A COMPARATIVE STUDY OF ANTIOXIDANT VITAMINS AND SIMVASTATIN IN HYPERCHOLESTEROLIMIC RABBITS

NAWAZISH-I-HUSAIN SYED*, SHAMAS ARSHAD, AMIR ALI RIZVI SYED, MAQSOOD AHMAD, FARRAKH ZIA KHAN AND BASHIR AHMAD

University College of Pharmacy, University of the Punjab, Lahore, Pakistan

ABSTRACT

The anti-lipidemic effects of orally administered antioxidant vitamins (vitamin A, vitamin C and vitamin E) individually and in combination were studied in cholesterol-fed rabbits and compared to the group of hypercholesterolemic animals that were treated with simvastatin. All treatment groups exhibited a decrease in serum total cholesterol, low density lipoprotein-cholesterol (LDL-C) and triglycerides concentrations, whilst vitamin C, vitamin E, the combination and simvastatin showed a more profound decrease in the lipid profile than vitamin A at different time intervals. The order of increase in high density lipoprotein-cholesterol (HDL-C) levels remained in favour of simvastatin, as none of the antioxidant vitamins treated group could exhibit a profound increase in the HDL-C.

Keywords: Antioxidants, dyslipidemia, hypocholesterolemia, simvastatin.

INTRODUCTION

Elevated levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides; as well as, decreased levels of high-density lipoprotein cholesterol (HDL-C) lead to atherosclerosis and/or related conditions, such as coronary heart diseases (Tulenko & Sumner, 2002). A variety of pharmacological agents including statins such as simvastatin (Todd & Goa, 1990; Vaughan *et al*., 1996) and nutrients, such as antioxidant vitamins (Husain *et al*., 2004; McRae, 2007; McRae, 2008) may therapeutically be used to treat dyslipidemia, in addition to recommending a modified life style.

Nutritional agents such as antioxidant vitamins (vitamin A, vitamin C, and vitamin E) have shown some activity in decreasing serum cholesterol levels (McRae, 2007; McRae, 2008). Present study was carried out to investigate the effects of antioxidant vitamins, individually and in combination in modulating serum total cholesterol, LDL-C, triglycerides and HDL-C in cholesterol-fed rabbits and compared with those obtained by administering simvastatin.

MATERIALS AND METHODS

Drugs

Cholesterol of analytical grade was obtained from E. Merck (Germany). Vitamins E (alpha-tocopherol) and A (retinol) in powder form were supplied by Irza Pharma and Schazoo Laboratories, Lahore, respectively. Vitamin C (ascorbic acid) tablets marketed as Ascorbon by Pfizer were purchased from local market. Simvastatin tablets (10 mg) marketed as Zocor by MSD was donated by their representative in Lahore, Pakistan.

**Corresponding author:* e-mail: snihusain@yahoo.com

Animals

Healthy albino male rabbits (*Caprolagus hispidus*) with an average body weight of 1.4 Kg were acclimatized for a week in the animal house of University College of Pharmacy, University of the Punjab, Lahore. During this period, fresh green fodder and tap water were provided *ad libitum*. The temperature of the animal house was maintained at 22 ± 2ºC. Rabbits were randomly divided into six groups, each of six animals. They were made hypercholesterolemic by administering cholesterol 250 mg/kg b.w. for one week prior to administration of experimental drugs. Group 1 received vitamin A (50 µg/kg body weight), group 2 received vitamin C (30 mg/kg), group 3 received vitamin E (30 mg/kg), group 4 received combination of vitamins A, C and E (50 mg, 30 mg and 30 mg respectively/ kg), group 5 was administered simvastatin (3 mg/kg) and group 6 received empty gelatin capsules and served as a control.

The drugs were administered orally once daily for 56 days. During this period, cholesterol supplementation was continued to all groups of rabbits. Blood samples were drawn from the marginal ear vein before administration of the dose and then at 7, 14, 28, 42 and 56 post-treatment days.

