J Food Sci Technol (November 2014) 51(11):3464–3469
DOI 10.1007/s13197-012-0859-9

ORIGINAL ARTICLE

Effect of supplementation of drumstick (*Moringa oleifera*) and amaranth (*Amaranthus tricolor*) leaves powder on antioxidant profile and oxidative status among postmenopausal women

Shalini Kushwaha · Paramjit Chawla · Anita Kochhar

Revised: 10 September 2012 / Accepted: 13 September 2012 / Published online: 5 October 2012

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 blood levels of antioxidant and marker of oxidative stress. 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 7 g drumstick leaves powder (DLP) and 9 g amaranth leaves powder (ALP), respectively for a period of 3 months in their diet. The subjects of group I was not given supplementation. Serum retinol, serum ascorbic acid, glutathione peroxidase, superoxide dismutase and malondialdehyde were analyzed before and after supplementation. Fasting blood glucose and haemoglobin level of the subjects were also analyzed. The data revealed that supplementation of DLP and ALP significantly increased serum retinol (8.8 % and 5.0 %), serum ascorbic acid (44.4 % and 5.9 %), glutathione peroxidase (18.0 % and 11.9 %), superoxide dismutase (10.4 % and 10.8) whereas decrease in marker of oxidative stress i.e. malondialdehyde (16.3 % and 9.6 %) in postmenopausal women of group II and group III, respectively. A significant ($p \leq 0.01$) decrease was also observed in fasting blood glucose level (13.5 % and 10.4 %) and increase in haemoglobin (17.5 % and 5.3 %) in group II and group III, respectively. The results indicated that these plants possess antioxidant property and have therapeutic potential for the prevention of complications during postmenopause.

S. Kushwaha (✉) · P. Chawla · A. Kochhar
Department of Food and Nutrition, Punjab Agricultural University, Ludhiana 141 004, India
e-mail: shalinikushwaha72@gmail.com

Keywords Amaranth leaves powder · Antioxidant · Drumstick leaves powder · Oxidative stress

Menopause is a gradual three-stage process that concludes with the end of periods and reproductive life. When woman's menstruation has ceased continously 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. Menopause is associated with a wide variety of physical and psychological symptoms. Typical symptoms at the time of menopause lasting 4–5 years are hot flushes, night sweats, vaginal dryness and sleep disturbance (Moilanen et al. 2010). In postmenopause, ovaries stop producing 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 (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 contain cytokinins in the form of zeatin as well as other beneficial phytochemicals such as vanillin, beta-sitosterol, caffeoylquinic acids, kaempferol, quercetin and carotenes (Andrews and Andrews 2009). 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). Red amaranth leaves tended to be associated with high total polyphenol and antioxidant activity. The high amount of betacyanin in amaranth, which gives its deep red hue, enhances antioxidant activity along with phenolic compounds (Khandaker et al. 2008). Hence, the present study was designed to see the effect of supplementation of dried drumstick and amaranth leaves powder on oxidative stress and antioxidant levels in postmenopausal women.

Materials and methods

Ninety healthy postmenopausal women aged between 45 and 60 years, who were not having their menstrual period from last 1–3 years were selected randomly from Punjab Agricultural University, Ludhiana. Women who had undergone hysterectomy or taken hormone replacement therapy were not included in 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: 7 g) and antioxidant powder II (Amaranth leaves powder: 9 g) in the recipes in daily diet for 3 months (February 2011 to April 2011), whereas group I was not given any supplementation. Information regarding general and reproductive history were recorded for all the subjects through an interview schedule.

Biochemical analysis Blood samples were analysed before and after supplementation for serum retinol (Bessey et al. 1946), plasma ascorbic acid (Raghuramulu et al. 2003) and malondialdehyde using thiobarbituric acid (Esterbauer et al. 1982). Glutathione peroxidase and superoxide dismutase were analysed by kit provided by RANDOX Laboratories Limited, United Kingdom. Blood was also analysed for haemoglobin (Dacie and Lewis 1975) and fasting blood glucose (Trinder 1969).

Statistical analysis The data on all the blood parameters was analyzed statistically in triplicates. The mean standard error, analysis of variance, paired *t*-test and their statistical significance was ascertained using a computer programme package.

Results and discussion

Ninety postmenopausal subjects were identified and divided into three groups. General information of the subjects showed that majority of the subjects of group I and group II belonged to the age group of 50–55 yrs whereas 45–50 years in group III. Majority of subjects of group I and group II were studied up to high school whereas subjects of group III were graduate. Majority of the subjects in were married and belonged to Hindu religion and nuclear family. Most of the subjects were housewives (Table 1).

