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A PHARMACODYNAMIC INTERACTIVE STUDY OF ATORVASTATIN AND GARLIC IN TREATMENT OF DYSLIPIDAEMIAS IN RATS.

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ABSTRACT

This experiment was aimed to study the interaction between atorvastatin and garlic in correcting dyslipidaemias. A total of 56 male Sprague dawley rats were randomly divided into seven groups to evaluate the interaction of atorvastatin with garlic in induced dyslipidaemia. Different proportions of fresh garlic in feed and atorvastatin orally were fed in different groups for 12 weeks. Plasma was analyzed for total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) for every two weeks. All the treatment groups exhibited significant improvement in dyslipidaemic condition from 2nd week of treatment by reducing the TC, TG and LDL-C levels with subsequent increase in HDL-C levels. It can be concluded that garlic and atorvastatin exhibited positive pharmacodynamic interaction in reducing dyslipidaemias.

KEYWORDS: **Atorvastatin, Garlic, Dyslipidaemias, Pharmacodynamic interaction**

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INTRODUCTION

One of the major risk factors for atherosclerosis and its associated conditions such as coronary heart disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease is dyslipidaemia[1,2]. HMG CoA reductase inhibitors namely statins are effective in controlling dyslipidaemias and reduce the risk of cardiovascular morbidity and mortality[1,3]. Generally the statins are safe but recent studies suggest their hepatic and renal toxicity[4,5,6] apart from known serious adverse effects like rhabdomyolysis[7]. The usage of herbal therapies along with prescription and over the counter medications is increasing day by day. Use of garlic, opium, castor oil, coriander, mint, indigo and other herbs has been reported in ancient Egyptian medicine as early as 1000 BC[8]. Garlic has been studied for its hypotensive and hypocholesterolaemic activity[9]. There are reports of hepato-protective action of allicin from garlic in experimentally induced hepatitis in rats[10]. Keeping the above facts in view, an experimental study was conducted to study the pharmacodynamic interaction of anti-dyslipidaemic herb (garlic) with statin.

MATERIALS AND METHODS

Fifty six male *Sprague dawley* rats of uniform age and weight were procured for the study. Feed and water was provided *ad lib* throughout the experiment. Animals were housed in polypropylene cages in a well ventilated animal house with 12 h – 12h light – dark cycles. Acclimatization period of 3 weeks was observed before the start of experiment.

i. Induction of dyslipidaemia and initiation of herb/drug treatment

After an acclimatization period, rats were randomly divided into 7 groups of 8 rats in each and blood samples were collected and plasma was separated for lipid profile to ascertain group differences, if any. Then, group 1 was kept as normal control throughout the experimental period. Remaining 6 groups were fed with the diet containing 14% beef tallow and 1% cholesterol (mixed in the diet on w/w basis) for 6 weeks (high fat and high cholesterol diet). After 6 weeks, groups 2 and 3 were fed with the same diet throughout the experimental period, while groups 4 to 7 received respective quantities of fresh garlic in addition to the high fat and high cholesterol diet.

ii. Experimental Design

After initiation of drug/herb treatment, all the groups were maintained as per the following drug and herb treatment schedule for 12 weeks.

Group – 1: Normal control
Group – 2: Dyslipidaemic control (DL)
Group – 3: DL + Statin (100%) control
Group – 4: DL + Statin (100%) + Herb (100%)
Group – 5: DL + Statin (50%) + Herb (50%)
Group – 6: DL + Statin (75%) + Herb (25%)
Group – 7: DL + Statin (25%) + Herb (75%)

iii. Treatment procedure

The atorvastatin 100% dose was 10 mg/kg body weight which was administered once daily to groups 3 to 7 in required dose orally as aqueous suspension in distilled water using oral gavaging needle and syringe. Distilled water was administered to control groups 1 and 2. The 100% dose of garlic was 1% w/w in the feed. Garlic was freshly homogenized every day in sufficient quantity of water and mixed in the feed of group 4 to 7 in required amounts and made into pellets and offered to respective groups *ad lib*. Garlic administration was initiated 2 weeks before atorvastatin administration. Feed offered to groups 1 to 3 was made into pellet by using sufficient quantity of water.

iv. Sample collection

Blood collection was carried out at every two weeks interval for plasma biochemical analysis after initiation of the drug administration till the end of the experiment (12 weeks). Feed was withdrawn 12 h before

