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CURRY LEAF POWDER AND CUCUMBER SLICES ATTENUATE HYPERLIPIDEMIA IN POST MENOPAUSAL WOMEN: A RANDOMIZED CONTROL SINGLE BLINDED STUDY

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ABSTRACT: Background: Woman's risk for developing heart disease rises after menopause may possibly correlated with the striking increase in cholesterol levels. Objective: A randomized controlled cluster group single blind study was conducted by treating with dried curry leaf powder and cucumber slices in hyperlipidemic post menopausal women in rural area. **Methods:** Menopausal healthy women of rural communities (n =40; 45–65 years of age) with hyperlipidemia who were on with or without hypolipidemic drug were treated with dried curry leaf powder (5 g) adding in main side dish during lunch. Similarly another forty who were on with or without hypolipidemic drug were given100-125g cucumber slices before lunch for 45 consecutive days. A group of forty women with or without hypolipidemic drug was kept as the control. The demographic variables were collected using questionnaire. Fasting blood samples were collected before and after the intervention and lipid profile. **Results:** Among the total 120 subjects recruited, most of them (46.66%) had total cholesterol (TC) 200-239 mg/dl and 26.66% had high cholesterol. The low density cholesterol (LDL–C) (60%) was 100-150 or borderline high and 36.66% had above 150 mg/dl. When the pre test and post test values were compared, the cucumber, and curry leaf were significantly (<0.001) effective to lower the TC level. Both curry leaf and cucumber slices lowered hyperlipidemia in post menopausal women with drug and without drug. **Conclusion**: Curry leaf and cucumber were effective in improving HDL-C and lowering of TC, LDL-C and triglyceride levels in post menopausal women with hyperlipidemia.

INTRODUCTION: Woman's risk for developing heart disease rises after menopause. As they approach the menopause, many women show a very striking increase in total cholesterol (TC) levels, which in turn increase the risk of cardiovascular diseases (CVD). Hyperlipidemia is highly prevalent in rural India which progresses with age.

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Estrogen may play a major role, both directly and indirectly, and cause lower the Low Density Lipoproteins (LDL) levels by up regulation of B100 E receptors.

The reduced estrogen levels in post menopausal women cause a relatively high concentration of testosterone which in turn increase the LDL levels and lower the High Density Lipoproteins (HDL) levels. Estrogen naturally protects women from heart diseases by promoting a favorable plasma lipid profile and healthy circulation [1]. Prevalence of hyperlipidemia in menopause is a known feature in women, which may lead to significant increase in the development of coronary artery disease (CAD).

Every year, CVD claims the lives of females more than males. More than 450,000 women succumb to heart disease annually, and 250,000 die of CAD. Cardiovascular risk is poorly managed in women, especially during the menopausal transition when susceptibility to cardiovascular events increases [2].

Murraya koennigii, is commonly known as curry leaf or curry patta in Indian dialects [3]. Curry leaves belongs to the family of Rutaceae. *M. koennigii* is a highly valued plant for its aroma and medicinal value. It contains a number of chemical constituents that interact to elicit their pharmacodynamic response. It has also have anti-oxidative, and cholesterol reducing activities [4]. It grows throughout the Indian subcontinent and has wide culinary effect and is one of the main components of formulations in the traditional Ayurvedic system. [3] To support the burden of cost, availability of the medical services and minimize the side effects of the drugs like statin, the present study was undertaken with curry leaf and cucumber which is available at natural habitat in rural community. This study was aimed to evaluate the effect of dried curry leaf powder and cucumber slices in hyperlipidemic post meanpausal women living in rural area.

MATERIALS AND METHODS:

Trial design, setting and selection of participants: A randomized control cluster group trial was designed. The block method of randomization of the subjects screened from block of 6 rural communities (20 from each) who diagnosed of hyperlipidemia was recruited. The inclusion criteria was menopausal women who were between the age of 45 - 65years, those who were diagnosed with hyperlipidemia and having abnormal TC (> 200 mg/dl) and also abnormalities in one of the lipid parameters (HDL < 40 mg/dl, LDL > 100 mg/dl, Triglycerides > 150 mg/dl) and subjects were not having co morbidity like auto immune diseases, liver and renal impairment, underwent surgical intervention in gastro intestinal diseases and irritable bowel syndrome. The purpose and procedure of the study were explained to each subject and written consent was taken from them before allocating to the groups. The study was approved by the ethics committee for research in human, Saveetha University, Chennai, India (003/01/2016/IEC/SU; dated 28 January -2016).