Biochemical analysis of blood

Blood samples were analyzed to estimate serum levels of total cholesterol, LDL-C, triglycerides and HDL-C by enzymatic kit method (Randox, U.K).

STATISTICAL ANALYSIS

The mean effects of vitamin A in group 1, vitamin C in group 2, vitamin E in Group 3, combination of vitamins in groups 4, simvastatin in group 5 and control in group 6 on various biochemical parameters, were recorded with their

effective ranges. The comparative relationship for the various parameters among the six groups at six time intervals (from 0 to 56 days) was determined by analysis of variance (ANOVA) and Duncan's multiple range test. $P<0.05$ was considered significant.

RESULTS

Table 1 shows the lipid profile levels in the six groups of rabbits, before and after receiving cholesterol supplementation. After seven daily doses 9-10, 10-11 and 3-3.5 fold increase was observed in serum total cholesterol, LDL-C and triglyceride levels respectively. However, serum HDL-C did not seem to change significantly.

Serum cholesterol levels of the various groups of hypercholesterolemic rabbits receiving different therapies are given in table 2. The day zero (0) levels of cholesterol prior to administration of any therapy were considered as 100%. A continuous and consistent decrease of serum cholesterol levels was observed in all the animal groups which were administered a therapy. Cholesterol levels in the vitamin C- and vitamin E- treated groups showed over 60% decrease after 56 days (table 2). Drop in cholesterol levels in the group treated with the combination of

Table 1: Serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels (mg/dl) of rabbits (n=6) that received cholesterol (250 mg/kg b.w. /day) daily for seven days. Serum lipid levels were measured two hours after administration of cholesterol on day zero and day seven.

CHOLESTEROL						
Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	34.54±2.97 (100%)	35.38± 3.45 (100%)	37.64±2.19 (100%)	36.61±2.97 (100%)	39.02±3.0 (100%)	44.53±1.20 (100%)
7	334.71±8.62 (969.05%)	368.11±23.18 (1040.44%)	370.28±16.92 (983.74%)	390.0±15.12 (1065.28%)	357.56±20.24 (916.35%)	421.24±3.32 (945.96%)
LDL-CHOLESTEROL						
0	28.49±2.39 (100%)	29.17±3.0 (100%)	31.05±1.87 (100%)	31.37±2.70 (100%)	32.20±2.70 (100%)	38.13±1.95 (100%)
7	309.94±7.48 (1087.89%)	341.48±26.37 (1170.65%)	350.48±17.96 (1128.72%)	359.57±12.72 (1146.22%)	335.20±19.37 (1041.0%)	403.56±5.01 (1058.96%)
HDL-CHOLESTEROL						
0	11.55±0.27 (100%)	13.60±0.87 (100%)	12.23±0.53 (100%)	13.62±0.64 (100%)	11.03±0.76 (100%)	13.10±0.81 (100%)
7	12.93±0.36 (111.94%)	13.89±0.63 (102.13%)	12.89±0.44 (105.39%)	13.84±0.42 (101.61%)	11.46±0.80 (103.89%)	12.73±0.57 (97.17%)
TRIGLYCERIDES						
0	54.56±4.08 (100%)	37.20±3.54 (100%)	48.62±2.84 (100%)	35.08±2.80 (100%)	43.60±3.82 (100%)	49.54±2.76 (100%)
7	168.72±8.75 (309.23%)	144.97± .59 (389.70%)	162.78±5.89 (334.80%)	156.16±2.75 (445.15%)	156.48±7.27 (358.90%)	158.19±4.70 (319.31%)

Each value presents Mean ± S.E.M. (mg/dl) (n=6). The values in parentheses present % increase or decrease in concentration of lipid levels considering the day zero value as 100%.

Table 2: The serum total cholesterol levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin A (50 µg/kg B.W./day), vitamin C (30 mg/kg b.w./day), vitamin E (30 mg/kg b.w./day), vitamin A+C+E (50µg/kg + 30mg/kg + 30mg/kg b.w./day, respectively), simvastatin 3mg/kg b.w./day) and vehicle. Serum cholesterol levels were measured two hours after administration of therapies on various days.