the blood collection and blood was collected through retro-orbital plexus after ether anaesthesia into heparinized containers and centrifuged at 3000 RPM for 15 min and plasma was separated and stored at -20^{0}C till analysis. The plasma samples were analyzed for the concentration of total cholesterol, HDL cholesterol and triglycerides by using commercial kits available. At the end of the experiment, 6 rats from each group were sacrificed to collect liver for tissue lipid estimation by the method described[11]; briefly to 1 gm of liver tissue added 20 ml of 2:1 chloroform: methanol. Then homogenized for 2 min and agitated the tubes for 10 min using orbital shaker. Then, contents were centrifuged at 3000 rpm for 5 minutes. Supernatant was transferred to another tube. To this, added 4 ml of distilled water and mixed well for a few seconds and again centrifuged at 3000 rpm for 5 min. Then the three phases were separated. After removing the upper phase, middle phase was rinsed carefully twice with 1:1 chloroform: methanol, without disturbing the lower phase. Then the lower chloroform phase containing lipids was transferred into round bottom flask. Chloroform was evaporated under vacuum using rotary evaporator. After adding 30 ml of hexane- isopropyl alcohol to each round bottom flask, kept it over night by sealing the round bottom flask with aluminium foil. After removing foil cover next day, round bottom flask was heated gently using a water bath (50-600C). Then, rinsed the flask with 2-3 ml of 2:1 chloroform: methanol to dissolve the precipitate. Transferred the contents of round bottomed flask to a measuring cylinder and made up the volume to 30 ml with 2:1 chloroform: methanol in all estimations to keep the final volume constant. From the final extracted portion, total cholesterol was estimated using the method as in case of plasma estimation. The percent decrease in different plasma lipid parameters was analyzed at different time intervals. The biochemical data were statistically analyzed by applying one way ANOVA using statistical package for social sciences (SPSS) 15.0 version. A difference between means was tested using Duncan's multiple comparison test by setting the significance level at 0.05.

RESULTS

1. Total cholesterol

The total cholesterol concentration (mg/dl) of normal control group was significantly ($p<0.05$) lower (ranged from 42.39 ± 2.82 to 52.99 ± 1.42) and that of dyslipidaemic control group 2 was significantly ($p<0.05$) higher (ranged from 101.22 ± 4.36 to 127.99 ± 4.99) throughout the experimental period when compared to treatment groups 3 to 7. All the treatment groups showed a significant ($p<0.05$) decrease in total cholesterol concentration from 2nd week of treatment when compared to dyslipidaemic control, though there was no significant difference among treatments till 6th week of treatment. The highest per cent decrease was observed in group 3 during 2nd and 4th week (20.28 and 26.65, respectively) and group 4 during 6th week (27.12). At the end of 8th week of treatment, group 4 (statin 100% + garlic 100%) exhibited a significant ($p<0.05$) decrease in total cholesterol concentration (70.15 ± 3.63) when compared to groups 3, 5, 6, 7 and dyslipidaemic control group 2 (78.97± 3.79, 89.71 ± 2.86, 82.35 ± 3.06, 88.97 ± 3.06 and 120.44 ± 6.85, respectively), though not comparable with that of normal control group 1 (46.91 ± 2.66). By the end of the 10th week, there was no significant difference among the treatments, though they displayed a significantly ($p<0.05$) lower total cholesterol concentration than that of group 2. The highest per cent decrease was observed in group 4 (45.14) followed by groups 6, 3, 5 and 7. At the end of the experiment after 12 weeks, group 7 (statin 25% + garlic 75%) exhibited a significantly ($p<0.05$) higher value of total cholesterol (75.00 ± 1.66) among all the treatments, while group 4 (100% statin + 100% garlic) showed the highest per cent decrease in total cholesterol (50.96) followed by groups 3, 5, 6 and 7 at the end of the experiment. The statin control group 3 displayed a significant ($p<0.05$) reduction in the total cholesterol by the end of 4th week of treatment (88.02 ± 2.61) when compared to its basal value at the beginning of the experiment (102.04 ± 6.64). Further significant ($p<0.05$) reduction was observed at the end of 10th week (69.82 ± 1.76) and reduced to 65.22 ±

1.96 at the end of the 12th week. Similar trend was observed with group 4 (100% statin + 100% garlic), where there was a significant ($p<0.05$) reduction in cholesterol level when compared to its basal level (101.09 ± 5.23) during the 4th week (100.57 ± 1.36) followed by still further decrease during 8th to 12th week. The groups 5 (statin 50% + garlic 50%), 6 (statin 75% + garlic 25%) and 7 (statin 25% + garlic 75%) showed similar trend initially, but the second significant reduction in the total cholesterol was observed after 10th week when compared to their base value at the time of the experiment (Table 1).