Sample size: The sample size was estimated based on the pilot study and assuming the following (i) 5 % decrease in the clinical lipid parameters. (ii) 30% as standard deviation, (iii) 90% power at 5% significant level. The estimated sample size was 90, (30 in each group). Expecting a drop outs of 25% the sample size was rounded to 40 in each group.

Interventions and out comes: They were asked to complete a questionnaire regarding baseline variables and the previous report of hyperlipidemia. Total 120 subjects were selected randomly and assigned to 40 participants in each; control group, experimental group 1(treated with curry leaves) and experimental group 2 (treated with cucumber). Each group carries participants with or without taking hypolipidemic drug (atorvastatin, 10 mg daily).

Experiment group was instructed to gather in the community in the allotted time with overnight fasting (8-10 hrs). After the initial screening blood samples were collected for the determination of lipid profile and ASL, ALT, urea and creatinine collected only from curry leaf intervention group and all the blood samples were given unique coding in order to blind the analyst about the participant group and sent to the laboratory which had adequate quality control.

Curry leaves were collected from the natural habitat. Cleaned the curry leaves with adequate care was oven dried for 3 minutes and produced a concentrated powder and tested for oregano phosphorous pesticides residues (Ref. NO.HO/ LRO:198662/15) in NABL laboratory before intervention and the result showed substances were free of oregano phosphorous pesticides residues. 100 gram of fresh curry leaves gave around 35 gram of powder which was packed separately in a food grade plastic bottle and distributed to the study participants after the necessary explanation. The 5 gm of powder contain 0.5 gm of carbohydrate, 0.4 gm of fiber, 0.34 mg µg of s – carotene, 31.4 mg of total chlorophyll and 5.86 mg of Vitamin C. The subject was instructed to consume 5gm of curry leave powder adding to the main side dish once daily during the lunch for 45 days. The powder was freshly prepared once in 7 days and given to the experimental study subjects.

Fresh cucumber's fruit were collected from organic farm without using any pesticides, and washed thoroughly in running water. Cut both ends 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 and instructed to take this once daily before the lunch for 45 consecutive days. Control group did not receive any treatment. All the participants were instructed to maintain the regular life style and exercise. Subjects were given a diary after explanation to document the intervention or adverse effect, if any daily. Blood samples were analyzed after 45 days in a fully automated clinical chemistry analyzer (Rayto-Lantwin 2000) using DiaTek reagent in a diagnostic lab which had adequate quality assurance programme.

Statistical analysis: The analysis was carried out by using SPSS 23 version. The data were expressed as mean and standard error of mean (SE). Paired 't' test and one way ANOVA was used to find the effect and significant difference between pre-test and post-test curry leaf and cucumber group with control group. Further, Chi- square test was used to find the homogeneity of three groups. A probability of 0.05 or less was taken as statistically significant.

RESULTS: The participant flow diagram according to the CONSORT guideline is depicted in **Fig. 1**. Total of 120 participants were included in the study. No participants were dropped out from the study. Among the participants who were taking drug (statin) in the control group was 22, curry leaf was 20 and cucumber group was 24. While the participants who were not taking drug were18, 20 and 16, respectively in the control, curry leave and cucumber treated group. **Table 1** showed the baseline demographic and clinical characteristics for each group.

