.49 ± 0.13	0.49 ± 0.13	0.56 ± 0.16	0.62 ± 0.14	0.59 ± 0.15
'*p*' value	0.005**	0.06	0.003**	0.01*	0.01*	0.002**

* Significantly different at $p < 0.05$.
** Significantly different at $p < 0.01$.

difference in serum HsCRP values in E Gp. However, all the subjects who were at risk (pre mean serum HsCRP values >0.3 mg/dl or 0.01 mmol/l) came in the normal range (post mean serum HsCRP values <3 mg/dl or 0.16 mmol/l) after supplementation. In C Gp only males showed significant increase in HsCRP values after two months ($p < 0.05$) [Pre values: 3.23 ± 2.11 mg/dl (0.179 mmol/l); Post values: 3.70 ± 2.21 mg/dl) (0.20 mmol/l)] (Table 8). There was no significant alteration in the total protein values and albumin levels in E Gp post supplementation ($p > 0.05$) but significant drop in the total globulin and A/G ratio levels in males of E Gp ($p < 0.05$). In C Gp there was significant increase in total protein among males and females ($p < 0.05$), and total group ($p < 0.0001$). Same trend was seen in total albumin levels in males ($p < 0.05$), females and total group ($p < 0.001$) in C Gp. There was no alteration in Globulin and A/G ratio in C Gp. But all the subjects were in the normal range of 6.0–8.3 g/dl. There was significant rise (17.7%) in mean serum FRAP values ($p < 0.0001$) and also in males and females (Table 8). However there was significant decrease ($p < 0.0001$) in serum FRAP values (15%) in all the subjects of C Gp ($p < 0.0001$), males ($p < 0.01$) and females ($p < 0.05$).

Overall AMLC leaf juice supplementation resulted in remarkable reduction in the following biochemical parameters as seen in Table 8.

FBG 20% (Expt. group: Mean pre/post FBS values – [174.7 ± 41 mg/dl (9.70 mmol/l)/140.1 ± 46.3 mg/dl (7.78 mmol/l); Cont. group (15% increase): 168.3 ± 37.3 mg/dl/(9.35 mmol/l) 193.7 ± 41.5], **HbA1c 20%** [Expt. group: Mean pre/post HbA1c values – 9.8 ± 1.2/7.9 ± 1.1%; Cont. group (7% increase): 8.5 ± 1.1/ 9.1 ± 1.1%], **PPBG 15%** [Expt. group: Mean pre/post PPBG values (mg/dl) – 212.40 ± 40.92 (11.8 mmol/l)/179.63 ± 49.98 (9.98 mmol/l); Cont. group (8.8% increase): 222.59 ± 36.98 mg/dl (12.36 mmol/l)/242.3 ± 36.67 mg/dl (12.36 mmol/l)] **LDL 15%** [Expt. group: Mean pre/post LDL values(mg/dl) – 140.7 ± 41.9 mg/dl (7.81 mmol/l)/119.7 ± 34.7 mg/dl (6.65 mmol/ l); Cont. group (7.5% increase): 120.6 ± 32.3 mg/dl (6.7 mmol/l)/ 129.7 ± 28.3 mg/dl (7.20 mmol/l)], **TG 10.9%** [Expt. group: Mean pre/post TG values – 152.8 ± 44.5 mg/dl (8.49 mmol/l)/ 137.4 ± 42.2 mg/dl (7.64 mmol/l); Cont. group (9.6% increase): 144.9 ± 4.8 (8.05 mmol/l)/158.9 ± 41.1(8.82 mmol/l)], **VLDL 12.6%** [Expt. group: Mean pre/post VLDL values – 31.0 ± 10.0 mg/dl (1.72 mmol/l)/27.2 ± 8.3(1.51 mmol/l); Cont. group (11.2% increase): 28.4 ± 8.1 (1.58 mmol/l)/31.6 ± 8.6 (1.75 mmol/l)], **Serum FRAP 17.7% rise** [Expt. group: Mean pre/post serum FRAP values (μmolTE/L) – 1.6 ± 0.3/1.9 ± 0.3; Cont. group (15% drop): 168.3 ± 37.3/193.7 ± 41.5], **SGPT 13%** [Expt. group: Mean pre/post SGPT values – 25.3 ± 7.8 IU/L/21.7 ± 5.7 IU/L; Cont. group (15% increase): 25.9 ± 8.9 IU/L/29.8 ± 8.5 IU/L], **SGOT 19%** Expt. group: Mean pre/post SGOT values – 29.0 ± 9.9 IU/L/23.3 ± 8.6 IU/L; Cont. group (14.4% increase): 26.3 ± 7.9 IU/L/30.1 ± 9.8 IU/L].

