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RESEARCH ARTICLE

Lipid Lowering Effect of a Herbal Mixture in Hyperlipidaemic Adult Male Albino Mice

Ijaz Javed[1]*, Muhammad Sarfraz[1], Faqir Muhammad[1], Bilal Aslam[1], Zia-ur-Rahman[1], Muhammad Zargham Khan[2], Tanweer Khaliq[1], Faqir Hussain Khan[3] and Mahmood Ahmad[4]

[1]Department of Physiology and Pharmacology [2]Department of Pathology, University of Agriculture Faisalabad; [3]The University of Faisalabad, Faisalabad; [4]Faculty of Pharmacy and Alternative Medicine, Islamia University, Bahawalpur, Pakistan
*Corresponding author:sandhu_drijaz@yahoo.com

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ABSTRACT

The present study was conducted to evaluate the lipid lowering effect of a herbal mixture (containing garlic, lemon, ginger, apple vinegar and honey) in hyperlipidemic adult male albino mice. Animals were divided into six groups. Except normal control group, which was kept on routine mice feed, the rest of the groups were provided with atherogenic diet for 0-15 days (lead-in period) to induce hyperlipidemia. After that period mixture was fed to hyperlipidemic albino mice at the dose level of 1.5, 2.5 and 3.5 ml/kg BW to three treated groups, respectively, for 15-60 days as cellulose replacement in atherogenic diet. Simvastatin (synthetic lipid lowering drug, as reference standard) at the dose rate of 0.6mg/kg BW was fed to the hyperlipidemic albino mice of treated control group for 15-60 days as cellulose replacement in atherogenic diet while untreated control group was kept on atherogenic diet as such. Blood samples were taken and serum was tested for lipid profile parameters at day 0, 15, 30, 45 and 60 after the initiation of experiment. The results suggested that the values of percentage reduction induced after administration of mixture, 3.5 ml/kg and simvastatin, 0.6 mg/kg, respectively, at post treatment day 60, are non-significantly ($P>0.05$) different (51.63 and 68.61 for TL, 49.61 and 60.26 for TGs, 59.54 and 64.72 for TC and 65.75 and 66.44 for LDL-c. Similarly, respective percentage increase, 20.38 and 26.25, for HDL-c were also mutually non-significant. Therefore, this is concluded that mixture, 3.5 ml/kg and simvastatin, 0.6 mg/kg, are equieffective in treating hyperlipidemia in male albino mice.

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INTRODUCTION

About 80% of world's population depends on indigenous medicinal plants. Sixty one percent of global population has been reported to use herbal therapy in various diseased conditions. Use of indigenous plants as remedy against various diseases is increasing because synthetic drugs possess many side effects (Javed *et al*., 2009).

The epidemiological studies showed that the consumption of fat rich diet and the animal source derived food products are appropriate key factor for coronary heart disease (CHD) and known cause of death, all over the world (Rajendran and Ekambaram, 2010). The risk factors of CHD may include an age, sex, obesity, diabetes, increase in blood cholesterol, triglycerol (TAG), and lipoproteins such as LDL (Nago *et al.,* 2011; Bui*et al.,* 2011).

Increased levels of serum total cholesterol, triglycerides, very low density lipoproteins (VLDLs), low density lipoproteins (LDLs) and decreased levels of high density lipoproteins (HDL-c) in blood is referred as hyperlipidemia (Javed *et al*., 2006). Cardiovascular disease (CVD) is the foremost cause of deaths in South Asia (Lim *et al.,* 2012). South Asians have higher evidence of heart diseases at younger age when compared to developed countries (Prashant *et al*., 2007).

There is certain cholesterol lowering functional foods and extract of traditional plants like Cydonia oblonga Mill which are getting more and more attention these days

because of their activity to reduce the total plasma cholesterol level. Such type of foods and plant extracts are possibly alternative therapy for treating the hypercholesterolemia patients whose blood cholesterol level is slightly raised rather, in treating the patients having very high cholesterol level and cardiovascular diseases (Chen*et al.,* 2008; Grundemann *et al.,* 2011). In herbal system of indigenous medicine about 2000 plants are identified and these are offer protection against various cardiac problems such as ischemic heart disease and hypercholesterolemia (Mahmood*et al*., 2010). Some of these plants such as *Allium sativum* (garlic) and *Zingiber officinale* (ginger), being frequently used as spice and *Citrus lemon* (lemon) and *Malus domestica* (apple) vinegar, used as food or part of food has been reported for having a variety of medicinal effects (Saraswat*et al.,* 2010).

