

Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesteremic rats

N. P. Visavadiya, A.V.R.L. Narasimhacharya

Department of
Biosciences, Sardar Patel
University, Vallabh
Vidyanagar – 388 120.
Gujarat, India

Received: 28.3.2005

Revised: 23.7.2005

Accepted: 26.7.2005

Correspondence to:
A.V.R.L. Narasimhacharya
E-mail: n.bs@spu.ernet.in

ABSTRACT

Objective: To study the efficacy of *Asparagus racemosus* in reducing the cholesterol levels and as an antioxidant in hypercholesteremic rats.

Materials and Methods: Hypercholesteremia was induced in normal rats by including 0.75 g% cholesterol and 1.5 g% bile salt in normal diet and were used for the experiments. Dried root powder of *Asparagus racemosus* was administered as feed supplement at 5 g% and 10 gm% dose levels to the hypercholesteremic rats. Plasma and liver lipid profiles, hepatic HMG-CoA reductase, bile acid, malondialdehyde, ascorbic acid, catalase and SOD, fecal bile acid, cholesterol and neutral sterols were estimated using standard methods.

Results: Feed supplementation with 5 g% and 10 g% *Asparagus racemosus* resulted in a significant decline in plasma and hepatic lipid profiles. The feed supplementation increased the HMG-CoA reductase activity and bile acid production in both groups (5 and 10 g% supplemented groups) with concomitant increase in fecal bile acid and fecal cholesterol excretion. The activities of catalase, SOD and ascorbic acid content increased significantly in both the experimental groups (5 and 10 g% supplemented groups). On the other hand, the concentration of malondialdehyde in these groups (5 and 10 g% supplemented groups) decreased significantly, indicating decreased lipid peroxidation.

Conclusion: The present study demonstrates that addition of *Asparagus racemosus* root powder at 5 g% and 10 g% level as feed supplement reduces the plasma and hepatic lipid (cholesterol) levels and also decreases lipid peroxidation.

KEY WORDS: Feed supplement, HMG-CoA reductase, lipid profile.

Introduction

Raised serum lipid levels, particularly of cholesterol along with generation of reactive oxygen species (ROS), play a key role in the development of coronary artery disease (CAD) and atherosclerosis.^[1] CAD is a serious medical problem that affects millions of people annually throughout the world. People who are predisposed to a combination of risk factors (dietary habits, genetic susceptibility, etc.) are more prone to develop atherosclerosis and CAD. Besides stress, sedentary habits, use of tobacco and alcohol are reported to have an additive effect in contributing to development of atherosclerosis and CAD.^[2] Dietary modification, physical exercise, abstinence from tobacco and alcohol, and changes in life-style have been proposed to reduce the incidence of CAD and other cardiac maladies by medical fraternity all over the world. Phytosterols and natural antioxidants have also been shown to be effective in reducing lipid profiles and also mitigate peroxidative modification of lipoproteins and atherosclerosis.^[3]

Asparagus racemosus Willd. (Liliaceae), commonly known

as 'satawar', 'satavari' or 'shatavari', has been used as antidiarrheal, antiulcerogenic, refrigerant, tonic, demulcent, diuretic, galactagogue, aphrodisiac and antispasmodic in Ayurvedia, Siddha and Unani systems of medicine.^[4] Besides, *Asparagus racemosus* has also been found to have antioxytocin, immunostimulant and hepatoprotective activities.^{[5], [6]}

As there have been no reports on the hypocholesteremic and antiperoxidative effects of *Asparagus racemosus*, the present study was undertaken to evaluate its ability to reduce the cholesterol profile and its antiperoxidative effects on body lipids.

Materials and Methods

Plant material

Fresh roots of *Asparagus racemosus* were harvested from Sardar Patel University Botanical garden and were dried at 37°C in an incubator. Then the dried roots were powdered in a mixer grinder and used as feed supplement.

Animals

Three-month-old male albino rats (Charles Foster,

150-200 g) were selected from the animal house, Department of Biosciences, and used with the approval of Animal Ethics Committee. Animals were housed individually in a well-ventilated animal unit with normal daylight. The animals were fed standard food (Pranav Agro-Industries Ltd.) and water *ad libitum*. After a 10-day adaptation period, the animals were divided into three groups (n=8) and the following treatments were given simultaneously to the concerned groups for four weeks.

Group-I: Standard diet mixed with 0.75 g% cholesterol and 1.5 g% bile salt to induce hypercholesteremia.