Table 1
Total cholesterol concentration (mg/dl) in different groups of rats

Groups	*0th Week*	*2nd Week*	*4th Week*	*6th Week*	*8th Week*	*10th Week*	12th Week
1.	42.39 ± 2.82^{aA}	44.78 ± 2.36^{aAB}	48.60 ± 1.26^{aABC}	46.51 ± 1.36^{aAB}	46.91 ± 2.66^{aABC}	49.70 ± 1.70^{aBC}	52.99 ± 1.42^{aC}
2.	101.22 ± 4.36^{bA}	115.37 ± 10.02^{cA}	120.00 ± 6.26^{cA}	116.63 ± 8.99^{cA}	120.44 ± 6.85^{dA}	124.85 ± 6.02^{cA}	127.99 ± 4.99^{dA}
3.	102.04 ± 6.64^{bE}	91.97 ± 2.40^{bDE} (20.28)	88.02 ± 2.61^{bCD} (26.65)	87.33 ± 3.50^{bCD} (25.13)	78.97 ± 3.79^{bcBC} (34.43)	69.82 ± 1.76^{bAB} (44.07)	65.22 ± 1.96^{bA} (49.04)
4.	101.09 ± 5.23^{bC}	100.57 ± 1.36^{bC} (12.83)	88.72 ± 2.69^{bB} (26.07)	85.00 ± 2.78^{bB} (27.12)	70.15 ± 3.63^{bA} (41.76)	68.49 ± 2.34^{bA} (45.14)	62.77 ± 2.72^{bA} (50.96)
5.	100.41 ± 2.08^{bC}	96.67 ± 4.37^{bBC} (16.20)	91.40 ± 2.71^{bB} (23.84)	88.49 ± 3.48 $^{b B}$ (24.13)	89.71 ± 2.86^{cB} (25.52)	71.75 ± 2.53^{bA} (42.53)	66.71 ± 2.16^{bcA} (47.88)
6.	102.31 ± 2.77^{bD}	96.90 ± 3.47^{bCD} (16.01)	92.91 ± 3.52^{bC} (22.58)	91.74 ± 3.26^{bC} (21.34)	82.35 ± 3.06^{bcB} (31.62)	69.38 ± 1.99^{bA} (44.43)	69.02 ± 3.56^{bcA} (46.07)
7.	104.48 ± 3.95^{bD}	99.19 ± 2.33^{bCD} (14.02)	94.42 ± 3.67^{bBC} (21.32)	91.86 ± 4.00^{bBC} (21.24)	88.97 ± 3.06^{cB} (26.13)	76.92 ± 1.76^{bA} (38.39)	75.00 ± 1.66^{cA} (41.40)

Values are mean ± standard error on mean of 8 observations
Means with different alphabets as superscripts differ significantly ($p<0.05$); Capital alphabets for horizontal comparison and small alphabets for vertical comparison.
Value in the parentheses indicates the per cent decrease in the plasma total cholesterol concentration when compared with corresponding dyslipidaemic control during that week.

2. HDL cholesterol

HDL cholesterol concentration (mg/dl) of normal control group (ranged from 23.60 ± 1.44 to 31.46 ± 2.05) was significantly ($p<0.05$) higher than that of dyslipidaemic control (ranged from 8.55 ± 0.70 to 13.76 ± 1.14) throughout the experimental period. The treatment groups showed a significant increase from 4th week onwards when compared to group 2. At the end of 4th and 6th week, group 4 showed a significant ($p<0.05$) increase in HDL cholesterol concentration (17.56 ± 0.83 and 19.77 ± 0.96, respectively) when compared with groups 3, 4, 6 and 7. At the end of 8th week, the treatment groups showed no significant difference when compared to a normal control group and the highest percent increase was seen in group 3 (174.94). The groups 5, 6 and 7 showed significant ($p<0.05$) decrease when compared to normal control (group 1) at the end of 10th week, while groups 6 and 7 showed significant ($p<0.05$) decrease (24.34 ± 1.76 and 24.16 ± 2.85, respectively) when compared to normal control (group 1; 31.46 ± 2.05) at the end of 12th week. The dyslipidaemic control group showed a significant ($p<0.05$) decrease in HDL cholesterol concentration at the end of 4th week of (8.55 ± 0.70) when compared to its basal value at the beginning of the treatment (13.76 ± 1.14). While groups 3, 4 and 6 displayed significant ($p<0.05$) increase towards the end of 6th week (16.52 ± 1.16, 19.77 ± 0.96 and 16.89 ± 0.72, respectively) and further increase was seen at the end of 8th week up to 12th week of treatment .The groups 5 and 7 displayed a significant ($p<0.05$) increase in the HDL concentration at the end of 8th week (25.20 ± 1.24 and 25.20 ± 1.73, respectively) of treatment when compared to basal level at the time of treatment (Table 2).

