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Effect of Supplementation of Drumstick (*Moringa Oleifera*) and Amaranth (*Amaranthus Tricolor*) Leaves Powder on Lipid Profile in Postmenopausal Women

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Abstract- Menopause is a gradual three-stage process that concludes with the end of periods and reproductive life. The antioxidant enzyme system get affected in postmenopause due to deficiency of estrogen, which has got antioxidant properties. The objective of the present study was therefore, to analyze the effect of supplementation of drumstick and amaranth leaves powder on lipid profile and blood pressure. Ninety postmenopausal women aged 45-60 years were selected and divided into three groups viz. Group I, II and III having thirty subjects in each group. The subjects of group II and III were supplemented daily with 7g drumstick leaves powder (DLP) and 9g amaranth leaves powder (ALP), respectively for a period of three months in their diet. The subjects of group I was not given supplementation. Total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C were analyzed before and after supplementation. Blood pressure of the subjects were also recorded. The data revealed that supplementation of DLP and ALP significantly decreased total cholesterol (14.2 and 8.2%), triglycerides (5.9 and 4.6%), LDL-C (10.9 and 5.3%), VLDL-C (5.8 and 4.6%) whereas increase in HDL-C (15.3 and 7.2%) in postmenopausal women of group II and III respectively. SBP and DBP decrease by 3.6 and 2.1% in group II whereas 4.3 and 6.7% in group III. The results indicated that these plants possess hypolipidemic and hypotensive properties.

Index Terms- Amaranth leaves powder, Drumstick leaves powder, Lipid profile

I. INTRODUCTION

Menopause is associated with a wide variety of physical and psychological symptoms. It is a gradual three-stage process that concludes with the end of periods and reproductive life. When woman's menstruation has ceased spontaneously at least for a year it is postmenopause. Most women experience menopause between 40 and 58 years of age, the median age being 51 years (Moilanen *et al* 2010). In postmenopause, ovaries stop making estrogen hormone. The antioxidant enzyme system seems to be affected in postmenopause due to deficiency of estrogen, which has got antioxidant properties. The beneficial effects of estrogen might be attributable to their free radical scavenging structures. Another benefit of estrogen is that it decreases low density lipoprotein (LDL) cholesterol and increases high density lipoprotein (HDL) cholesterol affecting lipid metabolism. Estrogen also plays a role in the increased production of neurotrophic growth factors, which modulate neuronal growth, survival and aging (Srivastava et al 2005).

Damage caused by oxygen radicals is responsible for many of the bodily changes that come with age. Antioxidant offers protection against a wide spectrum of diseases. Antioxidant scavenges free radicals, provides cellular protection and fights against human diseases. Drumstick (*Moringa oleifera)* and amaranth (*Amaranthus tricolor*) are such promising plants which have both a medicinal and a functional property. Drumstick leaves had the highest proportion of essential amino acids and significant quantities of minerals (Sreelatha and Padma 2009). Lako et al (2007) reported that drumstick leaves have a high total antioxidant capacity (260 mg/100 g) and are rich in total polyphenol content (260 mg/100 g), quercetin (100 mg/100 g), kaempferol (34 mg/100 g) and β-carotene (34 mg/100 g). Amarnath leaves contain dietary fibre, folic acid and perhaps other bioactive nutrients such as bioflavonoids. Further, amaranth leaves contain magnesium, an antimutagen and chlorophyllin, a proven efficient antimutagen and antioxidants (Anilakumar *et al* 2006). Hence, the present study was designed to see the effect of supplementation of dried drumstick and amaranth leaves powder on lipid profile and blood pressure in postmenopausal women.

II. METHODOLOGY

Procurement of antioxidant powders: Fresh leaves of Drumstick (*Moringa oleifera*) and amaranth (*Amaranthus tricolor*) were procured from Department of Vegetable Crops, Punjab Agricultural University, Ludhiana. Fresh leaves were sorted and washed. Washed leaves were spread and dried in oven at 40˚C for 4-6 hours and then powdered. Powdered drumstick leaves were named as Antioxidant powder I (DLP) and amaranth leaves as antioxidant powder II (ALP). All other ingredients were purchased from the local market.