Antioxidant levels and oxidative stress levels of the subjects before and after supplementation of Antioxidant powder I & II are presented in Table 2. Results indicated that before supplementation serum retinol level of subjects of control group I and experimental group II and III were 33.55, 34.12 and 33.55 μg/dl and after supplementation serum retinol level increased to 34.04, 37.12 and 35.23 μg/dl in group I (control), group II (DLP supplementation), group III (ALP supplementation) respectively. Change in serum retinol levels in group II was highly significant ($p \leq 0.01$) while significant in group III ($p \leq 0.05$). A non significant change was observed in control group I. Serum vitamin A concentrations were higher in the group supplemented with choline and carnitine which promote conservation of retinol in women (Sachan et al. 2005).

Present study revealed that the mean serum ascorbic acid levels of the subjects before and after supplementation was 0.83, 0.81, 0.84 mg/dl and 0.80, 1.17, 0.89 mg/dl in all the three group I (control), group II (DLP supplementation) and group III (ALP supplementation), respectively. A significant increase was obseved in serum ascorbic acid levels in group II and III after supplementation, whereas a non significant change was observed in control group I. The observed values were in the range of normal values. Increase in serum ascorbic acid may be due to high content of ascorbic acid in antioxidant powders. Arora et al. (2009) reported significantly lower level of serum vitamin C in postmenopausal women as compared to premenopausal women. *Moringa oleifera* (drumstick) leaves have a high total antioxidant capacity and are rich in total polyphenol content, quercetin, kaempferol and β-carotene (Lako et al. 2007).

The initial and final glutathione peroxidase values in group I, group II and group III were 20.76, 19.69 and 17.31 U/g Hb and 20.63, 23.24, 19.38 U/g Hb respectively. Increase in glutathione peroxidase level in experimental groups was statistically highly significant ($p \leq 0.01$) while non significant in control group I. The observed values were below the reference values. However Arora et al. (2009) reported lower value of 18.11 U/g Hb glutathione peroxidase level among postmenopausal women.

It was observed that the initial and final mean values of superoxide dismutase of the subjects in group I (control), group II (DLP supplementation) and group III (ALP supplementation) were 1080, 1097, 1076 U/g Hb and 1082, 1211, 1192 U/g Hb, respectively. There was a highly significant increase in experimental groups II and III of superoxide dismutase level. The observed values before supplementation

Table 1 General information of the subjects

S. No.	Characteristics	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)
1	Age (yrs)			
	45–50	14(46.7)	12(40)	17(56.7)
	50–55	16(53.3)	18(60)	13(43.3)
2	Religion			
	Hindu	19(63.3)	20(66.6)	11(36.7)
	Sikh	11(36.7)	10(33.4)	19(63.3)
3	Education			
	Illiterate	5(16.6)	5(16.6)	5(16.6)
	High School	15(50)	10(33.4)	3(10)
	Higher Secondary	4(13.3)	10(33.4)	10(33.4)
	Graduate	6(20.1)	5(16.6)	12(40)
4	Occupation			
	Service	10(33.4)	12(40)	15(50)
	Housewife	20(66.6)	17(56.7)	13(43.3)
	Entrepreneur	0(0)	1(3.3)	2(6.7)
5	Marital Status			
	Married	30(100)	29(96.7)	30(100)
	Widow	0(0)	1(3.3)	0(0)
6	Type of family			
	Nuclear	25(83.4)	21(70)	18(60)
	Joint	5(16.6)	9(30)	12(40)
7	Family Size			
	2 to 4	15(50)	17(56.7)	15(50)
	4 to 6	14(46.7)	6(20)	6(20)
	>6	1(3.3)	7(23.3)	9(30)

Figures in parenthesis are percentages

DLP drumstick leaves powder, *ALP* amaranth leaves powder

were below normal range and supplementation with antioxidant powders helped in increasing the level. Menopause is associated with oxidative stress as indicated by increase in lipid peroxidation. Kaya et al. (2005) reported superoxide dismutase level in postmenopausal women to be 1375 U/g Hb. Antioxidant enzymes like superoxide dismutase and glutathione peroxidase decreases in postmenopausal women showing oxidative stress in the cells (Srivastava et al. 2005).

Initial and final malondialdehyde values in group I, group II and group III were 285, 288, 250 nmoles/g Hb and 287, 241, 226 nmoles/g Hb. There was a highly significant decrease in experimental groups II and III in level of malondialdehyde whereas non significant decrease in control group I. Arora et al. (2009) reported significantly higher level of malondialdehyde (4.68 nmol/ml) in postmenopausal women as compared to premenopausal women.

