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Hypolipidemic Effects of Garlic (*Allium sativum*) and Vitamin E in Rabbits

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Abstract

The effects of orally administered crude garlic and vitamin E individually and in combination were studied on biochemical parameters of hyperlipidemia in rabbits. All the treatments exhibited lowering effect on serum cholesterol, serum low-density lipoproteins (LDL) and serum triglycerides concentrations. The serum high-density lipoproteins (HDL) were boosted to significant levels only by the combination therapy. Vitamin E used alone depicted lipid profile lowering activity in all parameters of the study. The combination therapy markedly increased the cardio- protective lipoproteins HDL which was not significantly raised by the effect of individual drugs.

Key words: *Allium sativum*, Allicin, Tocopherol, Hypolipidemic activity.

Introduction

Since the mid 1960's almost 50 clinical trials of cholesterol lowering to prevent coronary heart disease (CHD) have been reported. These trials have fairly consistently shown a reduction in CHD events (Gould *et al.*, 1995). There is a strong evidence which supports a direct relationship between plasma levels of low-density lipoprotein (LDL), cholesterol, and coronary heart disease risk. This relationship is well synchronized in countries whose inhabitants have high cholesterol and LDL levels (Diaz *et al.*, 1997). For reducing the levels of lipids in plasma, varied types of interventions have been employed including diet, and use of antioxidant vitamins like Vitamin E. 20,21(Morris and Carson, 2003; Dutta and Duttra, 2003). Garlic (*Allium sativum*), native to Europe and Central Asia is now cultivated worldwide. Garlic and garlic oil have been reported to exhibit numerous pharmacological properties, which, in addition to others, include hypoglycemic activities (Sitprija *et al.*, 1987; Kiesewetter *et al.*, 1991), lowering of serum cholesterol and other parameters of lipid profile (Warshafsky *et al.*, 1993; Silagy and Neil, 1994). The volatile sulfur compounds (especially allicin, diallyl disulfide and diallyl trisulfide) are generally considered to be responsible for these pharmacological activities (Simons, 1986; Evans, 2002). Vitamin E prevents the peroxidation of serum lipids, and is used to lower the serum cholesterol in the hypercholesterolemic patients. Cardiologists recommend vitamin E to prevent the ischemic heart diseases as it prevents the formation of atheromatous plaque by decreasing the elevated cholesterol; very low-density and low-density lipoproteins' levels to the optimal concentrations. (Olson, 1973; Stampfer *et al.*, 1993; Rimm *et al.*, 1993).The present

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investigations were carried out to evaluate and assess the hypolipidemic effects of garlic and vitamin E individually and in combination in hypercholesterolemic rabbits.

Materials and Methods

Drugs: Garlic *(Allium sativum)* bulbs were purchased from local market; peeled and cloves were finely ground. Vitamin E (Evion) marketed as sugar-coated tablets of 100 mg were obtained from E.Merck (Germany). Cholesterol of analytical grade was obtained from BDS Chemical Ltd. Poole, England.

Animals: Healthy albino rabbits of either sex of subspecies *caprolagus hispidus* with an average body weight of 1.2. kg.were acclimatized for a week in the animal house of Faculty of Pharmacy. During this period fresh green fodder and tap water were provided *ad libitum*. The temperature of the animal house was maintained at 22 $\pm$ 2°C. Rabbits were randomly divided into four groups each comprising 6 animals. The blood samples of all the rabbits were drawn to measure the normal levels of serum cholesterol, serum LDL, and serum HDL and serum triglycerides. All the rabbits were administered orally cholesterol 250 mg/kg/day for seven days and blood samples were tested for above-mentioned parameters. The four groups of hypercholesterolemic rabbits were given by oral route I. vitamin E (30 mg/kg/day), II. garlic paste in hard gelatin capsules (500 mg/kg/day), III. combination of garlic + vitamin E (500 mg + 30 mg respectively mg/kg/day) and IV. control (empty gelatin capsules) respectively for a period of one month. In the post-dosing period, all experimental animals did not receive cholesterol supplementation. The blood samples were drawn from the marginal ear vein at days 2,6,10,14,18,22,26 and 30 after the last dosing.

Biochemical analysis of Blood: Blood serum was analysed to estimate cholesterol, LDL, HDL and triglyceride contents by kits manufactured by Biosystem, Spain.