Table 2
HDL Cholesterol concentration (mg/dl) in different groups of rats

Groups	0th Week	2nd Week	4th Week	6th Week	8th Week	10th Week	12th Week
1.	23.84 ± 1.33^{bA}	24.83 ± 1.25^{bA}	24.27 ± 1.19^{dA}	23.60 ± 1.44^{dA}	29.76 ± 1.13^{bB}	29.95 ± 0.85^{dB}	31.46 ± 2.05^{cB}
2.	13.76 ± 1.14^{aD}	13.25 ± 0.55^{aCD}	8.55 ± 0.70^{aA}	11.86 ± 0.67^{aBCD}	10.60 ± 0.67^{aABC}	13.74 ± 0.99^{aD}	10.05 ± 1.61^{aAB}
3.	11.90 ± 1.06^{aA}	13.79 ± 1.02^{aAB} (4.06)	14.39 ± 1.21^{bAB} (68.25)	16.52 ± 1.16^{bB} (39.25)	29.14 ± 1.45^{bD} (174.94)	29.19 ± 0.72^{cdD} (112.44)	27.45 ± 1.68^{bcD} (173.13)
4.	13.69 ± 1.58^{aA}	14.76 ± 1.04^{aA} (11.41)	17.56 ± 0.83^{cAB} (105.41)	19.77 ± 0.96^{cB} (66.72)	28.26 ± 2.05^{bC} (166.61)	31.18 ± 0.92^{dC} (126.94)	29.19 ± 1.50^{bcC} (190.41)
5.	13.55 ± 1.60^{aA}	13.72 ± 0.92^{aA} (3.58)	14.39 ± 0.63^{bA} (68.25)	16.52 ± 0.64^{bA} (39.25)	25.20 ± 1.24^{bB} (137.77)	25.55 ± 0.89^{bB} (85.95)	27.15 ± 1.80^{bcB} (170.18)
6.	13.45 ± 1.53^{aA}	13.44 ± 0.36^{aA} (1.45)	14.14 ± 0.52^{bAB} (65.39)	16.89 ± 0.72^{bB} (42.44)	25.41 ± 0.93^{bC} (139.69)	26.72 ± 0.93^{bcC} (94.45)	24.34 ± 1.76^{bC} (142.21)
7.	13.94 ± 1.54^{aA}	14.29 ± 0.97^{aA} (7.85)	13.72 ± 0.57^{bA} (60.49)	15.19 ± 1.07^{bA} (28.07)	25.20 ± 1.73^{bB} (137.77)	25.21 ± 0.90^{bB} (83.45)	24.16 ± 2.85^{bB} (140.42)

Values are mean ± standard error on mean of 8 observations
Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets for horizontal comparison and small alphabets for vertical comparison.
Value in the parentheses indicates the percent increase in the plasma HDL-C concentration when compared with corresponding dyslipidaemic control during that week.

3. Triglycerides

The triglyceride concentration (mg/dl) of normal control was significantly (p<0.05) lower (ranged from 22.39 ± 2.67 to 27.25 ± 3.08) and that of dyslipidaemic control group 2 was significantly (p<0.05) higher (ranged from 165.45 ± 19.04 to 186.01 ± 8.14) when compared to treatment groups 3 to 7 throughout the experiment. All the treatment groups showed a significant (p<0.05) decrease in triglyceride concentration from 2nd to 12th week of treatment when compared to dyslipidaemic control, although there was no significant difference among treatments throughout the study. At the end of the 12th week, the highest percent decrease (71.23) in triglyceride concentration was observed in group 4 (statin 100% + garlic 100%). The statin control group (group 3) exhibited significant (p<0.05) reduction at the end of 4th week (111.36 ± 16.38) when compared to its base value (173.70 ± 8.06) and further significant (p<0.05) decrease was observed at the end of 10th week (72.24 ± 5.99). Group 4 showed significant (p<0.05) decrease at the end of 2nd week (159.42 ± 7.70) from its base level (183.70 ±12.67) at the beginning of the treatment and further subsequent significant reduction was recorded during 4th (130.19 ± 9.08), 6th (99.43 ± 5.97) and 12th week (53.38 ± 4.02). Similar trend was displayed by group 5, where there was a significant reduction (p<0.05) at the end of 2nd week (134.60 ± 6.59) from its base value (177.39 ±11.83) and further significant (p<0.05) reduction was observed at the end of 10th week (81.33 ± 5.11). In group 6, the first significant (p<0.05) reduction in the triglyceride level was observed at the end of 4th week (134.42 ± 17.12) and subsequent reductions at the ends of 6th and 12th week (95.51 ± 7.16 and 64.14 ± 4.19). Group 7 showed initial significant (p<0.05) reduction at the end of 4th week (109.10 ± 11.04) over its base value (176.74 ±12.12) and further reduction was observed at the end of the 12th week (66.50 ± 3.59) (Table 3).