Maximum difference was noted in Glycemic profile i.e. FBG, HbA1c, PPBG and serum antioxidant (Serum FRAP) values, lipid profile like TG, LDL and VLDL values and liver enzymes (SGPT and SGOT) while minimum difference was recorded in serum Creatinine and HsCRP values. There was no difference in total proteins values post supplementation.

4. Discussion

In the present study 20 g fresh AMLC (100 ml leaf juice) was supplemented to 30 confirmed type 2 diabetic subjects for 2 months and significant difference was recorded in blood pressure (reduction: SBP – 6.45%; DBP – 4.6%), glycemic profile (15–20%), lipid profile (8–15%), liver functions (13–19%), increase in antioxidant activity (18%) in the subjects of E Gp post supplementation. AMLC leaf juice supplementation has played a positive impact in reducing blood sugar levels, lipid profile and liver functions in Type 2 diabetic subjects

Until now, no studies have reported the anti-diabetic properties of fresh AMLC leaf juice. However some studies have reported the hypoglycemic and hypolipidemic effects of dry AMLC powder on human subjects (Table 9). However, the impact of the leaves on liver and kidney functions along with anti-inflammatory status, total proteins and antioxidant status of the subjects was not assessed in these studies.

The reduction in blood glucose level observed in our study may be due to presence of active component, aegelin 2 and scopoletin in leaf [31–33]. AMLC leaves produce a hypoglycemic effect possibly by enhancing the peripheral utilisation of glucose, correcting the impaired hepatic glycolysis and limiting its gluconeogenic formation similar to insulin [34].

Reduction of serum lipids observed in our study may be due to the decreased fat mobilisation from the peripheral depots as well as their synthesis. AMLC leaf extract activates hydrolysis of triglycerides and decreases circulatory level of blood cholesterol by decreasing fat mobilisation from the peripheral adipose tissues [31]. The reduction in total cholesterol could be due to beta-sitosterol present in AMLC leaf which is structurally similar to cholesterol helps in reducing serum concentration of cholesterol by reducing the absorption of cholesterol from the gut by competing for the limited space for cholesterol in mixed micelles [31]. A lower level of circulatory fatty acid may favour the restoration of normal functioning of Na+/K +-ATPase path, an essential gateway for proper burning of glucose at cellular level in diabetic animals [35]. Another mechanism for reduction in lipid level proposed that Aegline 2, active component present in AMLC leaf regulates lipid level [32]. The antioxidative phytochemicals such as flavonoids, alkaloids, sterols, tannins, phlobatannins, flavonoid glycosides present in the leaf extract possess this free radical scavenging activity.

4.1. Possible mechanism of action

Various possible mechanism of action for AMLC in diabetes treatment based on animal models studies are mentioned here. AMLC increases utilisation of glucose, either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion [36]. It decreases oxidative stress which indirectly simulates

Table 8
Impact of AMLC leaf juice supplementation on the bio-chemical parameters of experimental and control group (Mean ± SD).