Days	Vitamin A	Vitamin C	Vitamin E	Vitamin A±C±E	Simvastatin	Control
0	334.71±8.62 (100%)	368.11±23.18 (100%)	370.28±16.92 (100%)	390.0±15.12 (100%)	357.56±20.24 (100%)	421.24±3.32 (100%)
7	293.60±11.24 (12.28%)	291.34±22.06 (20.85%)	279.81±12.53 (24.43%)	296.36±10.30 (24.10%)	264.26±18.67 (26.09%)	421.50±3.50 (0.06%)
14	278.06±12.94 (16.92%)	258.22±20.65 (29.85%)*	262.15±11.47 (29.10%)*	226.30±12.11 (41.97%)*	241.44±17.72 (32.47%)	409.1±5.06 (2.88%)
28	261.65±11.86 (21.82%)	225.38±16.79 (38.77%)*	227.11±7.96 (38.66%)*	164.05±7.28 (57.93%)*	183.07±11.86 (48.81%)*	364.73±4.24 (13.41%)
42	257.33± 3.94 (23.12%)	195.03±14.98 (47.02%)*	183.93±9.71 (50.32%)*	107.95±2.64 (72.32%)**	122.62±9.52 (65.70%)**	317.52±4.57 (24.62%)
56	250.42±13.84 (25.18%)	145.07±11.05 (60.59%)	139.02±3.53 (62.45%)	82.05±3.38 (78.96%)**	72.37±3.24 (79.76%)**	289.18±2.72 (31.35%)

vitamins was found to be 79%, which was very similar to the simvastatin-treated group. Vitamin A therapy, however, showed only ~25% decrease in cholesterol levels, which was no better than the control group. Duncan's multiple range test showed simvastatin and the combination therapy more or less similar trends followed by Vitamins E, C, A and control in descending order.

The serum LDL-C concentration of hyperlipidemic rabbits after the drug therapies is shown in table 3. It is apparent from this table that vitamins C reduced serum LDL-C levels profoundly ($P<0.05$) on day 14 while, vitamin E, combination and simvastatin decreased LDL-C levels significantly ($P<0.05$) on almost all time intervals starting from day 14 to the conclusion of the study. However, vitamin A and control groups did not exhibit a profound reduction throughout the study. The combination therapy and simvastatin displayed a highly significant ($P<0.01$) decrease on day 56. Duncan's multiple range test showed similar trends to cholesterol in decreasing LDL-C concentration of different treatment-groups.

The serum triglycerides levels were markedly decreased in all the animal groups except control which has negligible decline (table 4). Vitamin A showed less marked decrease as compared to other drug therapies. Comparing the individual drugs by applying Duncan's

Table 3: The serum low density lipoprotein-cholesterol (LDL-C) levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin A (50 µg/kg b.w./day), vitamin C (30 mg/kg b.w./day), vitamin E (30 mg/kg b.w./day), vitamin A+C+E (50µg/kg + 30mg/kg + 30mg/kg b.w./day respectively), simvastatin 3mg/kg b.w./day) and vehicle. Serum LDL-C levels were measured two hours after administration of therapies on various days.