Selection of subjects and supplementation: Ninety healthy postmenopausal women aged between 45-60 years, who were not having their menstrual period from last 1-3 years were selected for the study. Women who had undergone hysterectomy or taken hormone replacement therapy were excluded from the study. The selected subjects were equally divided into three groups viz. group I, group II and group III i.e. 30 in each group. Subjects of group II and group III were supplemented with antioxiant powder I (Drumstick leaves powder: 7g) and antioxidant powder II (Amaranth leaves powder: 9g) in the recipes in daily diet for

three months, whereas group I was not given any supplementation. Information regarding physical activity and lifestyle related information were recorded for all the subjects through an interview schedule.

Analysis of blood samples: Blood samples were analysed before and after supplementation for total serum cholesterol (Richmond 1973), serum high density lipoprotein cholesterol (Lopes-Virella et al 1997) and serum triglycerides (Fossati and Principle 1982). Serum low density lipoprotein cholesterol was calculated based on the Friedwald equation (Frieldwalds et al 1972).

Blood pressure of the selected subjects was recorded with the Sphygmomanometer (Maclead 1984) before and after supplementation.

Statistical analysis: The data on all the blood parameters was analyzed statistically. The mean standard error, analysis of variance and their statistical significance was ascertained using a computer programme package (Cheema and Singh 1990).

III. RESULTS AND DISCUSSION

Ninety postmenopausal subjects were identified and divided into three groups. Information regarding physical activity and lifestyle related information revealed that walking was the most common physical exercise adopted by 70.0, 33.3 and 60.0 per cent of subjects of control group I and experimental group II and group III while 33.3, 50.0 and 40.0 performed yoga. Time spent on yoga was 15-30 minutes by 96.7, 73.3 and 93.4 per cent subjects of three groups. Karolkiewicz et al (2009) showed that an 8-week aerobic exercise enhanced insulin sensitivity, and improved the balance between oxidants and antioxidants in healthy, postmenopausal women. It was observed that majority of subjects 93.3, 96.7 and 96.7 per cent of control group I and experimental group II and III used to sleep more than 6 hours while 6.7, 3.3 and 3.3 per cent of subjects of three groups used to sleep for 6-8 hours. Campos et al (2006) reported that oxidative stress status of postmenopausal women, probably due to the lack of estrogen and due to sleep disturbances. Chandla (2006) reported that 35% postmenopausal women sleep more than 6 hours. It was observed in the present study that watching TV was the major relaxation mode adopted by all the subjects, while sitting idle was the second most popular way of relaxation among 73.3, 60.0 and 40.0 per cent of subjects (Table 1).

Lipid profile of the subjects before and after supplementation of Antioxidant powder I & II

Different blood lipid parameters of the subjects of the three groups were assessed before and after supplementation of Antioxidant powder I & II (Table 2) and their percentage distribution(Table 3).

The mean values of total cholesterol before and after supplementation period in group I, group II and group III were 217.56±8.39, 207.13±6.50, 210.70±7.29 mg/dl and 215.93±7.96, 177.80±5.03, 193.36±6.23 mg/dl. A highly significant ($p \leq 0.01$) decrease in TC was observed in experimental groups II and III whereas a non significant decrease in control group I. Distribution of subjects in group II and group III revealed that 46.6 and 40.0 per cent of subjects were in desirable range before supplementation which increased 83.4 and 60.0 per cent after supplementation. Drumstick leaves contain atenol which has profound hypolipidemic activity by increasing excretion of fecal cholesterol (Ara et al 2008, Jain et al 2010). Krishnamurthy et al (2011) also reported hypolipidemic effect of amaranth leaves.

The initial and final mean values of triglycerides were 173.56±10.07, 168.53±9.95, 145.73±9.69 mg/dl and 171.96±9.48, 158.63±8.69, 138.90±7.66 mg/dl in group I, group II and group III, respectively. A significant decrease of 5.9 per cent was observed in group II (DLP supplementation) whereas 4.6 per cent in group III (ALP supplementation). A non significant decrease was observed in control group I. Data revealed that before supplementation 30.0, 33.4 and 43.3 per cent of subjects were in desirable range which increased to 33.4, 60.0 and 70.0 per cent after supplementation in group I, group II and group III respectively. Kapoor (2010) also reported decrease in triglycerides by 3.8 per cent after supplementation in postmenopausal women.