	Experimental group					Control group			
	Male	Female	Total	Normal range	% Decrease (total subjects)	Male	Female	Total	% Increase (total subjects)
Fasting blood glucose (mg/dl)									
Basal	177.70 ± 37.46	172.99 ± 43.82	174.72 ± 50.06	70-110 mg/dl	↓ 20	171.11 ± 43.98	165.75 ± 31.73	168.25 ± 37.25	↑ 15.1
Final	134.18 ± 40.81	143.48 ± 50.06	140.07 ± 46.37			201.20 ± 49.65	187.01 ± 33.04	193.66 ± 41.50	
'*t*' value	7.06***	5.37***	7.14***			7.40***	3.00**	5.45***	
Post prandial blood glucose (mg/dl)									
Basal	221.19 ± 39.17	207.32 ± 42.08	212.40 ± 40.92	120-140 mg/dl	↓ 15.5	221.11 ± 42.55	223.00 ± 31.84		↑ 8.85
Final	186.98 ± 54.60	175.37 ± 48.12	179.63 ± 49.98			240.53 ± 44.54	243.29 ± 28.46	242.3 ± 36.67	
'*t*' value	4.11**	5.10***	6.64***			12.73***	7.80***	11.54***	
Glycosylated haemoglobin (HbA1c) (%)									
Basal	9.80 ± 1.33	9.93 ± 1.24	9.88 ± 1.25	<7%	↓ 20	8.57 ± 1.38	8.51 ± 0.96	8.54 ± 1.15	↑ 7.02
Final	7.93 ± 1.03	7.97 ± 1.17	7.96 ± 1.10			9.21 ± 1.23	9.08 ± 1.03	9.14 ± 1.11	
'*t*' value	6.06***	8.90***	10.92***			6.28***	3.90**	5.90***	
Total cholesterol (mg/dl)									
Basal	203.8 ± 63.84	212.9 ± 38.49	209.64 ± 48.43	<200 mg/dl	↓ 8.4	190.54 ± 36.10	191.79 ± 35.87	191.21 ± 35.36	↑ 6.85
Final	194.0 ± 46.21	190.86 ± 44.30	192.03 ± 44.24			199.47 ± 33.97	208.54 ± 29.71	204.31 ± 31.54	
'*t*' value	1.07	3.96***	3.58**			3.10**	3.78**	4.73***	
Serum triglycerides (mg/dl)									
Basal	154.86 ± 47.84	151.64 ± 43.90	152.80 ± 44.59	<150 mg/dl	10.9	136.47 ± 40.46	152.27 ± 41.09	144.90 ± 40.88	9.64
Final	142.00 ± 49.19	134.61 ± 38.87	137.40 ± 42.27			150.45 ± 42.98	166.23 ± 39.40	158.87 ± 41.17	
'*t*' value	1.47	2.19*	2.67*			2.96*	4.5***	5.17***	
Serum HDL (mg/dl)									
Basal	38.35 ± 4.62	41.03 ± 7.41	40.05 ± 6.57	>50 mg/dl	↑ 6.7	38.41 ± 6.11	40.49 ± 4.27	39.52 ± 5.22	↑ 0.07
Final	43.50 ± 6.04	42.28 ± 5.79	42.73 ± 5.81			39.82 ± 5.58	39.32 ± 6.38	39.55 ± 5.92	
'*t*' value	3.59**	0.58	1.80			1.37	0.59	0.02	
Serum LDL (mg/dl)									
Basal	134.50 ± 52.65	144.20 ± 35.43	140.7 ± 41.93	<130 mg/dl	↓ 15	121.41 ± 26.81	119.8 ± 37.42	120.6 ± 32.36	↑ 7.54
Final	118.50 ± 32.56	120.4 ± 36.79	119.7 ± 34.74			126.10 ± 29.18	132.8 ± 28.09	129.7 ± 28.31	
'*t*' value	1.61	4.54***	4.29***			1.60	2.18*	2.61*	
Serum VLDL (mg/dl)									
Basal	30.97 ± 9.56	30.97 ± 9.57	31.01 ± 10.0	<30 mg/dl	12.6	26.43 ± 7.76	30.21 ± 8.36	28.45 ± 8.17	11.28
Final	27.45 ± 9.74	27.45 ± 9.75	27.23 ± 8.30			29.29 ± 8.39	33.73 ± 8.56	31.66 ± 8.63	
'*t*' value	1.49	2.05	2.58*			3.05**	4.00**	5.08***	
Serum FRAP (Mmol TE/L)									
Basal	1.75 ± 0.44	1.59 ± 0.37	1.65 ± 0.40	0.5-2.0 mmol/l	↑1 7.7	2.12 ± 0.70	2.24 ± 0.97	2.18 ± 0.84	↓ 15.59
Final	2.04 ± 0.39	1.87 ± 0.39	1.93 ± 0.39			1.66 ± 0.36	2.00 ± 0.90	1.84 ± 0.71	
'*t*' value	3.49**	2.96**	4.27***			3.68**	2.74*	4.50***	
SGPT (IU/ml)									
Basal	21.97 ± 7.99	26.80 ± 7.34	25.02 ± 7.81	0-48 U/L	↓ 13	26.81 ± 10.09	25.19 ± 8.04	25.94 ± 8.93	↑ 15.03
Final	19.48 ± 4.11	23.13 ± 6.23	21.79 ± 5.75			31.05 ± 9.73	28.78 ± 7.43	29.84 ± 8.50	
'*t*' value	1.24	2.47*	2.75*			4.48***	4.17***	6.20***	
SGOT (IU/L)									
Basal	29.81 ± 11.59	28.58 ± 9.23	29.03 ± 9.98	0-42 U/L	↓ 19	25.93 ± 8.48	26.77 ± 7.78	26.38 ± 7.98	14.25
Final	22.35 ± 7.41	23.89 ± 9.44	23.32 ± 8.65			29.09 ± 10.72	31.07 ± 9.29	30.14 ± 9.86	
'*t*' value	1.89	2.29*	2.97**			2.04	3.24**	3.77***	
Serum creatinine (mg/dl)									
Basal	1.19 ± 0.32	1.11 ± 0.22	1.14 ± 0.26	0.7–1.4 mg/dl	2.6	1.34 ± 0.37	1.22 ± 0.44	1.27 ± 0.40	10.23
Final	1.19 ± 0.27	1.07 ± 0.13	1.11 ± 0.20			1.47 ± 0.36	1.34 ± 0.47	1.40 ± 0.42	
'*t*' value	0.048	0.79	0.75			3.93**	0.84	1.60	
HsCRP (mg/dl)									
Basal	3.14 ± 1.35	3.28 ± 1.18	3.23 ± 1.23	<3 mg/L	↓ 1.3	3.23 ± 2.11	4.40 ± 4.73	3.85 ± 3.73	↓ 3.63
Final	2.70 ± 1.11	2.87 ± 0.93	2.80 ± 0.98			3.70 ± 2.21	3.71 ± 1.31	3.71 ± 1.76	
'*t*' value	1.40	1.07	1.60			2.75*	0.53	0.21	
Total Protein (g/dl)									
Basal	6.34 ± 0.49	6.16 ± 0.47	6.23 ± 0.48	6.0–8.3 g/dl	↓ 0.6	6.45 ± 0.49	6.34 ± 0.65	6.39 ± 0.58	↑ 6.57
Final	6.27 ± 0.46	6.27 ± 0.57	6.27 ± 0.53			6.79 ± 0.48	6.82 ± 0.62	6.81 ± 0.55	
'*t*' value	0.58	1.27	0.60			2.79*	5.03***	5.45***	