TABLE 1: DEMOGRAPHIC VARIABLES OF PARTICIPANTS

Demographic variables		Control		Cucumber		Curry leaves		χ^2	p value
		n	%	n	%	n	%		
BMI	Normal	20	55.6	8	22.2	8	22.2	13.6	0.009
	Over weight	16	26.7	24	40.0	20	33.3		
	Obesity	4	16.7	8	33.3	12	50.0		
Habit of doing exercise	yes	18	34.6	11	21.2	23	44.2	7.39	0.025
	No	22	32.4	29	42.6	17	25.0		
Age	45 -50	10	32.3	11	35.5	10	32.3		
	51-55	10	32.3	11	35.5	10	32.	421	0.99
	56- 60	10	35.7	8	28.6	10	35.7		
	61-65	10	33.3	10	33.3	10	33.3		
Onset of	< 1 yr	12	30.0	15	37.5	13	32.5		
Hyperlipidemia	2 -5 yrs	9	33.3	9	33.3	9	33.3		0.95
	5 – 10 yrs	7	36.8	7	36.8	5	26.3		
	> 10 yrs	12	35.3	9	26.5	13	38.2	1.53	
Taking treatment	yes	22	33.3	24	36.4	20	30.3		0.66
	No	18	33.3	16	29.6	20	37.0	0.80	
Duration	1 - 5 yrs	13	31.0	16	38.1	13	31.0		
of menopause	5 – 10 yrs	13	37.1	9	25.7	13	37.1	1.38	0.84
	> 10 yrs	14	32.6	15	34.9	14	32.6		

The demographic characteristics were expressed in frequency and percentage. Data revealed that 31 (25.8%) subjects were between the age of 45-50 years, another 31(25.8%) were between 51-55 years, 28(23.3%) were between 56-60 years and 30 (25.0%) were in the age group of 61-65 years with (χ^2=0.41, not significant). Most of the control, cucumber and curry leaves groups had up to secondary education Most of them had onset of hyperlipidemia of above 5 years duration. Majority were taking drugs. That was Control 22 (33.3%), cucumber 24(36.4%) and curry leaves 20(30.3%) with (χ^2 =0.80, not significant). Majority of them had duration of menopause was above 10 years with (χ^2 =1.38, not significant).

Effect of curry leaf and cucumber compared was with TC in the post menopausal women. The pre test and post test was compared by paired 't' test and 'F' values by one way ANOVA. The TC in

curry leaf F= 7.823 and P <0.001; and in the cucumber F= 5.384 and P <0.001 both curry leaf and cucumber were significantly different from the pre test. The effect of curry leaf (2.463 and 0.018) and the cucumber (4.026 and < 0.001) was significantly different from pre test. The HDL on curry leaf and cucumber in post menopausal women in curry leaf F=5.975 and P<0.001, cucumber F= 3.326 and P= 0.002 both were significantly different from the pre test. The curry leaf and cucumber compared with control on LDL was also highly significant. The curry leaf F= 6.239 and P< 0.001; cucumber F= 6.267 and P <0.001. The effect of curry leaf and cucumber were compared with control on total cholesterol in post menopausal women. The decrease in curry leaf group on TC 31.4mg/dl, triglycerides 15.9 mg/dl, LDL 23.8mg/dl and the HDL was increased 8.9 mg/dl when compared with control group. In cucumber group the difference was in TC18.1 mg/dl, triglycerides 15.1 mg/dl, LDL 25.2 mg/dl and the HDL was increased 6.5 mg/dl. Which revealed both curry leaf and cucumber was effective in lowering cholesterol in post menopausal women. Assessment of HDL, LDL, TC and TG in post menopausal women with hyperlipidemia and were taking drug and not taking drug shown in (**Table 2** and **3**).

TABLE 2: ASSESSMENT OF HDL AND LDL IN WOMEN WHO WERE WITH DRUG, NO DRUG ON CURRY LEAF AND CUCUMBER EXPERIMENT

Sl. no	Parameter	Group	Mean ± SE	P value t value	One way ANOVA
1	Post HDL	Cont–Y	42.6 ± 1.6	P= 0.313	F= 2.987
		Cont -N	40.2 ± 1.7	t = 1.022	P= 0.014
		CL -Y	47.0 ± 1.5	P= 0.401	
		CL-N	49.1 ± 2.1[a]	t = 0.849	
		CU-Y	46.9 ± 2.5	P= 0.522	
		CU-N	49.3 ± 2.7[a]	t = 0.646	
2	Post LDL	Cont –Y	147.8 ± 8.6	P = 0.915	F= 5.867
		Cont - N	146.6 ± 5.6	t = 0.107	P= <0.001
		CL –Y	109.1 ± 5.5	P = 0.004	
		CL - N	139.7 ± 8.2[a]	t = 3.092	
		CU –Y	117.2 ± 4.9	P = 0.562	
		CU - N	122.1 ± 7.1[a]	t = 0.585	