Before supplementation mean values of HDL-C were 44.70±2.58, 45.30±2.52, 41.96±3.02 mg/dl which increased to 45.13±2.62, 50.26±2.67, 45.00±2.53 mg/dl in group I, group II and group III, respectively. Significant ($p \leq 0.01$) increae in HDL-C by 15.3 per cent was observed in experimental groups II and by 7.2 per cent in group III whereas a non significant increase in control group I. Data on distribution of subjects revealed that before supplementation 56.6, 60.0 and 43.3 per cent were in desirable range which increased to 63.4, 76.6 and 56.6 per cent after supplementation in group I, group II and group III, respectively. Srivastava (2009) also reported increase in HDL-C by 8.19 per cent after supplementation of amla powder.

The mean initial and final values of LDL-C in group I, group II and group III were 113.26±7.16, 154.60±8.29, 138.66±8.08 mg/dl and 115.16±6.72, 137.60±7.16, 131.30±6.77 mg/dl, respectively. A highly significant ($p \leq 0.01$) decrease in LDL-C was observed in experimental groups II (10.9 per cent) and group III (5.3 per cent) whereas a non significant decrease in control group I. Distribution of subjects revealed that before supplementation 30.0 and 33.3 per cent were in desirable range which increased to 43.4 and 40.0 per cent after supplementation in group II and group III. Kabiri et al (2010) reported that extract of Amaranthus decreased the most important risk factors (serum lipoproteins, apoB and oxdised-LDL) of cardiovascular diseases and inflammatory factors. Kim et al (2006) reported that supplementation of amaranth improves lipid metabolism. Drumstick and amaranth leaves contain β-sitosterol which may be responsible for its hypolipidemic effect as well as antioxidant properties (Rajanandh and Kavitha 2010, Baral et al 2011).

The initial mean values of VLDL-C were 34.71±2.01, 33.70±1.99 and 29.14±1.93 mg/dl and after supplementation period, the values decreased to 34.39±1.89, 31.72±1.73 and 27.78±1.53 mg/dl in group I, group II and group III respectively. A significant decrease was observed in group II and group III while non significant decrease was observed in control group I.

The initial and final TC:HDL-C ratio in group I, group II and group III were 6.00±0.77, 5.31±0.54, 6.59±0.85 and 5.80±0.67, 3.99±0.33, 4.94±0.44 respectively. A highly significant ($p \leq 0.01$) decrease in TC:HDL-C was observed in experimental groups II (24.9 per cent) and group III (25.0 per cent) whereas a non significant decrease in control group I.

The mean ratio of LDL-C:HDL-C before and after supplementation were 3.16±0.78, 3.81±0.36, 4.20±0.57 and 3.14±0.40, 3.03±0.28, 3.30±0.31 in group I, group II and group III respectively. A highly significant ($p \leq 0.01$) reduction was observed in group II (20.5 per cent) and group III (21.4 per cent), wheras a non-significant decrease was observed in control group I. Further data revealed that supplementation of DLP in group II, TC:HDL-C was reached upto desirable level.

The mean ratio of triglyceride:HDL-C before supplementation were 5.14±0.81, 4.63±0.68, 4.76±0.75 which decreased to 5.01±0.81, 3.77±0.48, 3.71±0.46 in group I, group II and group III respectively after supplementation. A highly significant ($p \leq 0.01$) reduction was observed in group II (18.5 per cent) and group III (22.1 per cent), wheras a non-significant decrease was observed in control group I.

Blood pressure of the subjects before and after supplementation of Antioxidant powder I & II

Blood pressure of the subjects recorded before and after supplementation (Table 4) and their percentage distribution (Table 5).

Data revealed that mean value for SBP before supplementation were 130.76±10.42, 136.26±8.35, 134.56±11.51 mm Hg which decreased to 130.26±10.39, 131.33±10.31, 131.76±7.60 mm Hg in group I, group II and group III, respectively. A significant ($p \leq 0.01$) decrease of 3.6 per cent was observed in experimental groups II (DLP supplementation) and 2.1 per cent in group III (ALP supplementation) whereas a non-significant decrease was observed in control group I. Drumstick leaves contain nitrile, mustard oil glycosides and thiocarbamate glycosides which have blood pressure lowering effect (Anwar et al 2007).

The data recorded revealed that the initial and final diastolic blood pressure recorded were 90.36±1.65, 90.43±1.11, 94.66±1.53 mm Hg and 88.23±1.23, 86.53±0.58, 88.30±1.15 mm Hg in group I, group II and group III respectively. A significant ($p \leq 0.01$) decrease of 4.3 per cent was observed in experimental groups II and 6.7 per cent in group III wheras a non-significant decrease in control group I. Morimoto et al (2008) explained that mental stress causes sustained diastolic blood pressure elevation in postmenopausal women, accompanied by heightened oxidative stress.