Table 3
Triglycerides concentration (mg/dl) in different groups of rats

Groups	0th Week	2nd Week	4th Week	6th Week	8th Week	10th Week	12th Week
1.	24.13 ± 2.80 ^{aA}	26.79 ± 3.04^{aA}	26.14 ± 3.29^{aA}	27.25 ± 3.08^{aA}	22.39 ± 2.67^{aA}	29.71 ± 2.42^{aA}	25.10 ± 2.17^{aA}
2.	175.87 ±11.09^{bA}	169.48 ± 9.90^{cA}	174.68 ± 13.55^{cA}	165.45 ± 19.04^{cA}	178.68 ± 11.84^{cA}	186.01 ± 8.14^{cA}	185.55 ± 9.61^{cA}
3.	173.70 ± 8.06^{bD}	158.44 ± 17.49^{bcD} (6.51)	111.36 ± 16.38^{bC} (36.25)	96.35 ± 14.18^{bBC} (41.77)	92.64 ± 7.73^{bBC} (48.15)	72.24 ± 5.99^{bAB} (61.17)	55.84 ± 2.94^{bA} (69.91)
4.	183.70 ±12.67^{bE}	159.42 ± 7.70^{bcD} (5.94)	130.19 ± 9.08^{bC} (25.47)	99.43 ± 5.97^{bB} (39.90)	84.36 ± 5.94^{bB} (52.79)	80.36 ± 6.00^{bB} (56.81)	53.38 ± 4.02^{bA} (71.23)
5.	177.39 ±11.83^{bD}	134.60 ± 6.59^{bC} (20.58)	125.00 ± 20.62^{bC} (28.44)	108.15 ± 17.84^{bBC} (34.64)	98.93 ± 3.56^{bBC} (44.63)	81.33 ± 5.11^{bAB} (56.28)	56.76 ± 3.01^{bA} (69.41)
6.	175.65 ±13.63^{bD}	161.63 ± 6.79^{bcCD} (4.63)	134.42 ± 17.12^{bC} (23.05)	95.51 ± 7.16^{bB} (42.28)	98.47 ± 6.37^{bB} (44.89)	79.06 ± 3.08^{bAB} (57.50)	64.14 ± 4.19^{bA} (65.43)
7.	176.74 ±12.12^{bC}	159.38 ± 10.49^{bcC} (5.97)	109.10 ± 11.04^{bB} (37.55)	101.69 ± 8.79^{bB} (38.54)	101.53 ± 6.80^{bB} (43.18)	84.25 ± 6.08^{bAB} (54.71)	66.50 ± 3.59^{bA} (64.16)

Values are mean ± standard error on mean of 8 observations
Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets for horizontal comparison and small alphabets for vertical comparison.
Value in the parentheses indicates the per cent decrease in the plasma triglycerides concentration when compared with corresponding dyslipidaemic control during that week.

4. LDL cholesterol

The LDL cholesterol concentration (mg/dl) of control group was significantly (p<0.05) lower (ranged from 12.68 ±1.93 to 19.11 ±1.59) and that of dyslipidaemic group was significantly (p<0.05) higher (ranged from 52.28 ±5.64 to 80.83 ±6.00) when compared to treatment groups 3 to 7 throughout the experiment. All the treatment groups showed a significant (p<0.05) decrease in LDL cholesterol concentration from 2nd week of treatment when compared to dyslipidaemic control, though there was no significant difference among treatment groups till the end of 4th week. The highest per cent decrease was observed in groups 3 and 4 (31.84 and 36.75, respectively) at the end of the 2nd and 6th week, respectively. At the end of 4th week, group 7 showed significant (p<0.05) increase (58.88 ± 3.47) when compared to other treatment groups. At the end of 8th week, group 5 (44.72 ± 3.21) showed a significant (p<0.05) increase when compared to other treatment groups, while group 7 showed significant increase (p<0.05) at the end of 10th and 12th week (34.87 ± 2.63 and 37.54 ± 3.71, respectively). The groups 3, 4, 6 and 7 showed significant (p<0.05) decrease in the LDL concentration at the end of 8th week onwards, while group 5 showed significant (p<0.05) decrease in LDL concentration at the end of 12th week (28.21 ± 2.72) (Table 4).

Table 4
LDL Cholesterol concentration (mg/dl) in different groups of rats

Groups	0th Week	2nd Week	4th Week	6th Week	8th Week	10th Week	12th Week
1.	13.72 ± 1.96^{aA}	14.59 ± 2.70^{aA}	19.11 ± 1.59^{aA}	17.46 ± 2.43^{aA}	12.68 ± 1.93^{aA}	13.82 ± 1.39^{aA}	16.51 ± 2.34^{aA}
2.	52.28 ± 5.64^{bA}	68.22 ± 9.83^{cA}	76.51 ± 7.11^{dA}	71.68 ± 9.03^{cA}	74.11 ± 6.34^{eA}	73.91 ± 6.53^{dA}	80.83 ± 6.00^{dA}
3.	55.40 ± 6.63^{bB}	46.50 ± 2.91^{bB} (31.84)	51.37 ± 2.93^{bcB} (32.86)	51.54 ± 2.82^{bB} (28.10)	31.30 ± 4.34^{bcA} (57.77)	26.18 ± 2.52^{bcA} (64.57)	26.60 ± 1.91^{bcA} (67.09)
4.	50.65 ± 5.50^{bB}	53.93 ± 3.00^{bB} (20.95)	45.12 ± 2.67^{bB} (41.03)	45.34 ± 2.89^{bB} (36.75)	25.02 ± 3.62^{bA} (66.25)	21.24 ± 3.62^{abA} (71.26)	22.91 ± 2.30^{abA} (71.66)
5.	51.38 ± 4.26^{bBC}	56.03 ± 4.56^{bcC} (17.87)	49.10 ± 2.89^{bcBC} (32.02)	53.25 ± 2.65^{bBC} (25.71)	44.72 ± 3.21^{dB} (39.66)	29.93 ± 3.52^{bcA} (59.50)	28.21 ± 2.72^{bcA} (65.10)
6.	53.73 ± 3.77^{bB}	51.14 ± 3.07^{bB} (25.04)	51.88 ± 2.97^{bcB} (32.19)	55.75 ± 3.67^{bB} (29.77)	37.25 ± 4.24^{bcdA} (49.73)	26.85 ± 2.88^{bcA} (63.67)	31.85 ± 3.68^{bcA} (60.59)
7.	55.20 ± 4.29^{bC}	53.02 ± 1.78^{bBC} (22.28)	58.88 ± 3.47^{cC} (23.04)	56.33 ± 3.99^{bC} (21.41)	43.46 ± 3.88^{cdAB} (41.36)	34.87 ± 2.63^{cA} (52.83)	37.54 ± 3.71^{cA} (53.56)