Group-II: Hypercholesteremic animals were given a 5 g% *Asparagus racemosus* root powder as feed supplement

Group-III: Hypercholesteremic animals were given a 10 g% *Asparagus racemosus* root powder as feed supplement.

Estimation of biochemical parameters

After the conclusion of the experiment, the animals were subjected to overnight fasting and killed under mild anesthesia.

Plasma

Blood samples were drawn by retro-orbital puncture using a fine sterile capillary tube and the plasma used for the estimation of total lipids,^[7] total cholesterol,^[8] triglycerides,^[9] HDL-cholesterol,^{[8], [10]} LDL-cholesterol, VLDL-cholesterol and the atherogenic index was calculated as described by Friedewald *et al.*^[11] The base line plasma lipid profiles and the fecal bile acid, cholesterol and neutral sterol profiles were determined prior to the treatment regime.

Liver

Hepatic lipids were extracted^[12] and estimated gravimetrically. Total hepatic cholesterol and triglycerides were extracted^[12] and estimated.^{[8], [9]} HMG-CoA reductase activity was assayed by the method of Rao and Ramakrishnan and expressed as the ratio of absorbance of HMG-CoA to mevalonate. This was taken as the index of HMG-CoA

reductase^[13] activity. Hepatic bile acid was estimated by the method of Snell and Snell.^[14] Malondialdehyde, catalase, superoxide dismutase and total ascorbic acid content were assayed using standard methods.^{[15]-[19]}

Fecal matter

Fecal bile acid, cholesterol and neutral sterols were extracted^[20] and estimated.^{[8], [14]}

Statistical analysis

Statistical evaluation was done using the one-way ANOVA. Duncan's test was performed for *post-hoc* analysis. Differences with $P < 0.05$ were considered significant. Data are presented as mean \pm SEM.

Results

Plasma and hepatic lipid profiles

A. racemosus as 5 g% feed supplementation to hypercholesteremic animals resulted in a decrease of total lipids (29%), total cholesterol (29%), triglycerides (39%), LDL-cholesterol (33%), VLDL-cholesterol (39%), atherogenic index (37%) and an increase in HDL-cholesterol content (11%). With 10gm% *A. racemosus* treatment, a further reduction occurred in total lipids (64%), total cholesterol (38%), triglycerides (52%), LDL-cholesterol (44%), VLDL-cholesterol (52%) and atherogenic index (49%). This reduction in total lipids, total cholesterol, triglycerides, LDL-, VLDL- cholesterol and atherogenic index was dose-dependent and significant. A further increase in HDL-cholesterol (21%) level was also noted as compared to Group-II animals. [Table 1] *A. racemosus* also reduced total lipids (26% and 36%, respectively), total cholesterol (46% and 57%, respectively) and triglycerides (38% and 57%, respectively) in the liver of treated groups as compared to control. [Table 2]

Cholesterol metabolism and excretion

A significant increase in hepatic HMG-CoA reductase activity was noted in Group-II (27%) and Group-III (37%) compared to the control hypercholesteremic animals. The

Table 1

Effect of *Asparagus racemosus* (root powder) on plasma lipid profiles in rats

Plasma	Treatment	TL	TC	TG	HDL-C	LDL-C	VLDL-C	AI
Baseline Values*	-	505.37 \pm 8.94	128.92 \pm 2.58	60.40 \pm 2.33	71.87 \pm 0.88	45.04 \pm 2.80	12.00 \pm 0.80	1.79 \pm 0.03
Group-I	HC	1444.16 \pm 20.64 ^a (+ 185.76)	505.64 \pm 11.5 ^a (+ 292.21)	149.38 \pm 8.18 ^b (+ 148.80)	49.95 \pm 1.74 ^c (- 30.49)	425.82 \pm 10.07 ^a (+ 845.42)	29.87 \pm 1.63 ^b (+ 148.91)	10.17 \pm 0.29 ^a (+468.15)
Group-II	HAR5	1030.83 \pm 27.93 ^b (- 28.62)	358.08 \pm 9.50 ^b (- 29.18)	91.55 \pm 7.27 ^c (- 38.71)	55.65 \pm 1.19 ^b (+ 11.41)	284.12 \pm 10.08 ^b (- 33.27)	18.30 \pm 1.45 ^c (- 38.73)	6.44 \pm 0.18 ^b (-36.67)
Group-III	HAR10	519.16 \pm 10.90 ^c (- 64.05)	315.23 \pm 9.11 ^c (- 37.65)	70.90 \pm 6.43 ^c (- 52.47)	60.51 \pm 0.52 ^a (+ 21.14)	240.52 \pm 10.11 ^c (- 43.51)	14.19 \pm 1.28 ^c (- 52.49)	5.21 \pm 0.17 ^c (- 48.77)
One-way ANOVA	F	488.737	98.135	30.622	17.747	30.848	92.677	131.819
	df	2, 21	2, 21	2, 21	2, 21	2, 21	2, 21	2, 21
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Values are mean \pm SEM; n=8 in each group. The lipid profiles are given in mg/dl. *Base line values represent pretreatment values. Group-I HC: Hypercholesteremic diet; Group-II HAR5: Hypercholesteremic diet with 5% *A.racemosus* root powder; Group-III HAR10: Hypercholesteremic diet with 10% *A. racemosus* root powder. Figures in parentheses indicate percent increase (+) or decrease (-) with reference to base line values Vs. group I, Group I Vs.II and group I Vs.III. Values in a column with different superscripts differ, $P < 0.05$. TL-total lipids; TC-total cholesterol; TG-triglycerides; HDL-C-HDL cholesterol; LDL-C-LDL cholesterol; VLDL-C-VLDL cholesterol; AI-atherogenic index.