IV. Conclusion

It was concluded that supplementation of drumstick leaves powder (7g) and amaranth leaves powder (9g) per day for three months significantly decreased total cholesterol (14.2 and 8.2%), triglycerides (5.9 and 4.6%), LDL-C (10.9 and 5.3%), VLDL-C (5.8 and 4.6%) whereas increase in HDL-C (15.3 and 7.2%) in postmenopausal women. SBP and DBP decrease by 3.6 and 2.1% in DLP supplemented group whereas 4.3 and 6.7% in ALP supplemented group. Hence, it is recommended to consume drumstick leaves and amaranth leaves as they are rich source of antioxidants and possess hypolipidemic and hypotensive properties.

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Table 1. Physical activity and lifestyle related information of the subjects

S.No.	Characteristics	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)
1	**Type of physical activity***			
	Walking	21(70)	10(33.3)	18(60)
	Yoga	10(33.3)	15(50)	12(40)
	Gym	2(6.6)	1(3.3)	-
2	**Time spent on physical activity(mins)**			
	15-30	29(96.7)	22(73.3)	28(93.4)
	30-45	1(3.3)	8(26.7)	2(6.6)
3	**Sleep hours**			
	<6	28(93.3)	29(96.7)	27(96.7)
	6-8	2(6.7)	1(3.3)	3(3.3)
4	**Relaxation technique***			
	Sitting idle	22(73.3)	18(60)	12(40)
	Listening music	1(3.3)	2(6.6)	1(3.3)
	Watching TV	10(33.3)	22(73.3)	24(80)
	Meditation	1(3.3)	-	-
	Reading	1(3.3)	-	-

Figures in parenthesis are percentages
*Multiple Responses

Table 2. Effect of supplementation of Antioxidant powder I (DLP) and II (ALP) on lipid profile of the subjects (n=30)

Parameters	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)	C.D. at 5%	Standard range
Total Cholesterol (mg/100ml)					
Baseline	217.56±8.39[a]	207.13±6.50[b]	210.70±7.29[c]	0.20	<200^
After Exp.	215.93±7.96[a]	177.80±5.03	193.36±6.23[c]	18.34	
% change	0.7	14.2	8.2		
Paired t-value	1.52[NS]	6.10**	7.00**		
Triglycerides (mg/100ml)					
Baseline	173.56±10.07	168.53±9.95	145.73±9.69	NS	<150^
After Exp.	171.96±9.48[a]	158.63±8.69	138.90±7.66[c]	24.30	
% change	0.9	5.9	4.6		
Paired t-value	1.74[NS]	3.00**	2.25*		
HDL-C (mg/100ml)					
Baseline	44.70±2.58	45.3±2.52	41.96±3.02	NS	>50^
After Exp.	45.13±2.62[a]	50.26±2.67[b]	45.00±2.53	3.53	
% change	1.0	15.3	7.2		
Paired t-value	1.11[NS]	6.76**	4.09**		
LDL-C (mg/100ml)					
Baseline	113.26±7.16[a]	154.60±8.29	138.66±8.08[c]	19.80	<130^
After Exp.	115.16±6.72[a]	137.60±7.16[b]	131.30±6.77[c]	5.12	
% change	1.7	10.9	5.3		
Paired t-value	1.72[NS]	4.08**	3.07**		
VLDL-C (mg/100ml)					
Baseline	34.71±2.01	33.70±1.99	29.14±1.93	NS	<30^
After Exp.	34.39±1.89	31.72±1.73	27.78±1.53	NS	
% change	0.9	5.8	4.6		
Paired t-value	1.74[NS]	3.00**	2.24*		
Total Cholesterol/ HDL-C					
Baseline	6.00±0.77	5.31±0.54	6.59±0.85	NS	<4[o]
After Exp.	5.80±0.67[a]	3.99±0.33	4.94±0.44	1.42	
% change	3.3	24.9	25		
Paired t-value	1.18[NS]	5.52**	3.69**		
LDL-C/HDL-C					
Baseline	3.16±0.78	3.81±0.36	4.20±0.57	NS	<3[o]
After Exp.	3.14±0.40	3.03±0.28	3.30±0.31	NS	
% change	0.6	20.5	21.4		
Paired t-value	0.78[NS]	3.42**	3.28**		