Days	Vitamin A	Vitamin C	Vitamin E	Vitamin A+C+E	Simvastatin	Control
0	309.94±7.48 (100%)	341.48±26.37 (100%)	350.47±17.96 (100%)	359.57±12.72 (100%)	335.20±19.37 (100%)	403.56±5.01 (100%)
7	284.57±9.78 (8.18%)	262.93±23.24 (23.0%)	261.52±12.03 (25.38%)	253.84±20.54 (29.40%)	237.85±18.42 (29.00%)	397.97±4.32 (1.38%)
14	271.15±13.74 (12.39%)	236.33±20.41 (30.80%)*	252.03±9.88 (28.08%)*	203.13±12.41 (43.50%)*	216.64±17.35 (35.36%)*	396.34±3.81 (1.80%)
28	252.11± 3.35 (18.65%)	207.82±15.50 (39.14%)	205.85±8.17 (41.26%)*	145.99±6.41 (59.39%)*	168.05± 0.39 (49.86%)*	358.08±4.36 (11.26%)
42	239.34±15.84 (5.35%)	169.13±14.63 (50.47%)	166.74±9.02 (52.42%)	86.93±5.47 (75.82%)*	92.32±9.51 (72.45%)*	294.99±7.31 (27.00%)
56	244.33± 3.79 (21.16%)	138.63±10.75 (59.40%)	131.84±3.49 (62.38%)*	74.69±3.63 (79.0%)**	63.33±3.11 (81.10%)**	289.63±3.08 (28.23±)

Each value presents Mean ± S.E.M. (mg/dl) (n=6).
Values in parentheses present %decrease in concentration considering the day zero value as 100%, *P<0.05, **P< 0.01
Day zero value is measured after seven days treatment with cholesterol 250mg/kg/day.

Table 4: The serum triglycerides levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin A (50 µg/kg b.w./day), vitamin C (30 mg/kg b.w./day), vitamin E (30 mg/kg b.w./day), Vitamin A+C+E (50µg/kg + 30mg/kg + 30mg/kg b.w./day respectively), simvastatin (3 mg/kg b.w./day) and vehicle. Serum triglycerides levels were measured two hours after administration of therapies on various days.

Days	Vitamin A	Vitamin C	Vitamin E	Vitamin A±C±E	Simvastatin	Control
0	168.72±8.75 (100%)	144.97±5.59 (100%)	162.78±5.89 (100%)	156.16±2.75 (100%)	156.48±7.27 (100%)	1.58.19±4.70 (100%)
7	160.27±8.05 (5.0%)	139.49±6.57 (3.84%)	154.56±4.72 (5.04%)	147.94±2.20 (5.26%)	150.01±6.91 (4.13%)	158.47±3.40 (0.17%)
14	155.01±7.81 (8.12%)	131.27±5.45 (9.45%)	137.43±2.38 (15.57%)	135.61± .34 (13.15%)	131.95±6.86 (15.67%)	151.71±3.15 (4.09%)
28	144.74±6.82 (14.21%)	111.40±2.26 (23.36%)	117.80±5.84 (27.63%)	95.66±1.33 (38.74%)*	95.65±4.96 (38.87%)*	150.79±3.55 (4.70%)
42	135.83±6.53 (19.50%)	92.69±5.18 (36.06%)*	96.34±0.91 (40.81%)*	65.75±1.73 (57.90%)*	74.88±3.34 (52.14%)*	148.68±2.05 (6.01%)
56	128.07±6.14 (24.09%)	84.93±4.47 (41.41%)*	86.30±1.11 (46.98%)*	60.27±2.20 (61.40%)**	66.66±3.31 (57.40%)**	153.0±1.43 (3.28%)

Each value presents Mean ± S.E.M. (mg/dl) (n=6).
Values in parentheses present %decrease in concentration considering the day zero value as 100%, *P< 0.05, **P<0.01
Day zero value is measured after seven days treatment with cholesterol 250 mg/kg/day.

multiple range test it was shown that simvastatin and combination therapy proved to be more effective in decreasing serum triglyceride levels in comparison to other individual vitamin products.

All the therapeutic regimens designed for this study could not produce any significant change in the serum HDL-C levels except simvastatin, which exhibited a significant ($P<0.05$) rise in the serum HDL-C levels on 2^{nd}, 3^{rd} and last time interval of study period (table 5). Two ways analysis of variance randomized complete blocks, showed an insignificant rise in all groups except simvastatin and order of increase in HDL-C levels remained in favour of simvastatin.