The drumstick leaves are found to be a potential source of natural antioxidants (Goyal et al. 2007). Bioactive compounds found in the leaves of drumstick are glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4′-O-acetyl- a -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B (Patel et al. 2010). These bioactive compounds helps to increase antioxidant level in body and decreases marker of oxidative stress i.e. malondialdehyde.

Percentage distribution of subjects based on antioxidant levels (Table 3) showed that 30 % and 33.3 % subjects of group II and group III were at risk for serum retinol level, which changed to 13.3 % and 20 %, respectively after supplementation. Likewise, 30 % and 33.3 % subjects of group II and III were at risk for serum ascorbic acid level. After supplementation only 6.6 % and 20 % subjects of group II and III were at risk. It was observed that 76.7 % and 70 % subjects of group II and III had glutathione peroxidase level below desirable range which decreased to 60 % in both the groups, respectively after supplementation. Table 3 showed that level of superoxide dismutase was below desirable level in 70 % and 76.7 % subjects of group II and III, after supplementation it decreased to 26.7 % and 33.3 %, respectively.

The mean initial fasting blood glucose levels of the subjects in group I, group II and group III were 125.66, 106.73 and 104.36 mg/dl and after supplementation period it decreased to 125.96, 91.56 and 93.46 mg/dl, respectively (Table 4). A highly significant ($p \leq 0.01$) decrease in blood

Table 2 Effect of supplementation of Antioxidant powder I (DLP) and II (ALP) on antioxidant levels and oxidative stress (n=30)

Parameters	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)	C.D. at 5 %	Standard range
Serum retinol (µg/dL)					
Baseline	33.5±0.99	34.1±1.09	33.5±0.99	NS	30–65 µg/dL[d]
After Exp.	34.0±1.00	37.1±1.05	35.2±0.96	NS	
% change	1.5	8.8	5.0		
Paired t-value	1.40[NS]	4.47**	2.09*		
Serum ascorbic acid (mg/dL)					
Baseline	0.8±0.07	0.8±0.05	0.8±0.04	NS	0.4–1.5 mg/dL[d]
After Exp.	0.8±0.07[a]	1.1±0.08[b]	0.8±0.04	0.19	
% change	3.6	44.4	5.9		
Paired t-value	2.00[NS]	5.89**	2.04*		
Glutathione peroxidase (U/g Hb)					
Baseline	20.7±1.29	19.6±0.73	17.3±0.81[c]	2.75	27.5–73.6 U/g Hb[e]
After Exp.	20.6±1.25	23.2±0.74[b]	19.3±0.77	2.69	
% change	0.6	18.0	11.9		
Paired t-value	1.77[NS]	7.98**	7.43**		
Superoxide dismutase (U/g Hb)					
Baseline	1080±212.69	1097±124.83	1076±142.08	NS	1102–1601 U/g Hb[f]
After Exp.	1082±223.07[a]	1211±247.46	1192±180.11[c]	61.01	
% change	0.2	10.4	10.8		
Paired t-value	1.15[NS]	4.70*	6.33**		
Malondialdehyde (nmoles/gHb)					
Baseline	285±11.35	288±12.18[b]	250±16.91[c]	29.26	
After Exp.	287±10.82[a]	241±12.74	226±16.35[c]	29.03	
% change	0.7	16.3	9.6		
Paired t-value	1.24[NS]	7.83**	4.38**		

Values represent Mean ± SE

NS non significant

Significant **P<0.01 Significant *P<0.05

Significant difference between group: [a]I and II, [b]II and III, [c]III and I

[d]Anonymous (2011)

[e]Ransod (2009)

[f]Ransel (2009)

For DLP, ALP refer Table 1

sugar levels was observed in experimental groups II and III, whereas a non significant change was observed in control group I. Hisa et al. (2008) reported 94 mg/dl fasting blood glucose level in postmenopausal women. Giridhari et al.

Table 3 Percentage distribution of subjects based on antioxidant levels (n=30)

Parameters	Group I (Control)		Group II (DLP supplementation)		Group III (ALP supplementation)	
	Baseline	After Exp.	Baseline	After Exp.	Baseline	After Exp.
Serum retinol (µg/dL)						
Desirable 30–65	19 (63.4)	20 (66.7)	21 (70)	26 (86.7)	20 (66.7)	24 (80)
Risk <30	11 (36.6)	10 (33.3)	9 (30)	4 (13.3)	10 (33.3)	6 (20)
Plasma ascorbic acid (mg/dL)						
Desirable 0.4–1.5	23 (76.7)	25 (83.4)	21 (70)	28 (93.4)	20 (66.7)	24 (80)
Risk <0.4	7 (23.3)	5 (16.6)	9 (30)	2 (6.6)	10 (33.3)	6 (20)
Glutathione peroxidase (U/g Hb)						
Desirable 27.5–73.6	3 (10)	3 (10)	7 (23.3)	12 (40)	9 (30)	12 (40)
Risk <27.5	27 (90)	27 (90)	23 (76.7)	18 (60)	21 (70)	18 (60)
Superoxide dismutase (U/g Hb)						
Desirable 1102–1601	5 (16.6)	6 (20)	9 (30)	22 (73.3)	7 (23.3)	20 (66.6)
Risk <1102	25 (83.4)	24 (80)	21 (70)	8 (26.7)	23 (76.7)	10 (33.3)