Table 2**Effect of *Asparagus racemosus* (root powder) on hepatic lipid profiles in rats**

Liver	Treatment	TL	TC	TG
		mg/g		
Group-I	HC	97.87±4.71 ^b	25.51±1.56 ^b	22.35±0.86 ^a
Group-II	HAR5	72.37±3.91 ^c (- 26.05)	13.66±0.84 ^c (- 46.45)	13.85±0.50 ^b (- 38.03)
Group-III	HAR10	62.50±2.91 ^c (- 36.13)	10.93±0.86 ^c (-57.15)	9.63±1.08 ^c (- 56.91)
One-way	F	21.825	46.338	57.924
ANOVA	df	2, 21	2, 21	2, 21
	P	0.0001	0.0001	0.0001

Values are mean ± SEM; n=8 in each group. Group-I HC: Hypercholesteremic diet; Group-II HAR5: Hypercholesteremic diet with 5% *A.racemosus* root Powder; Group-III HAR10: Hypercholesteremic diet with 10% *A.racemosus* root powder. Figures in parentheses indicate percent increase (+) or decrease (-). Comparisons for percentage were taken between Groups I Vs.II and I Vs.III. Values in a column with different superscripts differ, P<0.05. TL-total lipids; TC-total cholesterol; TG-triglycerides

hepatic bile acid production also increased [Table 3] in treated groups (12% and 25%, respectively). The fecal cholesterol metabolites excretion, such as bile acid (31% and 25%, respectively), cholesterol (14% and 28%, respectively) and neutral sterols (8% and 5%, respectively) increased in *A. racemosus* treated groups compared to control. [Table 4]

Antioxidant activities in hepatic tissue

The hepatic lipid peroxidation (malondialdehyde content) decreased significantly in *A. racemosus* treated groups (21% and 20%, respectively) when compared to control. The activities of catalase and superoxide dismutase also increased in both experimental groups (II and III 34%, 34% and 17%, 18%, respectively) as compared to the hypercholesteremic groups. The hepatic ascorbic acid content of both experimental groups (II and III) also exhibited a similar increase (25% and 24%, respectively) when compared to the values obtained for Group-I. [Table 5]

Discussion

Addition of *A. racemosus* dried root powder as a feed supplement at two levels, i.e., 5 g% and 10 g%, resulted in a dose-dependent reduction in lipid profiles in plasma and liver along with significant reduction in lipid peroxidation. The total lipids, total cholesterol and triglycerides in plasma and liver as well as plasma LDL- and VLDL-cholesterol were significantly reduced at both doses of feed supplementation. However, HDL-cholesterol level increased in both treated groups significantly. This observation indicates that *A. racemosus* root powder, as a feed component is effective in reducing plasma LDL- and VLDL-cholesterol levels. It is well known that increased HDL-cholesterol levels have a protective role in CAD.^[21] The decreased hepatic lipids including cholesterol and triglycerides in treated animals along with increased bile acid, cholesterol and neutral sterols content in fecal matter indicate that *A.racemosus* may reduce the absorption of dietary cholesterol

Table 3**Effect of *Asparagus racemosus* on the activity of HMG-CoA reductase and bile acid content in liver**