Values are mean ± standard error on mean of 8 observations
Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets for horizontal comparison and small alphabets for vertical comparison.
Value in the parentheses indicates the per cent decrease in the plasma LDL-C concentration when compared with corresponding dyslipidaemic control during that week.

5. Tissue total cholesterol

The total cholesterol concentration (mg/100mg tissue) of liver in the basal diet control was 543.05 ± 39.29, which was significantly (p<0.05) lower than that of dyslipidaemic control (1961.81 ± 49.90) and treatment

groups 3 to 7. The total cholesterol concentration in the treatment groups showed a significant ($p<0.05$) decrease when compared to dyslipidaemic control. However, there was no significant difference among treatment groups (Table 5).

Table 5
Tissue (liver) cholesterol concentration (mg/100g of tissue) in different groups of rats

Groups	*12th Week*
1.Control	543.05 ± 39.29[a]
2.Dyslipidaemic Control (DL)	1961.81 ± 49.90[c]
3. DL + Statin 100% (10mg/kg bw)	1154.41 ± 89.09[b]
4. DL + Statin 100% (10mg/kg bw) + Garlic 100% (1% w/w in feed)	1020.65 ± 82.16[b]
5. DL + Statin 50% (5mg/kg bw) + Garlic 50% (0.5% w/w in feed)	996.32 ± 40.93[b]
6. DL + Statin 75% (7.5mg/kg bw) + Garlic 25% (0.25% w/w in feed)	1030.32 ± 57.43[b]
7. DL + Statin 25% (2.5mg/kg bw) + Garlic 75% (0.75% w/w in feed)	1008.22 ± 38.35[b]
ANOVA with Duncan's multiple comparison test at 0.05 significance level.	F = 50.345 p = 0.000

Values are mean ± standard error on mean of 6 observations
Means with different alphabets as superscripts differ significantly (p<0.05)

DISCUSSION

When compared with dyslipidaemic control group, the treatment groups showed significant decrease in TC, TG and LDL-C concentration at the end of 2nd week and significant increase in HDL-C concentrations at the end of 4th week. At the end of 8th week, TC concentration of groups 4 (statin 100% + garlic 100%) and 5 (statin 50% + garlic 50%) was significantly reduced when compared to other treatment groups, while HDL-C concentration was significantly increased in group 4 than those of other treatments at the end of 4th and 6th week. By the end of 8th week, HDL-C concentrations of all treatment groups achieved the values that were comparable with that of control. Highest per cent increase in HDL-C was seen in group 3 (statin-100%). Total cholesterol concentrations were decreased significantly from their basal level in all the treatment groups at the end of 4th week, whereas treatment in group 4 could able to reduce TC further significantly at 8th week against 10th week with other treatments. Groups 4 and 5 could quickly reduce the basal TG levels significantly as early as in 2 weeks against 4 weeks for other treatment groups showing the synergistic action of the drugs in combination. Apart from this, group 4 also showed subsequent significant reductions in TG levels at 4th, 6th and 12th week, while remaining treatment groups showed only one significant reduction later on at the end of 12th week. Significant reduction in LDL-C concentration from their basal value was seen at the end of 8th week in groups 3, 4 and 6 against 10th week in groups 5 and 7. At the end of the treatment, TC, TG and LDL-C concentrations were significantly lowered and HDL-C concentration was significantly increased in treatment groups when compared to dyslipidaemic control. HDL-C concentration was quickly increased in statin control (group 3), statin 100 % + garlic 100% (group 4) and statin 50 % + garlic 50% (group 5) groups and the values were comparable to those of normal control (group 1). TC concentrations of treatment groups were not comparable with that of control but group 4 showed highest percent decrease with lowest concentration of cholesterol among all treatment groups suggesting the synergistic potential of statin and garlic. Similar trend was seen with TG in group 4, which showed highest percent decrease. Among treatments group 4 exhibited, lowest LDL-C concentration that was comparable with that of control with highest per cent decrease when compared to other treatment groups. The tissue cholesterol concentration in liver revealed a significant reduction in all the treated groups when compared to dyslipidaemic control.