Garlic contains allinase enzyme and sulphur containing compounds like alliin reported to be having cardioprotective and antihypertensive effect (Bhandari, 2012). Ginger and Lemon possesses antioxidant activity and hence lipid lowering effect due to polyphenoles, vitamin C, β carotene, flavonoids (flavonol glycosides), tannins and vitamin c (Adel and Prakash, 2010). Apple contains apple pectin and polyphenols which enhance lipid metabolism (Sheau, *et al*., 2011). Honey having flavonoids (apigenin, pinocembrin, kaempferol) and phenolic acids (ellagic, caffeic, p-coumaric and ferulic acids) proves to be a potent antioxidant (Alvarez-Suarez*et al*., 2010). Keeping in view the preceding lines, following study was carried out to evaluate the lipid lowering effect of an herbal mixture containing Garlic, lemon, ginger, apple vinegar and honey in hyperlipidemic mice.

MATERIALS AND METHODS

Animals: One hundred and fifty healthy adult male albino mice were purchased from the National Institute of Health (NIH) Islamabad, Pakistan. The animals were kept under the similar management conditions in animal room of Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The mice were kept in clean iron cages at ambient temperature with a 12/12 hour period of light/dark.

Experimental design: After seven days of acclimatization, mice were randomly divided in to six equal groups like normal control on routine feed, untreated control on atherogenic diet, treated control on synthetic lipid lowering drug, treated 1, treated 2 and treated 3 on three respective doses of the mixture. The mice were provided rat chow as normal routine feed till the completion of experiment. The diet was provided to mice twice a day usually in the morning and evening. However, drinking water was available throughout 24 hours. The experiments were conducted with the prior approval by the Directorates of Research and Advanced Studies and with the consent of the Society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan. The mice were sacrificed according to the rules laid down by the Society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan. Except normal control group which was kept on normal routine feed, the rest of groups were also provided with atherogenic diet for 0-60 days. The period of 0-15 days was considered as the lead-in period to induce hyperlipidemia in mice (Fig. 1). The atherogenic diet was comprised of cholesterol powder 0.5%, coconut oil 20% and cellulose 15%, mixed in the normal routine feed (Javed *et al.*, 2012). Tablets Survive® (Simvastatin 20mg, Werrick Pharmaceuticals, Pvt. Ltd, Islamabad, Pakistan) were used as synthetic anti-hyperlipidemic agent. Feeding schedule of normal routine feed, atherogenic diet, mixture and simvastatin in adult male albino mice during the experimental period of 0 to 60 days has been presented in Table 1.

Sample collection and analysis: Blood samples were collected on 0, 15, 30, 45 and 60 post treatment days. For the collection of blood samples five mice from each group were sacrificed at each sampling time and the blood of mice was collected individually. The samples were allowed to clot for 20 minutes at refrigeration temperature and then centrifuged for 5 min at 4000 rpm. Lipid profile parameters were determined spectrophotometerically in serum of mice with reagent kits (Randox laboratories Ltd, UK).

Statistical analysis: The significance of the difference between pre- treated and post- treated values was determined using student's 't' test, to assess the antihyperlipidemic effect of the mixture and respective percent reduction was computed. The level of significance was set to 5%. $P<0.05$ was considered significant while $P>0.05$ was considered as non-significant.

RESULTS AND DISCUSSION

In the present study, hyperlipidemia was induced in adult mice by feeding atherogenic diet for 0-60 days along with the normal routine feed. Antihyperlipidemic efficacy of the mixture at the dose rate of 1.5, 2.5 and 3.5 ml/kg BW has been shown in Tables 1-5.