Parameters	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)	C.D. at 5%	Standard range
Triglycerides/HDL-C					
Baseline	5.14±0.81	4.63±0.68	4.76±0.75	NS	1[o]
After Exp.	5.01±0.81	3.77±0.48	3.71±0.46	NS	
% change	2.5	18.5	22.1		
Paired t-value	1.022[NS]	3.59**	3.56**		

Values represent Mean ±SE

**Significant at 1% level of significance *Significant at 5% level of significance

NS-Non Significant

[^] Ghafoorunissa and Krishnamurthy (2007) [o] American Heart Association (2004)

[a] significant difference between group I and II

[b] significant difference between group II and III

[c] significant difference between group III and I

Table 3. Percentage distribution of subjects based on lipid profile (n=30)

Parameters	Group I (Control)		Group II (DLP supplementation)		Group III (ALP supplementation)	
	Baseline	After Exp.	Baseline	After Exp.	Baseline	After Exp.
Total Cholesterol (mg/dl)						
Desirable< 200	13 (43.3)	13 (43.3)	14 (46.6)	25 (83.4)	12 (40)	18 (60)
Border line high 200-240	11 (36.7)	11 (36.7)	11 (36.6)	5 (16.6)	12 (40)	10 (33.4)
High risk >240	6 (20)	6 (20)	5 (16.8)	0 (0)	6 (20)	2 (6.6)
Triglycerides (mg/100ml)						
Desirable< 150	9 (30)	10 (33.4)	10 (33.4)	18 (60)	13 (43.3)	21 (70)
Border line high 150-500	21 (70)	20 (66.6)	20 (66.6)	12 (40)	17 (56.6)	9 (30)
HDL-C (mg/100ml)						
Desirable >50	17 (56.6)	19 (63.4)	18 (60)	23(76.6)	13 (43.3)	17 (56.6)
High risk >35	13 (43.4)	11 (36.6)	12 (40)	7 (23.4)	17 (56.6)	13 (43.3)
LDL-C (mg/100ml)						
Desirable< 130	20 (66.6)	19 (63.4)	9 (30)	13 (43.4)	10 (33.3)	12 (40)
Border line high 130-160	4 (13.4)	5 (16.6)	11 (36.6)	9 (30)	10 (33.3)	13 (43.4)
High risk >160	6 (20)	6 (20)	10 (33.4)	8 (26.6)	10 (33.4)	5 (16.6)

Table 4. Effect of supplementation of Antioxidant powder I (DLP) and II (ALP) on blood pressure of the subjects (n=30)

Parameters	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)	C.D. at 5%	Standard range
Systolic BP (mmHg)					
Baseline	130.76±10.42[a]	136.26±8.35	134.56±11.51	4.02	120[@]
After Exp.	130.26±10.39[a]	131.33±10.31[b]	131.76±7.60[c]	0.23	
% change	0.3	3.6	2.1		
Paired t-value	1.77[NS]	7.47**	3.65**		
Diastolic BP (mmHg)					
Baseline	90.36±1.65	90.43±1.11	94.66±1.53	NS	80[@]
After Exp.	88.23±1.23	86.53±0.58	88.3±1.15	NS	
% change	2.3	4.3	6.7		
Paired t-value	3.31**	6.96**	5.83**		

Values represent Mean ±SE

**Significant at 1% level of significance

*Significant at 5% level of significance

NS-Non Significant

[@] Raghuram *et al* (2007)

[a] significant difference between group I and II

[b] significant difference between group II and III

[c] significant difference between group III and I

Table 5.Percentage distribution of subjects based on blood pressure (n=30)

Parameters	Group I (Control)		Group II (DLP supplementation)		Group III (ALP supplementation)	
	Baseline	After Exp.	Baseline	After Exp.	Baseline	After Exp.
Systolic BP (mmHg)						
Desirable 120	4 (13.3)	4 (13.3)	8 (26.6)	11 (36.6)	6 (20)	10 (33.3)
Risk >120	26 (86.7)	26 (86.7)	22 (73.4)	19 (63.4)	24 (80)	20 (66.7)
Diastolic BP (mmHg)						
Desirable 80	5 (16.6)	4 (13.3)	10 (33.3)	15 (50)	8 (26.6)	12 (40)
Risk >80	25 (83.4)	26 (86.7)	20 (66.7)	15 (50)	22 (73.4)	18 (60)