Statistical analysis: The mean effects of vitamin E, garlic, garlic + vitamin E and control in group I, II, III, and IV respectively on various biochemical parameters were recorded with their effective range. The comparative relationship among the four groups and time intervals, among various parameters were done by two way analysis of variance (ANOVA) (Jones, 2002)

Results and Discussion

The cholesterol levels of rabbits were increased nine to eleven fold after the administration of cholesterol 250 mg/kg/day for seven days (Table-1). All the four groups remained hypercholesterolemic on the next day before they received initial dose of either vitamin E, garlic or combined therapy. The results presented in Table-2 show a significant decrease in the serum cholesterol levels of each group. Vitamin E or garlic induced more than 90% decrease in the cholesterol levels while the combined therapy could not produce a potentiating, additive or synergistic effect. This might be due to saturation of some common mechanism(s) (Phelps and Harris, 1993; Kleinveld *et al.*, 1994). Although control group had shown a decreasing pattern, yet no significant decrease was observed. The independent effect of vitamin E and garlic in lowering the cholesterol levels are similar to the previous findings (Jain *et al.*, 1973; Phonpanichrasamee *et al.*,1990). However, in the present experimental design the reduction started from the very second day; might be due to the withdrawal of cholesterol supplementation before the start of therapy. Moreover, the green fodder diet and some other

physiological factors could be responsible for the sharp decline in serum cholesterol levels when compared to the normals. The data suggested that vitamin E was more potent when compared with garlic or combine therapy in lowering the serum cholesterol levels, which is in accordance with the results of Rahmani *et al.*, 1988 and Jain *et al.*, 1993. Two way analysis of variance randomized complete block had also displayed a highly significant ($P< 0.0000$) decline in the levels of cholesterol after administration of drugs when compared with control group of animals for thirty days. The serum low-density lipoproteins (LDL) concentrations of hypercholesterolemic rabbits are tabulated in Table-3. Vitamin E displayed a remarkable decrease in the LDL serum concentration where a significant decrease could be seen in all the intervals from day 10-30 ($P<0.01$). At day 30 6.65% of LDL levels could be detected in the experimental animals. An identical order was offered by garlic, garlic + vitamin E and control therapies, however, garlic and combined therapy showed highly significant reduction ($P<0.01$) in serum LDL concentration in the last days of therapy whereas control did not demonstrate any statistical significance (Table-3). The results of Tables-2 and 3 are in accordance with the known relationship of cholesterol with LDL. Both the factors can independently give rise to atherosclerosis. So the same drug is working against both the odds (Inkeles and Eisenbergh, 1971; Boyd *et al.*, 1989). Table-4 is indicative of the serum HDL Levels in treated hypercholesterolemic rabbits. It is evident from the table that an increase in serum HDL with vitamin E was not significant because fluctuations were observed on day eighteen (95%) and day twenty-two (84%). However apart from those fluctuations there was an increase in HDL concentration observed on other days and at the end of study it was 253mg/dl. Garlic and the combined therapy showed significant ($P<0.01$) increases but the latter was more pronounced in terms of statistical significance in boosting the serum HDL levels. The level of this protective lipoprotein could be quite encouraging for cardiovascular patients. The results are nearly in agreement with the studies of Herman *et al.*, 1979 and Barboriak *et al.*, 1982.

The serum triglyceride concentrations of hypercholesterolemic rabbits are given in Table-5 after either of the treatments. Serum triglyceride levels were dropped significantly in all the three groups to whom drugs were given; vitamin E remained the most potent compound. The control group had shown insignificant decrease in serum triglyceride concentration as on day thirty the concentration was 100.93%. Statistical analysis also proved that vitamin E was more effective in decreasing serum triglyceride levels while garlic and combined therapy were found comparatively less potent drugs. It is concluded that the above therapeutic agents may be helpful in lowering the lipid profile of cardiovascular and hyperlipidemic patients, so preventing atherosclerosis (Bordia and Verma, 1980; Williams *et al.*, 1992) The present findings fortified the fact that vitamin E and garlic both having ant oxidative molecules and possess antihyperlipidemic activity (Harris, 1992). These drugs are found to be useful in reducing cholesterol, LDL, triglyceride concentrations in serum. Moreover, the combination of vitamin E with garlic have clearly shown an increasing pattern of cardio protective lipoproteins the HDL.

Table1. Serum cholesterol levels (mg/d1) of rabbits (n=6) that received cholesterol (250 g/kg /day) daily for seven days. Serum levels were measured two hours after administration of cholesterol on day zero and day seven.

Days	Group I	Group II	Group III	Group IV
Zero	34.71±5.8	34.54±3.7	36.45±1.0	32.82±4.2
Seven	378.19±32.07* (1089.57%)	400.76±9.0* (1160.27%)	342.73±3.6* (940.27%)	322.30±29.6* (982.02%)

Each value presents Mean+ S.E.M. (mg/d1) concentration (n=6).

The values in parentheses present % mg/d1 concentrations of cholesterol levels considering the day zero value as 100%

* $P<0.05$ as compared to zero day value.

Table 2. Serum cholesterol levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin E (30mg/kg /day), garlic (500 mg/kg /day), garlic (500 mg/kg /day) + vitamin E(30 mg/kg /day), and vehicle (control). Serum levels were measured two hours after administration of drugs on various days.