Data are mean ± SD unless otherwise indicated, FBS, fasting blood sugar, HbA1C, haemoglobin A1C, hs-CRP, high-sensitivity C-reactive protein.
*Significantly different at $p<0.05$; **significantly different at $p<0.01$; ***highly significant at $p<0.001$.

Table 9
Studies (Human Clinical Trials) proving efficacy of various functional foods used in the management of type 2 diabetes.

Investigators	Functional food	Dose/duration	Results
Iyer et al. (2008)	Garden Cress Seeds	3 g/d (28 days) on 41 NIDDM subjects	↓ HbA1c-4.7%;TG-71%;TC,LDL,HDL-NS
Khan et al. (2003)	Cinnamon	1,3, 6 g/day (40 days)-short term on 60 NIDDM subjects	↓ FBS-18–29%
Ziegenfguss et al. (2006)	Cinnamon	500 mg/d (90 days) long term	↓ 83% subjects showed FBS-8%
Srivastava et al. (1993)	Momordica charantia	Dried powder and aq. extract of 5 g/d (1–3 times a day for 21 days	↓ extract-Av bl sugar-54% ↓ dry powder Av bl sugar-25%; HbA1c-27%
Iyer and Mani (1989)	Curry leaves	1 g/d for 1 month on 30 NIDDM	↓ Transient decrease in glycemic and other parameters
Rai et al. (1997)	Ocimum Sanctum	1 g/day for 1 month on 27 NIDDM	↓ FBS-21%, HbA1c- 11%,TC-11%,LDL-14%, VLDL-16%, TG-16%
Iyer and Desai (2008)	150 ml Panchatantra drink (ingr: Amla, tulsi, mint, cumin & turmeric)	150 ml/d for 45 days on 25 T2DM	↓ FBS-7%, HbA1c- 3%
Joshi and Iyer (2008)	Amla	35 g/d for 60 days	↓ Gly. profile-no change, TC-5.8%, LDL- 9.4%, non-HDL-8.3% HDL-5.5%
Venugopal and Iyer (2010)	Barley grass powder	1.2 g/d(capsules) for 60 days on 23 NIDDM subjects	↑ FBS-10.8%, HbA1c- ↓5.2%
Venugopal and Iyer (2010)	Kodari seeds	40 g/d for 28 days on 30 T2DM subjects	↓ HbA1c-1.2%, FBS-no change
Venugopal and Chug (2015)	Insulin plant leaf powder	1 g/d (4 capsules) for 45 days on 27 T2DM subjects	↓ FBS-13.8%, HbA1c-5.13%, PPBS-12.3%, ↑TG-16.2%, HDL-9.8%
Human studies on *Aegle marmelos (L.) Correa* leaves			
Yaheya and Ismail (2009)	5 g dry *Aegle marmelos* (L.) *Correa* leaf powder along with OHD	10 subjects for 30 days	↓ PPBS-31% (201 mg/dl-137 mg/dl) **Hypoglycemic effect**
Singh and Kochhar (2012)	2 g dry *Aegle marmelos* (L.) *Correa* leaf powder along with OHD	30 NIDDM subjects for 60 days	↑ FBS-9.8%, PPBS-5.6%, TC-4.5%, TG-6.2%, LDL-8.1%; ↑HDL-8.9% **Hypoglycemic effect**
Present study	100 ml Fresh juice containing 20 g fresh *Aegle marmelos* (L.) *Correa* leaves along with OHD	30 type II subjects for 60 days	↓ FBS-20%, HbA1c-20%,PPBS-15.5%, TC-8.4%, TG-10.9%, LDL-15%, VLDL-12.6%, SGPT-13%, SGOT-19%; ↑HDL-6.7%, Serum antioxidant value(Serum FRAP)-17.7% **Hypoglycemic effec**