Values in parenthesis represents percentage

For DLP, ALP refer Table 1

Table 4 Effect of supplementation of Antioxidant powder I (DLP) and II (ALP) on fasting blood glucose and haemoglobin levels (n=30)

Parameters	Group I (Control)	Group II (DLP supplementation)	Group III (ALP supplementation)	C.D. at 5 %	Standard range
Fasting blood glucose level (mg/dl)					
Baseline	125.6±9.15	106.7±7.23	104.3±5.30	NS	70–110[e]
After Exp.	125.9±8.19[a]	91.5±3.29	93.4±2.68[c]	14.97	
% change	0.2	13.5	10.4		
Paired t-value	0.81[NS]	3.26**	3.08**		
Haemoglobin (%)					
Baseline	11.6±0.35	11.0±0.28	11.4±0.20	NS	12–16[d]
After Exp.	11.2±0.33[a]	13.0±0.26[b]	12.0±0.21[c]	0.77	
% change	3.5	17.5	5.3		
Paired t-value	3.00[NS]	10.9**	5.06*		

Values represent Mean ± SE

Significant **P<0.01 Significant *P<0.05

NS non significant

Significant difference between group : [a] I and II, [b] II and III, [c] III and I

[d] Anonymous (2011)

[e] Raghuram et al. (2007)

For DLP, ALP refer Table 1

(2011) reported that antioxidants like carotenoids, vitamins C and E, and flavonoids had an important role in reducing the blood glucose. This is due to the improvement in the impaired glucose metabolism and decrease in insulin resistance.

It was observed that the initial and final mean values of haemoglobin of the subjects in group I (control), group II (DLP supplementation) and group III (ALP supplementation) were 11.63, 11.06, 11.40 % and 11.22, 13.00 and 12.01 % respectively. A highly significant ($p \leq 0.01$) increase in blood haemoglobin was observed in experimental groups II and III. Drumstick leaves and amaranth leaves are rich in ascorbic acid which may help to increase the absorbtion of iron. Gulati et al. 2009 reported that haemoglobin concentration was higher in women receiving micronutrients supplementation.

Percentage distribution of subjects based on fasting blood glucose and haemoglobin (Table 5) showed that 33.4 % and 20 % subjects of group II and group III had higher level of fasting blood glucose level which decreased to 10 % and 6.6 %, respectively after supplementation. It was observed that 63.4 % and 60 % subjects of group II and III had haemoglobin level below standard range, which decreased to 30 % and 46.7 %, respectively after supplementation.

After supplementation of antioxidant powder I (drumstick leaves powder) and antioxidant powder II (amaranth leaves powder) a significant improvement in antioxidant levels in blood as well as decrease in oxidative stress was observed in group II and group III. A significant reduction in fasting blood glucose levels and increase in haemoglobin was observed in groups supplemented with drumstick leaves powder and amaranth leaves powder. Similar to findings of the present study, a significant reduction in post prandial blood glucose and glycated haemoglobin was observed after administration of drumstick leaves powder (98.34 %) tablet for 90 days in non insulin dependent diabetics by Giridhari et al. (2011).

Table 5 Percentage distribution of subjects based on fasting blood glucose and haemoglobin levels (n=30)

Parameters	Group I (Control)		Group II (DLP supplementation)		Group III (ALP supplementation)	
	Baseline	After Exp.	Baseline	After Exp.	Baseline	After Exp.
Fasting blood glucose level (mg/dl)						
Desirable 70–110	18 (60)	18 (60)	20 (66.6)	27 (90)	24 (80)	28 (93.4)
Risk >110	12 (40)	12 (40)	10 (33.4)	3 (10)	6 (20)	2 (6.6)
Haemoglobin (%)						
Desirable >12	14 (46.6)	12 (40)	11 (36.6)	21 (70)	12 (40)	16 (53.3)
Risk <12	16 (53.4)	18 (60	19 (63.4)	9 (30)	18 (60)	14 (46.7)

Values in parenthesis represents percentage

For DLP, ALP refer Table 1

Conclusion

The investigation of present study revealed that supplementation of drumstick leaves powder (7 g) and amaranth leaves powder (9 g) per day for 3 months significantly improved the antioxidant levels by increase in serum retinol, serum ascorbic acid, glutathione peroxidase, superoxide dismutase whereas decrease in marker of oxidative stress i.e. malondialdehyde in postmenopausal women of group II and group III. A significant decrease was also observed in fasting blood glucose level and increase in haemoglobin in group II and group III. Hence, it is recommended to consume drumstick leaves and amaranth leaves as they are rich source of antioxidants and helps in improving nutritional status.

