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Original Research Article

Effect of curry leaves and cucumber fruit on lipid profile in menopausal women with hyperlipidaemia: a randomized controlled pilot study

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ABSTRACT

Background: Hyperlipidemia increases the risk of developing cardiovascular diseases (CVD). This study was aimed to determine the effect of curry leaves powder and slicing cucumber fruit on hyperlipidemia in the menopausal women of rural communities.
Methods: Thirty menopausal healthy women of rural communities (45–65 years of age) with mild hyperlipidemia were divided and randomly assigned into control group, subjects without any treatment and 2 experimental groups, one group treated with cucumber (100-125 g) and other group treated with curry leaves powder (5 g), once daily for 45 consecutive days. The demographic variables were collected using questionnaire. Fasting blood samples were collected before and after the intervention and determined total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein- cholesterol (HDL-C) and triacyl glycerol (TAG).
Results: TC, LDL-C and TAG were significantly ($p < 0.05$) reduced in the curry leaves powder and cucumber treated groups. HDL-C level (46.1±9.2 mg/dl) was significantly ($p < 0.05$) elevated (average 12%) only in the curry leaves powder treated group. Though the mean values of TC, LDL-C and TAG were elevated in the control group, the elevation was statistically non-significant. Comparing the efficacy of the test substances at the tested doses, HDL-C level in the curry leaves treated group showed statistically significant elevation ($p = 0.09$).
Conclusions: Curry leaves and cucumber were effective in improving HDL-C and lowering of LDL-C and TAG levels in menopausal women with hyperlipidemia, suggest the potential neutraceutical role in treating CVD.

Keywords: Hyperlipidemia, Curry leaves, Sliced cucumber, Cardiovascular diseases, Low-density lipoprotein, Coronary heart diseases

INTRODUCTION

Cardiovascular diseases (CVD), mainly coronary heart disease (CHD) are one the largest single leading causes of morbidity and mortality all over the world.[1] Hyperlipidaemia refers to the increased levels of lipids including total cholesterol (TC) and triacyl glycerol (TAG) in blood. Although hyperlipidaemia does not cause symptoms, raised TC and TAG can significantly increase the risk of developing CHD. An adult with level more than 240 mg/dl was considered to carry twice the cardiovascular risk than those individuals at the desired level (<200 mg/dl).[2] Elevated low density lipoprotein cholesterol (LDL-C) and TAG were considered detrimental of cardiovascular outcomes.[3,4] Studies from India had shown high prevalence of CHD, approximately

11% in urban and 7% in the rural populations from the southern rural area of Kerala, India.[5] In women, CHD remains a greater diagnostic challenge than men and the HDL-C and TAG levels were considered as the appropriate predictor of risk.[6,7] HDL levels <50 mg/dl found to be strongly associated with cardiovascular mortality in women.[6] In women, LDL-C and TC levels increase after the age of 45 years and peak between 55 and 65 years of age further enhances the risk.[7] Even at TC levels <200 mg/dl, low HDL levels were found to be associated with increased myocardial infarction rates in both men and women.[9] This emphasizes the need for other therapies, especially targeting to elevate the HDL-C.

Despite the statin therapy, approximately 70% of cad was found to occur.[10] Medicinal plants or their bioactive compounds have been utilized by developing countries for primary and traditional healthcare system since ancient time. Curry leaf, *Murraya koenigii* (l.) Spreng. Belongs to *Rutaceae* family and is one of the most commonly used flavoring agent in India. Antimicrobial, antioxidant, anticancer and renal protective effects of *M. Koenigii* had been reported.[11-15] leaves of *M. Koenigii* significantly decreased both tc as well as tag in high fatty diet fed rats.[16] Further, Gamphire et al demonstrated the hypolipidemic effect of curry leaf powder in normal and alloxan induced diabetic rats.[17] *Cucumis sativus* l. Of *Cucurbitaceae* family is commonly known as cucumber and is used traditionally for headaches, diuretic and as a nutritive agent. The fruit of this plant has antioxidant, lipid lowering, antidiabetic and analgesic activities.[18-21] However, the efficacy on lipid profile in clinical trials is not yet been reported. Hence, this study was aimed to determine the effect of curry leave and cucumber on hyperlipidemia among the menopausal women in rural communities.