The literature is scanty on the interaction of herbs with statins with respect to pharmacodynamics, though there is a

progressive increase in the usage of herbs around the globe. Although it is believed that herbal products are natural and safe, they require attention for risk to the biological system as they are pharmacologically active. Also, they can interact with other drugs with resultant increase or decrease in the efficacy and toxicity of drugs. Herbs like garlic have been reported to compete with certain other drugs for metabolism by CYP 450 enzymes or inactivate the CYP enzymes and therefore affect the concentration of certain co-administered drugs leading to clinically significant pharmacodynamic and pharmacokinetic interactions[12,13]. Reduced metabolism of either drug in combination following co-administration leads to significantly elevated concentrations with eventual toxicity. Alternatively, reduced concentration due to pharmacokinetic interaction results in therapeutic failure.

Cholesterol and triglycerides circulate in the blood stream as part of lipoprotein complexes. With centrifugation, these complexes separate into HDL, IDL, LDL and VLDL fractions. Triglycerides and cholesterol in the liver are incorporated into VLDL and released into the plasma for delivery to the peripheral tissues. LDL is formed from VLDL and is catabolized primarily through the high affinity LDL receptors. Elevated plasma levels of total cholesterol, LDL-C and ApoB promote atherosclerosis and are risk factors for developing cardiovascular disease, while reduced levels of HDL-C and its transport complex ApoA are associated with development of atherosclerosis. HMG CoA reductase inhibitors are the most often prescribed drugs for reducing plasma cholesterol in patients with hypercholesterolaemia, though they have limited ability to reduce triglycerides[14]. Atorvastatin is a selective and competitive inhibitor of HMG Co A reductase, a rate limiting enzyme that converts HMG CoA to mevalonate, a precursor of sterols. It also acts by increasing the number of hepatic LDL receptors on the cell surface to enhance uptake and catabolism of LDL, besides reducing LDL production and the number of LDL particles[15]. It has been reported that co-administration of HMG CoA reductase inhibitors with an agent such as nicotinic acid or fibrate can reduce both cholesterol and triglycerides more efficiently, but this combination is uneconomical and has an increased risk of myositis and renal failure[16]. Therefore, in the present investigation, garlic was used along with atorvastatin at different dose proportions to assess the pharmacodynamic interaction and the extent of efficacy. The overview of results on lipid profile revealed that full dose combination (100% atorvastatin + 100% garlic) was superior in normalizing concentration of TC, TG and LDL-C, besides increasing HDL-C but the safety profile of the combination was reported to be less with respect to nephotoxicity[6] and hepato toxicity[5]. However 100% statin control (group 3) found superior in normalizing lipid profile during the early stages of treatment and was found more effective in reducing only TC and LDL-C. The other treatment groups viz., 50% statin + 50% garlic (group 5) and 75% statin + 25% garlic (group 6) were found better in normalizing the lipid profile. Group 7 (25% statin + 75% garlic) was found inferior to other treatment groups in reducing TC and LDL-C. Several clinical studies have established cholesterol reducing action of garlic in hypercholesterolaemic subjects[17]. Direct measurement of enzyme activity has indicated that garlic and its constituents inhibit HMG CoA reductase[18,19], which may be the key mechanism in antidyslipidaemic action of garlic. Garlic has also been shown to suppress the hepatic activities of other lipogenic, cholesterologenic enzymes such as malic enzymes, fatty acid synthase, glucose 6 phosphate dehydrogenase etc.[20] There are reports that garlic extract could significantly reduce the serum TC, LDL-C and TG after 6 weeks of treatment along with a significant increase in HDL-C[21]. One study reported that raw garlic was more beneficial than the cooked form in normalizing the lipid profile[22]. Another study[23], concluded that decrease in the liver cholesterol and plasma cholesterol in garlic-fed animals was due to increased excretion of cholesterol and its metabolites. Some of the sulfur compounds such as allicin, ajoene, S-allyl cysteine (SAC), diallyl disulfide (DADS), S-methyl cysteine sulfoxide and S-allyl

cysteine sulfoxide may be responsible for the therapeutic properties of the garlic[20]. In this study, the group that was treated predominantly with major proportion of garlic (group 7- garlic 75% + atorvastatin – 25 %), though revealed normalized dyslipidaemic profile, was found inferior to other groups This finding suggests that addition of herb to statin is synergistic in countering dyslipidaemia, which is a positive pharmacodynamic interaction. One possible hypothesis for this result may be that inhibition of CYP 450 enzymes and inhibition of P-gp by garlic may be responsible for elevated concentration (C_{max} and AUC) of atorvastatin[13], which is a potent inhibitor of HMG CoA reductase that is responsible for cholesterol synthesis.