Liver	Treatment	HMG-CoA reductase*	Bile acid (mg/g)
Group-I	HC	5.26 ± 0.34 ^b	4.06 ± 0.12 ^c
Group-II	HAR5	3.82 ± 0.34 ^c (+ 27.37)	4.54 ± 0.11 ^b (+ 11.82)
Group-III	HAR10	3.31 ± 0.12 ^c (+ 37.07)	5.09 ± 0.20 (+ 25.36)
One-way	F	11.949	11.097
ANOVA	df	2, 21	2, 21
	P	0.0001	0.001

*HMG-CoA reductase activity is expressed as the ratio of HMG-CoA/Mevalonate and its activity is inversely proportional to the ratio HMG CoA/Mevalonate. Values are mean±SEM; n=8 in each group. Group-I HC: Hypercholesteremic diet; Group-II HAR5: Hypercholesteremic diet with 5% *A.racemosus* root powder; Group-III HAR10: Hypercholesteremic diet with 10% *A.racemosus* root powder. Figures in parentheses indicate percent increase (+) or decrease (-); Comparisons for percentage were taken between Groups I Vs.II and I Vs.III. Values in a column with different superscripts differ, P<0.05.

Table 4**Effect of *Asparagus racemosus* on bile acid, cholesterol and neutral sterols of fecal matter**

Fecal	Treatment	Bile acid	Cholesterol	Neutral sterols
		mg/g		
Baseline Values*	-	4.65±0.08	0.36±0.01	5.01±0.05
Group-I	HC	10.90±0.62 ^c (+134.40)	4.10±0.15 ^c (+ 1038.88)	13.77±0.42 ^c (+ 174.85)
Group-II	HAR5	14.32±0.68 ^b (+ 31.37)	4.68±0.17 ^b (+ 14.14)	14.88±0.42 ^c (+ 8.06)
Group-III	HAR10	13.65±0.64 ^b (+ 25.22)	5.23±0.10 ^a (+ 27.56)	14.52±0.71 ^c (+ 5.44)
One-way	F	7.785	14.096	1.103
ANOVA	df	2, 21	2, 21	2, 21
	P	0.01	0.0001	NS

Values are mean ± SEM; *Baseline values are the values obtained prior to treatments. Group-I HC: Hypercholesteremic diet; Group-II HAR5: Hypercholesteremic diet with 5% *A.racemosus* root Powder; Group-III HAR10: Hypercholesteremic diet with 10% *A.racemosus* root powder. Figures in parentheses indicate percent increase (+) or decrease (-) with reference to base line values Vs. group I, group I Vs.II and group I Vs.III. Values in a column with different superscripts differ, P<0.05.

and enhance its excretion. A similar result was reported when soy protein was used as feed supplement.^[22] The increased HMG CoA reductase activity noted in both experimental groups (II and III) as compared to control could be due to an increased cholesterol excretion and decreased cholesterol absorption through the gastrointestinal tract. Thus the decreasing cholesterol levels in the body under the influence of *A.racemosus* could have enhanced the enzymatic activity by a positive feedback mechanism. Further, increased bile acid

Table 5
Concentrations of malondialdehyde (MDA), total ascorbic acid and activities of catalase and superoxide dismutase in liver

Liver	Treatment	Lipid peroxidation nm MDA/g	Total ascorbic acid µg	Catalase nm H ₂ O ₂ decomposed/ sec /g	Superoxide dismutase unit /mg protein
Group-I	HC	22.58 ± 0.61 ^b	42.76 ± 0.43 ^c	12.82 ± 0.49 ^c	3.04 ± 0.10 ^c
Group-II	HAR5	17.91 ± 0.65 ^c (-20.68)	53.62 ± 0.90 ^b (+ 25.39)	17.20 ± 0.50 ^b (+ 34.16)	3.57 ± 0.08 ^b (+ 17.43)
Group-III	HAR10	17.98 ± 0.42 ^c (- 20.37)	53.17 ± 0.86 ^b (+ 24.34)	17.24 ± 0.44 ^b (+ 34.47)	3.60 ± 0.10 ^b (+ 18.42)
One-way	F	21.698	64.085	27.800	9.046
ANOVA	df	2, 21	2, 21	2, 21	2, 21
	P	0.0001	0.0001	0.0001	0.001

Values are mean ± SEM; Group-I HC: Hypercholesteremic diet; Group-II HAR5: Hypercholesteremic diet with 5% *A.racemosus* root powder; Group-III HAR10: Hypercholesteremic diet with 10% *A.racemosus* root powder. Figures in parentheses indicate percent increase (+) or decrease (-); n=8/group. Comparisons for percentage were taken between Groups I Vs.II and I Vs.III. Values in a column with different superscripts differ, P<0.05.