METHODS

Preparation of curry leaves powder and cucumber slices

Fresh curry leaves collected from own field (without using any pesticides) and washed thoroughly using water and dried under sunshade and fried on gas stove without adding oil. The fried dried leaves were powered under clean condition using kitchen grinder and stored in several food grade plastic container as small samples. Curry leaves powder was tested for heavy metals, pesticides and for other toxins.

Fresh slicing cucumber's fruits were collected from organic farm without using any pesticides, and washed thoroughly in running water. Cut both ends using sharp knife and rub the ends to remove sticky fluid like oozing substances in order to lessen the bitter taste at either ends. After removing the skin, small slices of approximately 100 -125 gram/slice was prepared.

Participants

A total of 50 menopausal women of rural communities (age of 45–65 years) with history of hyperlipidaemia were evaluated for the eligibility. They were asked to complete a questionnaire regarding base line variables and medical history. Women having hyperlipidaemia but suffering from auto immune diseases, liver and renal impairment, thyroid disorders, underwent surgical intervention in GI diseases and irritable bowel syndrome or those who were not willing to participate in study were excluded. After the initial screening, fasting (overnight fasting of 8-10 hours) blood samples were collected for the determination of lipid profile. Thirty healthy women with mild hyperlipidaemia such as elevation of TC of average of 200-230 mg/dl with LDL-C >130 mg/dl or HDL-C <50 mg/dl, who are not undergoing any cholesterol lowering drug treatment were selected for the study. The purpose and procedure of the study were explained to each subject and written consent was taken from them. The study design was according to the national guidelines prescribed for conducting trial in human and also complied with the Declaration of Helsinki. The study was approved by the Ethics Committee for research in human, Saveetha University, Chennai, India (Approval no: 06/06/2015/IEC/SU).

Trial design

The subjects were allocated to control and experimental groups of 10 each, by simple random technique. Group without any treatment was considered as control group; one experimental group was administered with cucumber and the other group with curry leaves powder. The subjects had been instructed to consume 5 g of curry leaves powder adding in the main side dish of lunch, while the participants in other experimental group were instructed to have the cucumber slices, 100-125 g before lunch. The test substances were consumed once daily for 45 consecutive days. The subjects were instructed to maintain the regular life style and exercise. The use of alcohol and other lipid metabolism affecting drugs were prohibited. The demographic variables such as age, education, occupation, or duration of hyper-cholesterolemia were collected using questionnaire. A telephone call was performed at every day to remind and visited the subjects every week to comply with consumption as well as to enquire any adverse event from the consumption. Fasting blood samples were collected after the intervention and determined the lipid profile. All the blood samples were given unique coding in order to blind the analyst about the subject group or type of sample. TC, HDL-C and TAG in pre- and post-intervention serum samples were determined in fully automated clinical chemistry analyzer in a diagnostic lab which had adequate quality assurance programme, while LDL-C was calculated using the Friedewald's equation.[22]

Statistical analyses

Paired t-test was used to determine the significant difference between the mean values of lipid profile parameters in the pre- and post-interventional samples using SPSS software (SPSS- version 16, IBM, Chicago, USA). Comparison of the effect of curry leave with cucumber in the post treated lipid profile result was tested using the independent t-test. Significant association between any of the socio-demographic variables was done using Chi-Square test. P <0.05 was considered as significant.

RESULTS

The experiment was conducted in the rural community during the period of August, 2015- December, 2015. Flow chart for the study design according to the consolidated standards of reporting trials (CONSORT) was given in Figure 1. The nutrient content of fresh and dehydrated curry leaves as well as that of raw fruit of cucumber is depicted in Table 1 and 2, respectively. Among the 30 healthy subjects enrolled in the study, 29 subjects completed the study. One subject 1/30 (3.3%) had allergies and hence excluded from the study. None of the socio-demographic variables were found to be significantly different.