DISCUSSION

The role of antioxidant vitamins in lowering serum total cholesterol and/or in decreasing atherosclerosis or preventing cardiovascular diseases have been demonstrated in human and animal models (Luo *et al*., 2004; McRae, 2008). However, lipid lowering effects of these antioxidant vitamins individually and in combination needed to be compared with widely used lipid lowering drugs, such as simvastatin. Current study demonstrates a significant lipid lowering effect of vitamin C, vitamin E, the combination therapy in comparison to simvastatin. Antioxidants have been shown to reduce or delay the progression of atherosclerosis, thus preventing the cardiovascular diseases (Odeh & Cornish, 1995; Brude *et al*., 1997; Diaz *et al*., 1997; Sun *et al*., 1997; Sutken *et al*., 2006; Meerten *et al*., 2008). Antioxidants have also exhibited a profound decrease in serum total cholesterol and triglycerides levels in rabbits (Luo *et al*., 2004), which is in agreement to our data. Vitamin C has exhibited a significant reduction in serum LDL-C and triglycerides (McRae, 2008) as well as demonstrated a decline in the incidence of cardiovascular diseases (Knekt *et al*., 2004) in human subjects. However, Dai & McNeill (1995) showed that vitamin C did not modify the total cholesterol and triglycerides concentrations in rats. Though vitamin E deficiency is related to low levels of total cholesterol, LDL-C and triglycerides, possible risk factors for cardiovascular diseases (Meertens *et al*., 2008), some studies may not suggest its role in regression of atherosclerosis (Prasad, 2009). Husain *et al*., (2004) have shown a significant decline in total cholesterol, LDL-C and triglyceride levels after administration of vitamin E in cholesterol-fed rabbits which is inline with the results of this study. These results may suggest a possible role of vitamin C, vitamin E and the combination in lowering lipid parameters in hypercholesterolemic rabbits, but the mechanism through which these agents do so need to be investigated. The contrast in the results in modulating lipid profile or preventing atherosclerosis and cardiovascular disease by vitamin E may be specie-dependent.

The combination therapy and simvastatin showed a more profound decline in serum cholesterol levels and LDL-C and triglycerides which may indicate their role in preventing atherosclerosis and decreasing morbidity and mortality from cardiovascular diseases. These data are in agreement with the role of vitamin C and E in improving hyperlipidemia and cardiac functions in rats (Manimegalai *et al*., 1997 and Dai *et al*., 1995). Moreover, a combination of vitamin C, vitamin E, B-carotene and co-enzyme Q10 had been shown to prevent the LDL oxidation, which is thought to lead towards atherosclerosis (Brude *et al*., 1997). The present study

Table 5: The serum high density lipoprotein cholesterol (HDL-C) levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin either vitamin A ((50 µg/kg b.w./day), vitamin C (30 mg/kg b.w./day), vitamin E (30 mg/kg b.w./day), Vitamin A+C+E (50µg/kg + 30mg/kg + 30mg/kg b.w./day respectively), simvastatin (3 mg/kg b.w./day) and vehicle. Serum HDL-C levels were measured two hours after administration of therapies on various days.

Days	Vitamin A	Vitamin C	Vitamin E	Vitamin A±C±E	Simvastatin	Control
0	12.93±0.36 (100%)	13.89±0.63 (100%)	12.89±0.44 (100%)	13.89±0.42 (100%)	11.46± .80 (100%)	12.73±0.57 (100%)
7	11.05±0.50 (14.53%)	13.37±0.55 (3.74%)	11.91±0.45 (7.60%)	12.41±0.14 (10.33%)	12.20±0.31 (6.45%)	10.24±0.47 (16.56%)
14	10.27±0.81 (20.57%)	15.63±0.72 (12.52%)	13.53±0.82 (4.88%)	14.24±1.45 (2.89%)	14.78±0.78 (28.97%)*	9.55±0.30 (24.89%)
28	10.45± .27 (19.18%)	12.53±1.16 (9.79%)	11.51±0.60 (10.70%)	12.25±0.80 (9.539%)*	15.57±0.66 (35.86%)*	12.48±0.47 (1.96%)
42	9.22±0.25 (28.69%)	10.73±0.83 (22.75%)	10.23±0.77 (20.63%)	12.98±0.93 (6.21%)*	15.98±0.49 (39.44%)	12.27±0.27 (3.61%)
56	8.51±0.42 (34.18%)	11.56±0.81 (16.77%)	10.81±0.56 (21.02%)	12.91±0.62 (6.71%)	17.0±0.37 (48.37%)*	10.36±0.16 (18.61%)