	Vitamin E	Garlic	Garlic+ Vitamin E	Control
#Hyper-cholester-olemic: Day Zero	378.19±32.07 (100%)	400.76±9.07 (100%)	342.33±3.64 (100%)	322.30±29.68 (100%)
Day Two	277.96±65.15 (73.49±10.2)	322.58±16.66 (80.49±5.3)	310.82±3.34 (90.79±0.7)	242.66±4.74 (75.33±6.5)
Day Six	119.75±14.07 (31.66±2.8)	208.35±30.04 (51.98±8.3)	251.75±6.02 (73.54±1.7)	189.26±33.45 (58.72±13.3)
Day Ten	79.97±11.71* (21.14±2.5)	138.70±11.76 (34.60±3.4)	194.61±2.64 (56.84±0.9)	171.17±40.62 (53.10±15.50)
Day Fourteen	62.39±7.32* (16.49±1.6)	88.25±4.43 (22.02±1.4)	109.25±1.39 (31.91±0.4)	176.49±47.74 (54.75±18.8)
Day Eighteen	47.86±3.17* (12.65±3.8)	72.35±1.67 (18.05±0.5)	80.92±0.88 (23.63±0.3)	87.16±17.22 (27.04±3.6)
Day Twenty-two	40.02±1.94** (10.58±0.7)	44.65±1.43* (11.14±0.4)	50.90±0.84 (14.86±0.2)	53.14±2.36 (16.48±0.9)
Day Twenty-six	31.25±1.50** (8.26±0.6)	32.15±0.94** (8.02±0.3)	40.26±0.31** (11.75±0.1)	62.36±4.22 (19.34±3.2)
Day Thirty	26.01±1.08** (6.87±0.5)	27.07±0.64** (6.75±0.2)	32.75±0.72** (9.56±0.2)	56.97±4.72 (17.67±3.0)

Each value presents Mean ± S.E.M.(mg/dl)(n=6).
Values in parentheses present Mean ± S.E.M. (mg/dl) concentrations considering the day zero value as 100%
* $P<0.05$; ** $P<0.01$.
# Day zero considered after seven days prior treatment with cholesterol 250 mg/kg/day.

Table3. Serum low-density lipoprotein(LDL) levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin E (30 mg/kg/day), garlic (500 mg/kg /day),garlic (500 mg/kg/day) + vitamin E (30 mg/kg/day) and vehicle (control). Serum levels were measured two hours after administration of drugs on various days.

	Vitamin E	Garlic	Garlic + Vitamin E	Control
# Hyper-cholester-olemic: Day Zero	371.03±32.43 (100%)	373.10±9.61 (100%)	334.44±2.99 (100%)	316.58±29.69 (100%)
Day Two	241.00±74.71 (64.95±15.4)	334.85±24.67 (89.74±5.8)	305.40±3.22 (91.31±0.8)	238.01±4.43 (75.18+6.9)
Day Six	98.39±19.88 (26.51±5.1)	205.12±29.85 (54.97±7.6)	246.19±5.68 (73.61±1.7)	184.37±33.17 (58.23±13.6)
Day Ten	42.38±4.71* (11.42±1.8)	136.70±10.14 (36.63±2.3)	188.22±1.72 (56.27±0.8)	166.26±40.97 (52.51±16.0)
Day Fourteen	47.40±7.65* (12.77±0.8)	85.91±3.72* (23.02±1.1)	103.27±1.35 (30.88±0.5)	170.85±48.06 (53.96±19.4)
Day Eighteen	42.24±4.40* (11.38±0.5)	66.85±3.05* (17.91±1.2)	76.34±0.84 (22.83±0.3)	81.57±17.65 (25.76±3.8)
Day Twenty-two	37.61±2.63* (10.13±0.7)	42.48±0.76* (11.38±0.4)	45.50±1.20 (13.60±0.4)	48.18±3.11 (15.21±0.5)
Day Twenty-six	29.20±1.16* (7.86±0.7)	30.78±0.62** (8.24±0.2)	35.33±0.50** (10.57±0.2)	57.26±3.52 (18.08±3.0)
Day Thirty	24.61±0.82* (6.63±0.3)	23.53±1.74** (6.30±0.5)	29.52±0.23** (8.81±0.1)	52.56±4.85 (16.60±3.0)

Each value presents Mean ± S.E.M.(mg/dl)(n=6).
Values in parentheses present Mean ± S.E.M. (mg/dl) concentrations considering the day zero value as 100% .
* $P<0.05$; ** $P<0.01$.
# Day zero considered after seven days prior treatment with cholesterol 250 mg/kg/day.