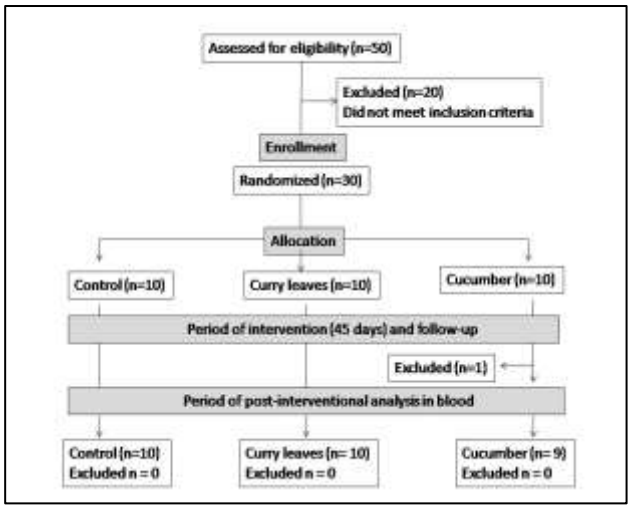

Figure 1: Flowchart of study according to consolidated standards of reporting trials (consort) guidelines.

Among the participants, 11/29 (37.9%) women were between 60-65 years and 7/29 (23.3%) were below 50 years. The socio-economic status revealed that most of the subjects (43.3%) had secondary education, 10% were higher education and only 2/29 was studied up to primary class. All the subjects were non vegetarians and 13/29 (44 %) were taking non vegetarian 2-3 times a week. Most of the women 24/29 (82%) were home makers and 33.3% were in very low income group. Body mass index of the subjects showed that 4/29 (17%) women had obesity. Only 9/29 (31%) had diabetes mellitus but were well controlled. While 33.3 % were doing regular exercise of 30 min duration, 66.6% were doing exercise at least 5 days in a week. Majority 13/29 (44%) of women had onset of mild hyperlipidaemia since 1-2 years and 11/29 (37%) had above 5 years.

The mean values for TC in the pre-and post-tests were 229.6 mg/dl and 197.6 mg/dl, respectively in the curry leaves powder consumed group. The difference was statistically highly significant (paired t=3.7; p <0.05). Similarly, the mean difference for HDL-C, LDL-C and TAG were statistically significant as given in Table 3.

Table 1: Comparative nutrient content of fresh and dehydrated curry leaves.

Nutrients	Value of fresh	Value of dehydrated
	Curry leaves (100g)	Curry leaves (100 g)
Protein	6.0 g	12.0 g
Fat	1.0 g	5.4 g
Carbohydrate	18.7g	64.3 g
Calcium	2040 mg	830.0 mg
Iron	0.93 mg	12.0 mg
Beta-carotene	7560µg	5292µg

Table 2: Nutritive value/100 g of raw Cucumber.

Constituent	Quantity	RDA (%)
Energy	15 Kcal	<1
Carbohydrates	3.63 g	3
Protein	0.65 g	1
Total Fat	0.11 g	0.5
Cholesterol	0 mg	0
Dietary Fibre	0.5 g	1

Table 3: Effect of curry leaves and cucumber on the lipid profile values.

Groups	Treatment	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TAG (mg/dl)
Curry leaves (5 g)	Pre	229.6 ± 30.9	40.5 ± 7.4	161.2 ± 23.2	138.3 ± 34.3
	Post	197.6 ± 30.0**	46.1 ± 9.2*	134.9 ± 21.9*	97.8 ± 22.3**
Control	Pre	207.4 ± 33.4	48.7 ± 7.0	143.2 ± 29.2	125.0± 42.1
	Post	222.1 ± 27.5*	46.0 ±7.8^{NS}	148.7 ± 25.8^{NS}	130.2 ± 53.3^{NS}
Cucumber (100-125 g)	Pre	202.2 ± 31.9	39.4 ± 6.8	139.1 ± 22.8	132.6± 38.4
	Post	188.3 ± 32.3*	39.4 ± 6.8^{NS}	127.2 ± 23.2*	106.2± 27.7**

Values are mean ± SD, n= 10 in control and curry leaves; n= 9 in cucumber ; * p<0.05, ** p<0.01, ^{NS} p>0.05 (paired t test).

Table 4: Comparison of the effect of curry leaves with cucumber on the post intervention lipid profile values.