production also indicates the turnover of endogenous cholesterol into bile acid that could be under the influence of supplementary feeding with *A.racemosus*. A similar modulator activity was observed when guar gum was used as feed supplement.^[23]

Hypercholesteremia, high cholesterol diet and oxidative stress increase serum LDL levels resulting in increased risk for development of atherosclerosis.^[24] Besides, malondialdehyde a secondary product of lipid peroxidation is a major reactive aldehyde; higher levels can lead to peroxidation of biological membranes.^[25] The antioxidant enzymes, mainly superoxide dismutase and catalase are first line defensive enzymes against free radicals and, ascorbic acid is also known to control oxidative damage.^[26] The present work shows that the *A.racemosus* treated groups have higher levels of antioxidative parameters (ascorbic acid, catalase and superoxide dismutase) and decreased level of lipid peroxidation indicating its efficacy to reduce the LDL-cholesterol oxidation. The quantitative analysis of *A. racemosus* root powder indicated the presence of flavonoids, polyphenols and ascorbic acid (4.7 ± 0.32 mg/g, 16.9 ± 1.1 mg/g and 7.6 ± 0.06 mg/g, respectively, our unpublished data). It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD and catalase activities.^[27-34] Further, it was shown that these compounds act as promoters for SOD and catalase^[31] and cause the expression of SOD and catalase.^[33] The currently noted elevated levels of both SOD and catalase with *A.racemosus* root powder could be due to the influence of flavonoids and polyphenols. A significantly elevated ascorbic acid content in the hepatic tissues of treated groups due to dietary supplementation with *A.racemosus* root powder could have reduced the hepatic MDA levels leading to a significant decrease in lipid peroxidation.^[26]

To conclude, feed supplementation with *A. racemosus* dried root powder reduced the hyperlipidemic and hypercholesteremic conditions. *A. racemosus* appeared to ameliorate hypercholesteremia probably by decreasing the exogenous cholesterol absorption and increasing the

endogenous cholesterol conversion to bile acid, though to know the exact mechanism further studies are needed.

References

- Ross R. Atherosclerosis: An inflammatory disease. *N Eng J Med* 1999;340:115-26.
- Ashakumary A, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. *Indian J Exp Biol* 1993;31:270-4.
- Ikeda I, Sugano M. Inhibition of cholesterol absorption by plant sterols for mass intervention. *Curr Opin Lipido* 1998;9:527-31.
- Kapoor LD. Hand book of Ayurvedic medicinal plants. Herbal Reference Library Edition. New York: CRC Press; 2001.
- Ravikumar PR, Soman R, Chetty GL, Pandey RC, Sukh Dev. Chemistry of Ayurvedic crude drugs: Part VI-(Shatavari-I). Structure of Shatavarin -IV. *Indian J Chem* 1987;26:1012-7.
- Maruganandan S, Garg H, Lal J, Chandra S, Kumar D. Studies on the immunostimulant and antihepatotoxic activities of *Asparagus racemosus* root extract. *J Med Arom Plant Sci* 2001;23:49-51.
- Fringe CS, Fendley TW, Dunn RT, Owen CA. Improved determination of total serum lipids by sulphosphovanillin reaction. *Clin Chem* 1972;18:673-4.
- Wybenga DR, Pileggi VJ, Dirstine PH, Di Giorgio J. Direct manual determination of serum total cholesterol with a single stable reagent. *Clin Chem* 1970;16:980-4.
- Mc Gown MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 1983;29:538-42.
- Burstein M, Scholnic HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanion. *J Lipid Res* 1970;11:583-95.
- Friedewald WT, Levy RT, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
- Rao AV, Ramakrishnan S. Indirect assessment of hydroxy-methylglutaryl-CoA reductase (NADPH) activity in liver tissue. *Clin Chem* 1975;21:1523-5.
- Snell FD, Snell CT. Colorimetric Methods of Analysis. 3 ed. Vol. 3. Canada: D.Van no Strand Company, Inc.; 1953.
- Niehaus Jr. WG, Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Europ J Biochem* 1968;6:126-30.
- Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extract. *Anal Biochem* 1970;34:30-8.
- Aebi H. Methods of Enzymatic Analysis. 2 ed. Vol. 2. (Ed Bergmeyer). New York: Academic Press; 1974.