Each value presents Mean ± S.E.M. (mg/dl) (n=6).
Values in parentheses present % increase or decrease in concentration considering the day zero value as 100%,*P<0.05,**P< 0.01
Day zero value is measured after seven days treatment with cholesterol 250mg/kg/day.

may well demonstrate the role of vitamin C, vitamin E and the combination therapy to reduce the dyslipidemia; responsible for atherosclerosis and heart ailments – the major cause of deaths in whole world.

It has clearly been demonstrated that a relationship exists between increased concentration of HDL-C and decreased morbidity- and mortality-rate in cardiovascular patients (Abbott *et al*., 1988; Franceschini, 2001). Present study, could not exhibit a substantial increase in HDL-C levels in all the treatment groups except simvastatin. These results are in compliance with the randomized controlled trials, where vitamin C failed to elevate serum HDL-C levels (Johnson & Obenshain, 1981; Joshi *et al*., 1981; McRae, 2008). Similarly, vitamin E-treatment to cholesterol-fed rabbits did not show an increase in serum HDL-C levels (Husain *et al*., 2004). Some studies have indicated that vitamin E did not cause any regression in the progression of hypercholesterolemic atherosclerosis, but exact mechanism is still unknown (Knekt *et al*., 2004; Prasad, 2009). It is difficult to establish a linkage between unaltered levels of HDL-C with no effect on atherosclerosis lesions after administration of vitamin E. It is not known why these antioxidant vitamins could not modify this cardio-protective lipoprotein in rabbits.

Although simvastatin remained the most potent of all therapeutic regimens employed in the study yet the combination therapy could not remain far behind, in achieving the same anti-hyperlipidemic affects except the HDL-C levels. Perhaps an advantage may be associated with the combination therapy in terms of low-toxicity. Moreover, anti-oxidant vitamins are suggested to inhibit LDL-C from oxidizing into dangerous oxidized cholesterol which is responsible for plaque formation and ultimately atherosclerosis. Future studies should aim to investigate lipid lowering effect of these vitamins in conjunction with simvastatin. A continuous and prolonged use of the combined vitamin therapy and simvastatin may be more beneficial for hyperlipidemic patients to avoid atherosclerosis and fatal heart diseases.

REFERENCES

Abbott R.D, Wilson PW, Kannel WB and Castelli WP (1988). High-density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Farmingham study. *Arteriosclerosis*, **8**: 207-211.

Brude IR, Drevon CA, Hjermann I, Sljeflot I, Lund-Katz S, Saarem K, Sandstad B, Solvoll K, Halvorsen B, Arnesen H and Nenseter MS (1997). Peroxidation of LDL from combined-hyperlipidemic male smokers supplied with -3 fatty acids and antioxidants. *Arteriorscler. Thromb. Vasc. Biol*., **17**: 2576-2588**.**

Chang WH and Liu JF (2009). Effect of kiwi fruit consumption on serum lipid profile and antioxidative status in hyperlipidemic subjects. *Int. J. Food Sci. Nutr*., **60**: 709-716.

Dai S and McNeill JH (1995). Ascorbic acid supplementation prevents hyperlipidemia and improves myocardial performance in streptozotocin-diabetic rats. *Diabetes Res. Clin. Pract*., **27**; 11-18**.**

Diaz MN, Frei B, Vita JA and Keaney JF Jr. (1997). Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.*, **337**: 408-416.