Tested agents (g)	Lipid parameters	N	Mean ± SD	Independent t	P value
Curry leaves (5)	TC	10	197.6±29.6	0.65	0.52
Cucumber (100-125)		9	188.3±32.3		
Curry leaves (5)	HDL-C	10	46.1±9.2	1.76	0.09
Cucumber (100-125)		9	39.4±6.8		
Curry leaves (5)	LDL-C	10	134.9±21.9	0.74	0.47
Cucumber (100-125)		9	127.2±23.2		
Curry leaves (5)	TAG	10	97.8±22.8	0.73	0.47
Cucumber (100-125)		9	106.2±27.7		

Values are expressed as mean ± SD.

The mean values for TC in the pre- and post-tests were 202.2 mg/dl and 188.3 mg/dl, respectively in the cucumber administered group as in Table 4. The difference was statistically significant (paired t=2.38; p <0.05). Similarly, the mean difference for HDL-C, LDL-C and TAG were statistically significant. Though the mean values of TC, LDL-C and TAG were increased and that of HDL-C was decreased in the control group. However, no statistically significant difference was evidenced.

Comparison of the lipid lowering efficacy of both test substances on the difference in the mean values for TC, LDL-C and TAG in the post samples was not statistically significant (p >0.05) as shown in Table 4. But, the HDL-C elevation was statistically significant. The mean LDL-C/HDL-C ratio, before and after the intervention was given in the Figure 2.

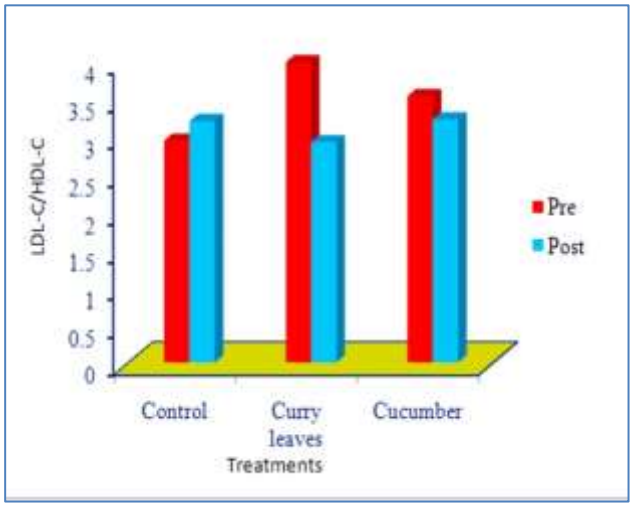

Figure 2: The mean LDL-C/HDL-C ratio, before and after the intervention.

Group without any treatment was considered as control group; Among the two experimental groups, one group was administered with curry leaves powder (5 g) added in the main side dish of lunch and other group was taken cucumber slices (100-125 g), before the lunch. The test substances were consumed once daily for 45 consecutive days, and analyzed the blood sample for lipid profile.

After the period 45 days, 1.09 fold increases in the ratio was found in the control groups, whereas in the intervention groups the ratio was decreased. The fold decreases were 1.33 and 1.09 in the curry leaves and cucumber treated groups, respectively.

DISCUSSION

The results of this study revealed that the consumption either curry leaves powder or cucumber slice was effective in improving the HDL-C and thus lowering the TC. Both the interventions were equally effective. This was evidenced from comparing the lipid profile parameters before and after the intervention. The mean values of TC, LDL-C and TAG were lowered while that of HDL-C was elevated in both experimental groups. Since increase in the reverse cholesterol transport will contribute an influx of excess peripheral cholesterol to liver in order to excrete in bile, an elevated HDL-C level could support the efficacy of the tested compounds. In this study, both interventions were effective to favor the reverse cholesterol transport and hence could lower the TC. Elevated HDL-C level was found to be protector of CHD.[23] It was found that for every 1 mg/dl increase in HDL-C, the risk of CHD in women decreased by 3%.[24]

Further, LDL-C/HDL-C ratio was considered as a more clinically relevant measurement of CHD risk.[25] The cut-off values of LDL-C/HDL-C ratio for initiating the primary prevention of CHD as described by Millan et al was <3.0 in women or low as 2.5 suggested by Chen et al in Chinese adults.[26,27] The ratio was decreased in both the interventions when compared to the control group. Though there are various recommendations for the normal ratio, it was lowest among the curry leaves group, supported the efficacy.