18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984;21:130-2.
19. Schaffert RR, Kingsley GR. A rapid simple method for the determination of reduced, dehydro, and total ascorbic acid in biological material. *J Bio Chem* 1955;212:59-68.
20. Kalek HD, Stellaard F, Kruis W, Paumgartner G. Detection of increase bile acid excretion by determination of bile acid content in simple stool samples. *Clin Chem Acta* 1984;140:85-90.
21. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham heart study. *Arterioscler Thromb Vasc Biol* 1988;8:737-41.
22. Lin Y, Meijer GW, Mario A, Vermeer, Trautwein EA. Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol-fed hamsters. *J Nutr* 2004;134:143-8.
23. Moundras C, Behr SR, Remesy C, Demigne C. Fecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. *J Nutr* 1997;127:1068-76.
24. Warnholtz A, Mollnau H, Oelze M, Wendt M, Munzel T. Antioxidants and endothelial dysfunction in Hyperlipidemia. *Curr Hypertension Reports* 2001;3: 53-60.
25. Tiwari AK. Natural product antioxidants and their therapeutic potential in mitigating peroxidative modification of lipoproteins and atherosclerosis: recent development. *J Med Arom Plant Sci* 1999;21:730-41.
26. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Oxidant and antioxidants in atherogenesis: an appraisal. *J Lipid Res* 1999;40:2143-57.
27. Fang YZ, Yang S, Wu G. Free radicals, Antioxidants and Nutrition. *Nutrition* 2002;18:872-9.
28. Badami S, Gupta MK, Suresh B. Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. *J Ethanopharmacol* 2003;85:227-30.
29. Frei B, Higdon JV. Antioxidant activity of tea polyphenols *in vivo*: Evidence from animal studies. *J Nutr* 2003;133:3275-84.
30. Soto C, Recoba R, Barron H, Alvarez C, Favari L. Silymarin increases antioxidant enzymes in alloxan induced diabetes in rat pancreas. *Comp Biochem Physiol C Toxicol Pharmacol* 2003;136:205-12.
31. Toyokuni S, Tanaka T, Kawaguchi W, Fang NR, Ozeki M, Akatsuka S, *et al.* Effects of the phenolic contents of Mauritian endemic plant extracts on promoter activities of antioxidant enzymes. *Free Radic Res* 2003;37:1215-24.
32. Jung SH, Lee YS, Lim SS, Lee S, Shin KH, Kim YS. Antioxidant activities of isoflavones from the rhizomes of *Belamcanda chinensis* on carbon tetrachloride induced hepatic injury in rats. *Arch Pharm Res* 2004;27:184-8.
33. Ranaivo HR, Rakotoarison O, Tesse A, Schott C, Randrianisoa A, Lobstein A, *et al.* *Cedrelopsis grevei* induced hypotension and improved endothelial vasodilation through an increase of Cu/Zn SOD protein expression. *Am J Physiol Heart Circ Physiol* 2004;286:775-81.
34. Sudheesh S, Vijayalakshmi NR. Flavonoids from *Punic granatum*- potential antiperoxidative agents. *Fitoterapia* 2005;76:181-6.

HOW TO KILL A SOCIETY

1. Do not go to meetings - let "them" handle things. Then complain that members have no voice in the management.
2. If you go, arrive late and limit your remarks to destructive comments.
3. Don't pay your dues. Don't participate in membership drive.
- 4 Decline offices or appointments. But become indignant if you are not nominated or appointed.
5. If you are appointed or elected, fail to attend meetings. When you do attend a meeting, pretend to be active, volunteer for something, then forget all about it after the meeting.
6. Refuse to speak out, and then complain that no one listens to you and you have learnt nothing.
7. Don't read the organization's newsletter. Then complain that you're never kept informed.
8. Don't volunteer your talents - that's ego fulfillment. Then complain that you're never asked and never appreciated.
9. Do not work if you can avoid it. When others roll up their sleeves and do their very best, complain that the association is run by a group of ego trippers.
8. Oppose all banquets, parties, and social events as being a waste of members' money.
9. When everything is strictly business, complain that meetings are dull and the organizers a bunch of old sticks.
10. Complain that the official publication is of low standard but submit your good quality work to foreign journals.
11. When the society dies say you saw it coming years before.

And, if by chance the organization grows in spite of your contributions, grasp every opportunity to tell the youngsters how tough it was and how hard you worked in the old days to bring the organization to it's present level of success.

(From the Net)