CONCLUSION

From this study it can be concluded that garlic and atorvastatin exhibited positive pharmacodynamic interaction in reducing dyslipidaemias. The toxicological and pharmacokinetic studies reported earlier[5,6,13] indicated that high dose of atorvastatin with high dose of garlic has a negative safety profile, while the groups having moderate dose of statin and equal or low dose of garlic revealed better safety profile in rats. From this study, it can be postulated that the combination having either equal or major proportion of statin would be beneficial in improving the dyslipidaemias.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Mahley RW and Bersot, TP, Drug therapy for hypercholesterolemia and dyslipidemia. In: L.L. Brunton, J. S. Lazo, and K.L. Parker, (eds.), *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 11th ed. McGraw Hill, NewYork, 2006, pp.933–960.
2. WHO, Memorandum on classifications of Hyperlipidemias and Hyperlipoproteinemias 43, 891, (1970).
3. Heart Protection Study Collaborative Group, MRC/BHF Heart ProtectionStudy of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. Lancet 360: 7–22, (2002).
4. Dormuth CR, Hemmelgarn BR, Paterson, JM, James MT, Teare GF, Raymond CB, Lafrance J-P, Levy A, Garg A X and Ernst P, Use of high potency statins and rates of admission for acute kidney injury: multicenter, retrospective observational analysis of administrative databases. British Medical Journal, BMJ 346:f880, (2013).
5. Reddy GD, Reddy AG, Kumar AA and Anilkumar B, Pathological studies on interaction of atorvastatin with garlic in induced dyslipidaemia in rats. Inventi Rapid: Molecular Pharmacology, 2012(4):1-4, (2012).
6. Reddy GD, Reddy AG, Rao GS, Haritha C and Jyothi K, Interaction study on garlic and atorvastatin with reference to nephrotoxicity in dyslipidaemic rats. Toxicology International, 17: 90-93, (2010).
7. Law MR, Wald NJ, and Rudnicka AR, Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: Systematic review and meta-analysis. British Medical Journal, 326:1423-1429, (2003).
8. Anonymous. Herbalism -role of herbal medicine in modern human society. Accessed on 20-11-2008 from http://en.wikipedia.org/wiki/Herbalism#Role_of_herbal_medicine_in_modern_human_society, (2008).
9. Reuter HD, *Allium sativum* and *Allium ursinum*, part 2: pharmacology and medicinal applications. Phytomedicine, 2: 73-91, (1995).
10. Vimal V, and Devaki T, Hepatoprotective effect of allicin on tissue defense system in galactosamine/endotoxin challenged rats. Journal of Ethnopharmacology, 90:151-154, (2004).

11. Folch J, Lees M, and Stanley GHS, A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226:497-509, (1957).
12. Jani A, and Mehta A, Pharmacology of *Allium sativum* in relation to Cytochrome P450 and possible drug interactions. Pharmacognosy Reviews, 2: 222-226, (2007).
13. Reddy GD, Reddy AG, Rao GS, and Vijaykumar M, Pharmacokinetic interaction of garlic and atorvastatin in dyslipidemic rats. Indian Journal of Pharmacology, 44: 246-252, (2012).
14. Endo A, The discovery and development of HMG-CoA reductase inhibitors. Journal of Lipid Research, 33:1569–1582, (1992).
15. Nawrocki JW, Weiss SR, Davidson MII, Sprecher DL, Schwartz SL, Lupien PJ, Jones PH, Haber HE, and Black DM, Reduction of LDL cholesterol by 25 to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. Arterioscler Thromb Vasc Biol, 15:678–682, (1995).
16. East C, Bilheimer DW, and Grundy SM, Combination drug therapy for familial combined hyperlipidemia. Ann Intern Med, 109:25–32, (1988).
17. Rahman K, and Lowe GM, Garlic and cardiovascular disease: a critical review. The Journal of Nutrition, 136: 736S–740S, (2006).
18. Gupta N, and Porter TD, Garlic and garlic derived compounds inhibit human squalene monooxygenase. The Journal of Nutrition, 131:1662–1667, (2001).
19. Augusti KT, Chackery J, Jacob J, Kuriakose S, George S, and Nair SS, Beneficial effects of a polar fraction of garlic (*Allium sativum* Linn) oil in rats fed with two different high fat diets. Indian Journal of Experimental Biology, 43:76–83, (2005).
20. Yeh YY, and Liu L, Cholesterol-lowering effects of garlic extracts and organosulfur compounds: human and animal studies. The Journal of Nutrition, 131:989S–93S, (2001).
21. Nwanjo HU, and Oze GO, Changes in serum lipid profiles and heart rate in rats treated with aqueous garlic extract. The Internet Journal of Nutrition and Wellness, 4: Number 1, (2007).
22. Thomson M, Al-Qattan KK, Bordia T, and Ali M, Including garlic in the diet may help lower blood glucose, cholesterol, and triglycerides. The Journal of Nutrition, 136: 800S–802S, (2006).
23. Chi MS, Koh ET, and Stewartt AJ, Effects of garlic on lipid metabolism in rats fed cholesterol or lard. The Journal of Nutrition, 112: 241-248, (1982).