The TAG level was also effectively reduced by the intervention. A long-standing association between elevated TAG levels and CVD were reported.[28] Hence, the TAG lowering effect further contributes to the protective role of the tested substance against CVD. While studying the effect of selected socio-demographic variables such as age, education, occupation, or duration of hypercholesterolemia of the menopausal women on the consumption of curry leaves and cucumber, we found no

significant association between any of such variables. All subjects included in the study were doing exercise with almost equal total duration/week hence eliminated the role of the effect of exercise over the intervention. Further, the simple randomization of the subjects did not show any interference in the studied lipid profile parameters. No adverse effect could find in the study except a subject was removed from the study due to allergy of unknown etiology.

The exact mechanism of the exhibited activity could not be explained in this study. The phytochemical constituents of *M. koenigii*, had been reported for various pharmacological activities. Some of them have potential role as important neutraceutical for diabetes and cardio-protection. A wide range of phytochemicals such as carbazole alkaloids, essential oil, carotenoids-lutein, phenolics, terpenoids, α-tocopherol, minerals, fibers, nicotinic acid, vitamin C etc. were reported in curry leaves.[29] Many of these constituents were proved to be effective in lowering the lipid level. The antioxidant activities of vegetables and leaves have gained an importance in preventing dreaded disease caused by oxidants. The antioxidants present in the curry leaves might be involved in the elevation of HDL-C and thus could reduce the LDL-C and TC. The antioxidant activity can prevent the oxidation of LDL which was considered as the early event in the atheroma formation and hence offer protection from the CHD. Gajaria et al demonstrated the protective effect of curry leaves against the *in-vitro* LDL-C oxidation and oxidized LDL-C-induced apoptosis in macrophages due to the antioxidant potentials of flavonoids.[30] Birari et al reported the potent antilipase activity of curry leaves which might be involved in the decreased digestion and thus the absorption of lipids from intestine.[31] Decreased absorption of cholesterol found to increase the LDL receptor on cell surface to uptake the LDL which will lead to decrease blood cholesterol.[32]

The phytochemical screening of cucumber fruit was reported to contain flavonoids such as quercetin, apigenin, kaempferol, luteolin, lignans, triterpenes, vitamins like biotin, vitamin B1, vitamin K, and pantothenic acid; and minerals like copper, magnesium, potassium, manganese, and phosphorus, sterols, saponins and tannins.[18] The fruit also contains proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases.[21] Phytosterols are effective in lowering TC and LDL-C by inhibiting the absorption of cholesterol from small intestine. Therefore, the constituents present in the cucumber might have involved in the exhibited effect. The amount of test substances used in this study was well tolerated by the participants and the amount was very easy to achieve through the daily food.

National Cholesterol Education Program guidelines emphasized the lifestyle modifications such as moderate exercise, smoking cessation, and monounsaturated fat in the diet as first-line therapy for the elevation of HDL-C.[33]

There are substantial evidences about the use of herbal based agents to lower the risk of number of chronic diseases including hyperlipidemia. Several previous studies had demonstrated the use of substances like garlic, artichoke leaf, plant stanolols, psyllium, soluble fibre, orange juice, and red yeast rice in the modest reduction of serum lipids.[34-36] However, efficacy and safety data from controlled trials using these herbal supplements are limited.[37] This is the first study that conducted in menopausal healthy women with hyperlipidaemia in rural communities to suggest the possible therapeutic potential of curry leaves and cucumber in lowering the lipid. The major limitations of the study were small number of subjects enrolled in the group as it was a pilot study. Furthermore, the short intervention period of 45 days used to find the changes in lipid profile.

CONCLUSION

The results of this study concluded that consumption curry leaves and cucumber by the menopausal women with mild hyperlipidemia daily for 45 days as a part of lunch was effective in improving HDL-C and lowering TC, LDL-C and TAG. The potent hypolipidemic effects of this short term pilot study however further warrant a long-term study in various communities.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee for research in human, Saveetha University, Chennai, India (Approval no: 06/06/2015/IEC/SU).

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