After the initiation of the experiment, administration of atherogenic diet during lead-in period of 0-15 days showed almost 2 fold increase in total lipids, triglycerides, total cholesterol and low density lipoprotein cholesterol inserum of mice, while high density lipoprotein level reflected decreasing trend (Fig. 1) ascertaining a parallel

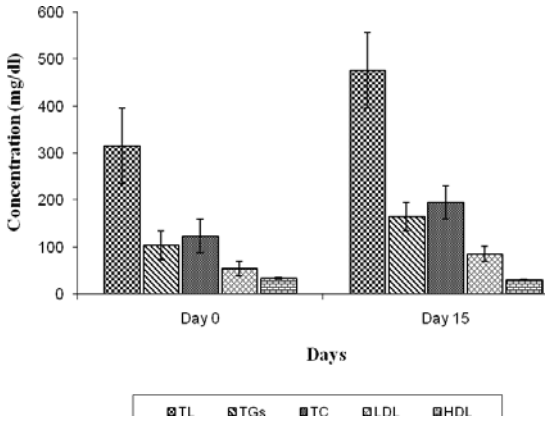

Fig 1: Serum lipid profile (Mean±SE) in adult male albino mice fed with atherogenic diet from day 0-15.

Table 1: Feeding schedule of normal routine diet, atherogenic diet, mixture and simvastatin in adult male albino mice during the experimental period of 0-60 days.

Group 1: Control on normal routine feed.	Routine diet 0-60 days.
Group 2: Untreated control on atherogenic diet.	Atherogenic diet (Routine diet + cholesterol 0.5%, coconut oil 20 %, Celllulose 15 %) 0-60 days.
Group 3: Treated control on synthetic lipid lowering drug; Tablet survive®	Atherogenic diet 0-15 days, atherogenic diet + Tablets Survive® (Simvastatin, 20mg, 0.6mg/kg) 15-60 days as cellulose replacement.
Group 4: Treated with mixture of *A. sativum, C. lemon, Z. officinale*, *M. domestica* vinegar and honey.	Atherogenic diet 0-15 days, atherogenic diet + Mixture (1.5 ml/kg BW) for 15-60 days as cellulose replacement.
Group 5: Treated with mixture of *A. sativum, C. lemon, Z. officinale*, *M. domestica* vinegar and honey.	Atherogenic diet 0-15 days, atherogenic diet + Mixture (2.5 ml/kg BW) for 15-60 days as cellulose replacement.
Group 6: Treated with mixture of *A. sativum, C. lemon, Z. officinale*, *M. domestica* vinegar and honey.	Atherogenic diet 0-15 days, atherogenic diet + Mixture (3.5 ml/kg BW) for 15-60 days as cellulose replacement.

Average consumption of feed for a 25 g mice = 33 g/day.

Table 2: Serum total lipids (Mean±SE; mg/dl) and their percent reductions in hyperlipidemic adult male albino mice (n=25) after administration of mixture and simvastatin.

	Lead-in-Period	Post treatment days			Percentage reduction on post treatment days		
Groups	Day 15	30	45	60	30	45	60
1	315.60±5.48	317.90±2.79	318.41±3.08	320.08±4.29	-	-	-
2	476.51±3.38	535.30±1.89	555.12±41.18	659.70±7.79	-	-	-
3	478.54±3.04	260.33±1.31	200.26±1.53	150.21±1.14	45.60±1.71	58.15±2.03	68.61±1.82
4	476.49±2.57	440.47±0.98	418.52±1.68	375.42±3.21	7.56±2.77	12.17±1.47	21.21±2.25
5	483.37±2.59	356.55±2.93	283.68±1.76*	271.99±0.82*	26.24±1.75	41.31±2.95	43.73±2.65
6	476.37±1.60	331.01±3.35	276.43±2.47*	230.41±1.70*	31.53±2.35	42.81±1.95	51.63±2.73

*=Significantly less (P<0.05) than the pretreatment value at 15 day.

Table 3: Serum triglycerides (Mean±SE; mg/dl) and their percent reductions in hyperlipidemic adult male albino mice (n=25) after administration of mixture and simvastatin.