Cont = Control; CL= curry leaf; CU = cucumber; Y = with drug; N= without drug (Cont- Y =22; Cont- N= 18), (CL-Y = 20; CL-N= 20); (CU-Y= 24; CU- N = 16)

a = statistically significant compared with treatment and without treatment of the respective groups

TABLE 3: ASSESSMENT OF TOTAL CHOLESTEROL (TC) AND TRIGLYCERIDES (TG) IN WOMEN WHO WERE WITH DRUG, NO DRUG, CURRY LEAF AND CUCUMBER EXPERIMENT

Sl. no	Parameter	Group	Mean ± SE	P value t value	One way ANOVA
1	Post TC	Cont –Y	218.5 ± 7.8	P= 0.947	F= 5.325
		Cont - N	219.2 ± 5.6	t = 0.066	P= <0.001
		CL -Y	177.3 ± 7.2[a]	P= 0.012	
		CL- N	205.2 ± 7.8	t = 2.644	
		CU - Y	196.3 ± 5.9	P= 0.166	
		CU - N	208.1 ± 5.1	t = 1.413	
2	Post TG	Cont –Y	160.2 ± 9.4	P= 0.096	F= 2.582
		Cont - N	138.2 ± 8.4	t = 1.709	P= 0.030
		CL-Y	126.9 ± 7.0	P= 0.493	(Other statistical
		CL- N	135.6 ± 9.8	t = 0.725	details given in
		CU-Y	131.5 ± 5.0	P= 0.995	Figure 1.2)
		CU - N	131.5 ± 3.3	t = 0.006	

Cont = Control; CL= curry leaf; CU = cucumber; Y = with drug; N= without drug (Cont- Y =22; Cont- N= 18), (CL-Y = 20; CL-N= 20); (CU-Y= 24; CU- N = 16)

a = statistically significant compared with treatment and without treatment of the respective groups

The effect of curry leaf and cucumber with control with drug and without drug in menopausal women were compared by one way ANOVA. The 'F' and 'P' values of HDL- 2.987 and 0.014. LDL –

F=5.867, P <0.001; triglycerides F=2.582, P= 0.030 and Total cholesterol F= 5.325, P <0.001. The results showed statistically significant difference compared with control without drug group. Which revealed the cucumber and curry leaf were effective in improving HDL and lowering the hyperlipidemia. The ratio between control, curry leaf and cucumber with TC/HDL, LDL/HDL and TG/HDL in pre and post test were highly significant. The ratio revealed cucumber and curry leaf were effective in reducing the cholesterol by increasing HDL (**Fig. 3**). No harms or any unintended effects were found in any of the treated groups.