Franceschini G (2001). Epidemiological evidence for high density lipoprotein cholesterol as a risk factor for coronary artery disease. *Am. J. Cardiol.*, **88**: 9N-13N.

Husain SN, Omar KA, Khan FZ, Ahmad M and Ahmad B (2004). Hypolipidemic effect of garlic (*Allium sativum*) and vitamin E in rabbits. *Acta Pharm. Sci*., **46**: 223-232

Inkeles S, and Eisenbreg. D. (1981). Hyperlipidemia and coronary atherosclerosis. *Medicine*, **70**: 110.

Johnson GE and Obenshain SS (1981). Non-responsiveness of serum high density lipoprotein-cholesterol to high dose ascorbic acid administration in normal men. *Am. J. Clin. Nutr.*, **34**: 2088-2091.

Joshi VD, Joshi LN and Gokhale LV (1981). Effect of ascorbic acid on total and high density lipoprotein cholesterol of plasma in normal human subjects. *Indian J. Physiol. Pharmacol.*, **25**: 348-350.

Knekt P, Ritz J, Pereira MA, O'Reilly EJ, Augustsson K, Fraser GE, Goldbourt U, Heitmann BL, Hallmans G, Liu S, Pietinen P, Spiegelman D, Stevens J, Virtamo J, Willett WC, Rimm EB and Ascherio A (2004). Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am. J. Clin. Nutr*., **80**: 1508-1520.

Luo Q, Cai Y, Yan J, Sun M and Corke H (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sci*., **76**: 137-149.

Manimegalai R, Geetha A and Rajalakshmi K (1997). Effect of vitamin E on high fat diet induced hyperlipidemia in rats. *Indian J. Exp. Biol*., **17**: 2576-2588.

McRae MP (2007). Vitamin C supplementation for treating hypercholesterolemia: a meta-analysis of 16 randomized controlled trials. *J. Am. Nutraceut. Ass*., **10**: 21-28.

McRae MP (2008). Vitamin C supplementation lowers serum low-density lipoprotein cholesterol and triglycerides: a meta-analysis of 13 randomized controlled trials. *J. Chiropr. Med.*, **7**: 48-58.

Meertens L, Ruido T, Diaz N, Naddaf G, Rodriguez A and Solano L (2008). Relationship between serum lipids and status of vitamin C and E as antioxidants in Venezuelan elderly people. *Arch. Latinoam Nutr*., **58**: 363-370.

Odeh RM and Cornish LA (1995). Natural antioxidants for the prevention of atherosclerosis. *Pharmacotherapy*, **15**: 648-659.

Pauciullol P, Lirato C, Sapio C and Mancini M (1993). Treatment of hyperlipidemia in patients with ischemic heart disease. *Cardiologia*, **38**: 345-348.

Prasad K (2009). Vitamin E does not regress hypercholesterolemic atherosclerosis. *J. Cardiovasc. Pharmacol. Ther*., **14**: 231-241.

Sun J, Giraud DW, Moxley RA and Driskell JA (1997). Beta-carotene and alpha-tocopherol inhibit the development of atherosclerotic lesion in hypercholesterolemic rabbits. *Int. J. Vitam. Nutr. Res*., **67**: 155-163.

Sutken E, Inal M and Ozdemir F (2006). Effect of vitamin E and gemfibrozil on lipid profiles, lipid peroxidation and antioxidant status in the elderly and young hyperlipidemic subjects. *Saudi Med. J*., **27**: 453-459.

Todd PA and Goa KL (1990). Simvastatin a review of its pharmacological properties & therapeutic potential in hypercholesterolemia. *Drugs*, **40**: 583-607.

Tulenko TN and Sumner AE (2002). The physiology of lipoproteins. *J. Nucl. Cardio.*, **9**: 638-649**.**

Vaughan CJ, Murphy MB and Buckley BM (1996). Statins do more than just lower cholesterol. *Lancet*, **348**: 1079-1082.