References

Andrews DA, Andrews K (2009) Nutraceutical Moringa composition. US Patent 0098 230

Anilakumar KR, Khanum F, Santhanam K (2006) Amelioration of hexachlorocyclohexane-induced oxidative stress by amaranth leaves in rats. Plant Foods Hum Nutr 61:169–173

Anonymous (2011) Blood test results - normal ranges. Bloodbook.com. Cited from: http://www.bloodbook.com/ranges.html. Accessed 2 Oct 2012

Arora KS, Gupta N, Singh RA, Nagpal S, Arora D (2009) Role of free radicals in menopausal distress. J Clin Diagn Res 3:1900–1902

Bessey OA, Lowry OH, Brock J, Lopez JA (1946) The determination of vitamin A and carotene in small quantities of blood serum. J Biol Chem 166:177–188

Dacie JV, Lewis SM (1975) Practical haematology. English Language Book Society and Churchill Ltd, London, p 321

Esterbauer H, Chesseman KH, Dianazani MU, Poli G, Slater TF (1982) Separation and characterization of aldehydic products of LPO stimulated by ADP-Fe2+ in rat liver microsomes. Biochem J 208:129–140

Giridhari VVA, Malathi D, Geetha K (2011) Anti diabetic property of drumstick (*Moringa oleifera*) leaf tablets. Int J Health Nutr 2(1):1–5

Goyal BR, Agrawal B, Goyal RK, Mehta AA (2007) Phytopharmacology of *Moringa oleifera* Lam.o An overview. Nat Prod Radiance 6(4):347–353

Gulati R, Bailey R, Prentice AM, Brabin BJ, Owens S (2009) Haematological effects of multimicronutrients supplementation in non-pregnant Gambian women. Eur J Clin Nutr 63(8):970–977

Hisa I, Kaji H, Inoue Y, Sugimoto T, Chihara K (2008) Fasting plasma glucose levels are related to bone mineral density in postmenopausal women with primary hyperparathyroidism. Int J Clin Exp Med 1(4):319–326

Kaya H, Ozkaya O, Sezik M, Arslanoglu E, Yilmaztepe A, Ulukaya E (2005) Effect of raloxifene on serum malondialdehyde, erythrocyte superoxide dismutase, and erythrocyte glutathione peroxidase levels in healthy postmenopausal women. Maturitas 50:182–188

Khandaker L, Ali MB, Oba S (2008) Total polyphenol and antioxidant activity of Red Amaranth (*Amaranthus tricolor* L.) as affected by different sunlight level. J Japan Soc Hortic Sci 77(4):395–401

Lako J, Trenerry VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R (2007) Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem 101:1727–1741

Moilanen J, Aalto AM, Hemminki E, Aro AR, Raitanen J, Luoto R (2010) Prevalence of menopause symptoms and their association with lifestyle among Finnish middle-aged women. Maturitas 67:368–374

Patel S, Thakur AS, Chandy A, Manigauha A (2010) Moringa oleifera: a review of their medicinal and economical importance to the health and nation. Drug Interv Today 2(7):339–342

Raghuram TC, Pasricha S, Sharma RD (2007) Diet and diabetes. ICMR, Hyderabad

Raghuramulu N, Nair KM, Kalyanasundaram S (2003) A manual of laboratory techniques. National Institute of Nutrition, Hyderabad

Ransel (2009) Randox Laboratories Ltd, United Kingdom, pp. 1–4

Ransod (2009) Randox Laboratories Ltd, United Kingdom, pp.1–3

Sachan DS, Hongu N, Johsen M (2005) Decreasing oxidative stress with Choline and Carnitine in women. J Am Coll Nutr 24(3):172–176

Srivastava V, Singh S, Neelima S, Shaila S (2005) Status of antioxidant enzymes and trace metals in postmenopausal women. J Obstet Gynecol India 55(1):64–66

Trinder P (1969) Practical clinical biochemistry, vol X, 5th edn. William Heinnemann Medical Books Limited, New York