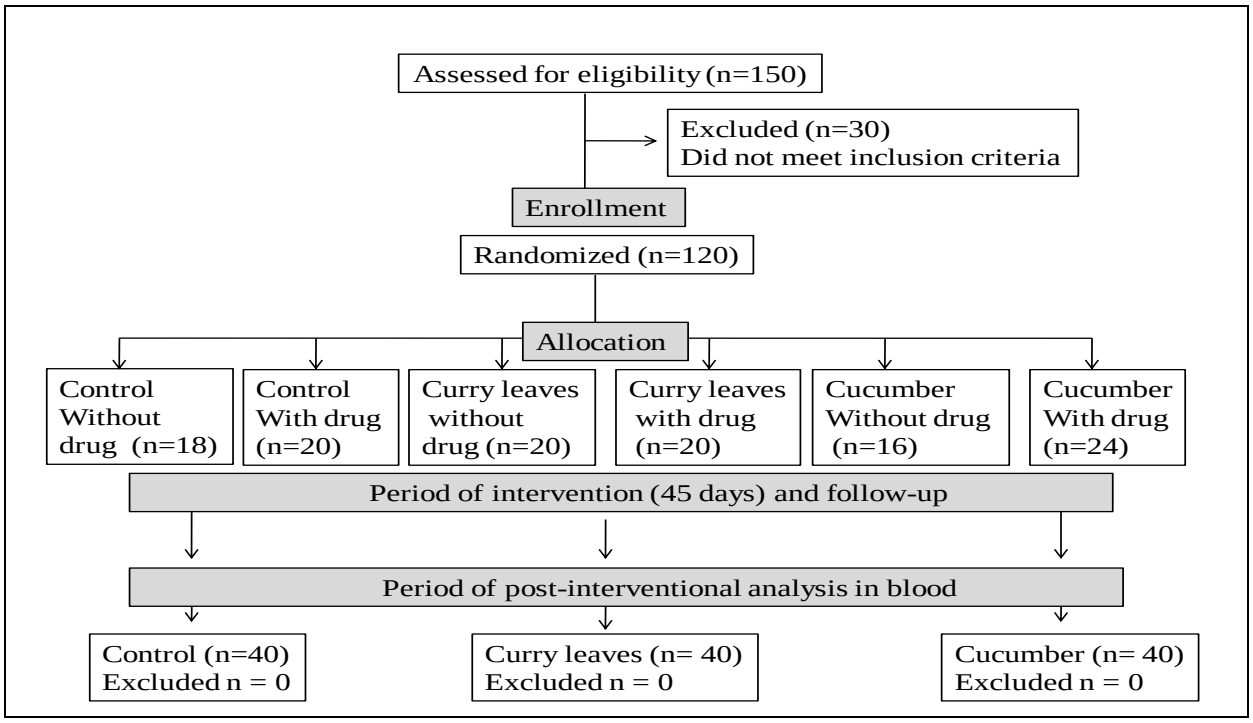

FIG. 1: THE PARTICIPANT FLOW DIAGRAM ACCORDING TO THE CONSORT GUIDELINE

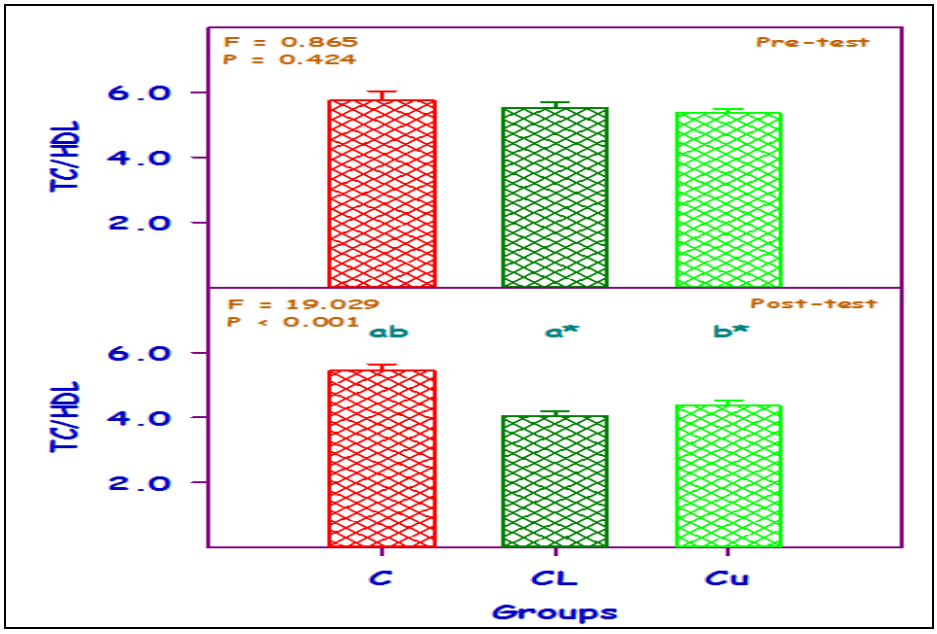

FIG. 2: EFFECTIVENESS OF CURRY LEAF POWDER (CL) AND CUCUMBER SLICES (Cu) COMPARED WITH CONTROL (C) ON THE RATIO OF SERUM TOTAL CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN (TC/HDL) IN MENOPAUSAL WOMEN. Values are mean ± SE (n = 40 each). The 'F' and 'P' values are by one way ANOVA comparing the respective pre-test and post-test. Same alphabetical characters are mutually significant. The pre-test and post-test are compared by paired 't' test. The 't' and 'P' values in the control are 0.851 and 0.400; in the curry leaf are 8.427 and < 0.001 and in the cucumber are 4.807 and < 0.001, respectively.