glycation of proteins and inactivation of enzymes [37]. AMLC stimulates the β cells to increase insulin secretion. It increases the receptor responsiveness of the insulin receptors [23]. AMLC may also contain some biomolecules that may sensitise the insulin receptor to insulin or stimulates the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolising enzymes towards the re-establishment of normal blood glucose level [38]. It modulates the activity of enzymic and non-enzymic antioxidants and enhances the defence against ROS-generated damage in diabetic rats [39]. It works like an insulin sensitiser which can be used in the treatment of diabetes. It improves the glycemic control by enhancing the insulin sensitivity in liver and muscle [40]. AMLC leaves produce hypoglycemic effect probably by enhancing. the peripheral utilisation of glucose, correcting the impaired hepatic glycolysis and limiting its gluconeogenic formation similar to insulin [19,24,41].

4.2. Cost of available commercial formulations of Aegle marmelos *(L.) Correa leaves versus cost of fresh leaf juice*

AMLC is one of the 10 most highly traded medicinal plants in India and it has huge demand in foreign markets [42]. However this leaf powder available commercially costs around 200 Rupees (USD 2.80; GBP 2.18) per 100 g as shown in Figs. 3 and 4 (2000 Rupees

Fig. 3. AMLC Leaf Powder Cost Rs. 204/100 g Naturmed.

Fig. 4. AMLC Leaf Powder Cost Rs. 195/100 g Nature Herbal Products.

[28 USD; 21.80 GBP] per kg). In comparison to our fresh leaf juice which costs only around 40–80 Rupees per kg.

The low cost (and potenial cost-effectiveness)of fresh AMLC leaf juice can be very important factor particularly for low and middle income group and tribal belts of India which do not have an access to conventional medicine or more expensive herbal supplements available commercially in India and abroad.

4.3. Diabetes a public health problem and Aegle marmelos *(L.) Correa juice*

Diabetes mellitus is a growing public health problem in both developed and developing countries. According to the report of World Health Organisation, 346 million people have diabetes worldwide [43]. It is also estimated that in 2004, about 3.4 million patients died from diabetes-related complications. Without urgent action, this number is likely to double by 2030.

Conventional treatment comprises of oral hypoglycemic drugs like including insulin sensitisers (biguanides, thiazolidinediones), insulin secretagogues (sulfonylureas, meglitinides), α-glucosidase inhibitors, incretin agonists and dipeptidyl peptidase-4 inhibitors [44]. These are costly, linked with unpleasant side effects and many a times inaccessible in remote areas.

This study focused on AMLC herb, the proposed hypoglycemic actions of which (uncovered in animal studies) appear to have been supported by this clinical trial. The intervention has numerous beneifts regarding accessibility: AMLC juice can be prepared at home; it can easily manage the leaves from local sources like Shiva temples, local market and adjoining gardens; it can be easily grown in patient homes as it a plant of arid horticulture and can be grown in virtually any soil. AMLC is a cheap domestic technology which may help diabetic individuals control their blood sugar. The form used in this study can avoid complex and laborious preparations such as extract formation or decoction, and can be taught to those with low health literacy. These advantages suggest AMLC leaf juice may be a practically useful adjunct to combat diabetes, a growing public health problem in Indian and worldwide.

5. Conclusions

In this study, it can be concluded that supplementation of 20 g AMLC leaf juice had beneficial impact on blood sugar iii values and lipid profile along with liver functions significantly improved the nutritional status of the diabetic patients. This effect can be attributed to the synergistic effect of good nutrient and phyto-chemicals profile of AMLC leaves. AMLC may be supplemented along with oral hypoglycemic drugs to keep the above parameters in control. Further trials are needed to confirm the potential role of AMLC in diabetes control and management.

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