Table4. Serum high-density lipoprotein (HDL) levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin E (30 mg/kg/day), garlic (500 mg/kg/day), garlic(500 mg/kg/day) + vitamin E (30 mg/kg/day) and vehicle (control). Serum levels were measured two hours after administration of drugs on various days.

	Vitamin E	Garlic	Garlic + Vitamin E	Control
# Hyper-cholester-olemic: Day Zero	19.01±5.87 (100%)	11.52±1.34 (100%)	18.90±0.29 (100%)	29.12±5.93 (100%)
Day Two	15.30±2.71 (80.48±16.31)	13.41±1.88 (116.40±14.5)	23.86±0.40** (126.24±2.9)	18.58±1.68 (63.80±11.4)
Day Six	11.91±1.54 (62.65±16.10)	15.97±1.54 (138.62±26.7)	26.97±0.34** (142.69±3.4)	12.77±3.2 (43.85±13.9)
Day Ten	25.87±6.09 (136.08±14.09)	16.35±0.92* (141.92±23.5)	22.45±0.38** (118.78±2.8)	10.39±2.46 (35.67±8.0)
Day Fourteen	21.99±5.35 (115.67±16.44)	15.43±0.81 (133.94±23.3)	21.30±0.20* (112.69±1.7)	11.58±2.02 (39.76±10.3)
Day Eighteen	11.27±1.76 (59.28±13.23)	15.66±1.23* (135.93±60.4)	20.75±0.27** (109.78±1.1)	8.47±2.69 (29.08±10.1)
Day Twenty two	9.55±1.74 (50.23±12.90)	14.67±1.53 (127.34±22.8)	21.25±0.28** (112.44±1.3)	13.28±0.88 (45.60±10.4)
Day Twenty six	14.37±3.33 (75.43±18.15)	16.41±1.4 (142.44±23.8)	21.77±0.22** (115.18±2.5)	12.65±1.58 (43.44±8.7)
Day Thirty	17.99±6.06 (94.63±12.32)	17.61±1.5 (152.86±24.3)	22.15±0.22** (117.19±1.4)	21.35±4.92 (73.31±2.3)

Each value presents Mean ± S.E.M.(mg/dl)(n=6).
Values in parentheses present Mean ± S.E.M. (mg/dl) concentrations considering the day zero value as 100%.
* $P<0.05$; ** $P<0.01$.
# Day zero considered after seven days prior treatment with cholestero250mg/kg/day.

Table5. Serum triglyceride levels (mg/dl) of hypercholesterolemic rabbits that have received either vitamin E (30 mg/kg/day), garlic (500 mg/kg/day), garlic (500 mg/kg/day) + vitamin E (30 mg/kg/day) and vehicle (control). Serum levels were measured two hours after administration of drugs on various days.

	Vitamin E	Garlic	Garlic+ Vitamin E	Control
# Hyper-cholester-olemic: Day Zero	144.88±10.40 (100%)	163.69±17.24 (100%)	171.0.4±2.49 (100%)	149.67±19.07 (100%)
Day Two	124.69±9.73 (86.06±4.4)	151.49±19.42 (92.54±2.1)	150.33±1.34 (87.89±0.9)	154.67±11.10 (103.34±7.20)
Day Six	107.09±5.41** (73.91±5.3)	130.29±16.67 (79.59±4.4)	115.90±1.43* (67.76±1.2)	148.84±8.23 (99.44±9.50)
Day Ten	98.45±4.37** (67.95±4.1)	108.00±15.13 (65.97±5.5)	107.93±0.60 (63.10±0.9)	116.76±9.90 (78.01±15.60)
Day Fourteen	89.08±3.49** (61.48±3.0)	94.06±6.41* (57.46±7.0)	98.27±1.07** (57.45±0.6)	127.30±4.90 (85.05±8.9)
Day Eighteen	77.22±7.19** (53.29±3.2)	77.74±3.74** (47.49±7.5)	80.81±0.75** (47.24±0.9)	118.32±7.82 (79.05±5.5)
Day Twenty-two	48.17±8.53** (33.24±2.7)	74.00±3.79** (45.20±7.2)	71.33±0.76 (41.71±0.7)	126.29±4.84 (84.37±15.39)
Day Twenty-six	46.07±2.66** (31.79±1.8)	62.19±7.34** (37.99±8.4)	55.80±1.20** (32.62±0.4)	127.89±12.50 (85.44±21.73)
Day Thirty	42.53±2.22** (29.35±1.6)	56.69±7.22** (34.63±7.2)	50.35±0.79** (29.43±0.2)	141.94±17.29 (94.83±21.0)

Each value presents Mean ± S.E.M. (mg/dl) (n=6).
Values in parentheses present Mean ±S.E.M. (mg/dl) concentrations considering the day zero value as 100%.
* $P<0.05$; ** $P<0.01$.
# Day zero considered after seven days prior treatment with cholesterol 250 mg/kg/day

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