DISCUSSION: The results of the study revealed that both curry leaf and cucumber were equally effective (no statistical difference was showed comparison between curry leaf and cucumber) to lower the TC and TG levels. The mean values of the TC, LDL-C and TG-C 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 it in bile, an elevated HDL-C level could support the efficacy of the tested compounds. 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.

An elevated HDL-C level in both curry leaf and cucumber experimental groups was found to be protector of CVD. It was found that for every 1mg/dl increase in HDL-C, the risk of CHD in women decreased by 3% [6]. Another study found out that curry leaves have the antioxidant effect to protect against the oxidation of cholesterol and further favor a slow release of glucose to the blood stream during digestion [5]. The antioxidant activity of curry leaves might have contributed its importance in preventing the hyperlipidemia. It was previously demonstrated that the protective effect of curry leaves against the *in-vitro* LDL-C oxidation and oxidized LDL-C-induced apoptosis in macrophages was due to the antioxidant potentials of flavonoids [6].

Pharmacologic treatment for hyperlipidemia may be control CVD. Hypolipidaemic drugs such as statins can lower the levels of lipids in the blood. A high-dose statins should be initiated in patients with acute coronary syndrome which may not be tolerated by all patients. Other cholesterol-lowering medications used for primary or secondary prevention of CVD have not been shown to improve patient-oriented outcomes [7]. Similarly study was also demonstrated the pathophysiologic effects of lipid and neuroprotective effects of statins therapy [8]. All statins are remarkably well tolerated but some notable side effects of statin are rise in serum transaminase, muscle tenderness and rise in CPK levels and few fatalities due to rhabdomyolysis were reported [8]. In addition to the drug treatment, in order to lower the cholesterol life style modification and dietary management was also recommended. This may keep medicines as low possible to lower the side effects [9].

Cucumber is very low calorie vegetable which contains no saturated fat or cholesterol and good source of dietary fiber. However a statistically significant decrease was observed in TC, LDL-C and TG-C in paired ‘t test. Previous study found the cucumber has a greater impact on hyperlipidemia. One glass of cucumber juice (125 ml) for the period of 4 months was statistically ($P< 0.05$) significant in decreasing the TC, LDL and TG experimental group [10].

The results of the current study revealed that the consumption both, curry leaf powder and cucumber slices irrespective of the statin treatment were effective in improving the HDL-C and, thereby, lowering the TC. This was evidenced from comparing the lipid profile parameters before and after the intervention.

The easy availability of natural antioxidants, their non toxic nature and inexpensiveness make them an alternative source for the disease management. Non pharmacological medicinal plant sources prevent the oxidation of LDL-C as it is packed with antioxidants. One of the previous studies evaluated the nano particles of almond in hyperlipidemia in human. Supplementation of 2 g of almond nano paste (almonds were fabricated into nano particles and also grounded to form almond paste) for a period of two months brought reduction in lipid profile of hyperlipidemic subjects [10-11]. In a non pharmacological study, participants were given flaxseed powder 15 g and 20 g for two groups respectively for 60 days. A highly significant ($P< 0.01$) result was observed in both groups [11-12].

The result of the study concluded that both Curry leaf and cucumber were effective in improving HDL-C and lowering of TC, LDL-C and triglyceride levels in post menopausal women with hyperlipidemia irrespective of the statin therapy. A short duration, single blinded study and the limited number of participants were the main limitations of this study.

Hence, a multicentric double blinded controlled study covering a wide population is warranted to evaluate the effect completely.

CONCLUSION: Both Curry leaf and cucumber were effective in improving HDL-C and lowering of TC, LDL-C and triglyceride levels in post menopausal women with hyperlipidemia irrespective of the statin therapy. A short duration, single blinded study and the limited number of participants were the main limitations of this study. Hence, a multicentre double blinded controlled study covering a wide population is warranted to evaluate the effect completely.

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