	Lead-in-Period	Post treatment days			Percentage reduction on post treatment days		
Groups	Day 15	30	45	60	30	45	60
1	105.03±1.40	105.00±0.80	105.31±1.19	106.35±1.35	-	-	-
2	164.60±1.07	192.52±2.02	217.24±0.72	235.17±2.28	-	-	-
3	166.70±1.36	101.47±1.32	83.72±1.93	66.26±1.99	39.13±2.75	49.78±1.85	60.26±3.09
4	165.50±1.43	149.99±1.98	141.07±1.73	109.37±2.96	9.37±2.17	14.76±2.85	33.92±1.95
5	168.80±2.20	146.34±2.43	121.27±1.78	104.84±1.81*	13.32±3.05	28.18±2.15	37.90±1.76
6	165.80±1.38	134.11±1.86	112.92±2.07*	83.60±2.43*	19.16±2.35	31.93±1.96	49.61±2.87@

*=Significantly less (P<0.05) than the pretreatment value at 15 day; @=Non-significantly (P>0.05) different from percent reduction values of simvastatin.

Table 4: Serum total cholesterol (Mean±SE; mg/dl) and their percent reductions in hyperlipidemic adult male albino mice (n=25) after administration of mixture and simvastatin.

	Lead-in-Period	Post treatment days			Percentage reduction on post treatment days		
Groups	Day 15	30	45	60	30	45	60
1	126.06±1.13	124.70±1.30	125.67±0.87	126.55±0.64	-	-	-
2	195.46±1.55	210.05±1.28	186.45±40.25	249.64±3.62	-	-	-
3	195.05±1.14	120.26±1.65	87.62±2.78	68.81±2.30	38.34±3.15	55.08±1.85	64.72±2.35
4	193.86±1.03	169.78±3.15	157.53±2.66	138.82±2.01	12.42±1.92	18.74±2.94	28.39±2.19
5	196.12±1.06	147.67±2.57	118.61±2.70	83.05±2.53*	24.70±2.83	39.52±1.97	57.65±3.12
6	195.33±1.34	148.56±2.00	102.78±2.01*	79.03±3.53*	23.94±3.04	47.38±2.91	59.54±2.27@

*=Significantly less (P<0.05) than the pretreatment value at 15 day; @=Non-significantly (P>0.05) different from percent reduction values of simvastatin.

Table 5: Serum LDL-cholesterol (Mean±SE; mg/dl) and their percent reductions in hyperlipidemic adult male albino mice (n=25) after administration of mixture and simvastatin.

	Lead-in-Period	Post treatment days			Percentage increase on post treatment days		
Groups	Day 15	30	45	60	30	45	60
1	50.23±3.28	52.70±2.48	52.86±2.03	52.27±3.35	-	-	-
2	85.73±1.78	103.74±0.70	125.71±1.33	152.39±2.87	-	-	-
3	86.08±1.20	50.50±2.18	40.68±2.88	28.88±1.92	41.32±1.87	52.74±3.11	66.44±2.65
4	86.58±1.31	62.50±2.75	56.42*±2.63	50.63*±4.59	27.81±2.87	34.83±1.97	41.52±2.93
5	86.72±0.97	61.05±2.71	44.41*±1.94	36.26*±1.91	29.60±2.83	48.79±3.17	58.18±2.76
6	84.32±0.66	70.08±2.81	47.88*±1.56	28.90*±1.90	16.88±2.13	43.21±2.71	65.72±1.94@

*=Significantly less (P<0.05) than the pretreatment value at 15 day; @=Non-significantly (P>0.05) different from percent reduction values of simvastatin.

Table 6: Serum HDL-cholesterol (Mean±SE; mg/dl) and their percent increase in hyperlipidemic adult male albino mice (n=25) after administration of mixture and simvastatin.

	Lead-in-Period	Post treatment days			Percentage increase on post treatment days		
Groups	Day 15	30	45	60	30	45	60
1	34.28±1.31	35.40±0.77	34.57±0.86	34.91±0.99	-	-	-
2	30.72±0.75	29.00±0.71	25.72±0.97	22.50±1.06	-	-	-
3	30.70±0.83	31.52±1.57	35.65±2.73	38.76±2.84*	2.67±2.19	16.12±1.93	26.25±2.61
4	30.55±0.67	31.01±2.09	31.17±3.10	32.33±3.14	1.5±2.54	2.03±1.04	5.83±1.91
5	31.73±0.74	33.35±2.68	33.72±2.94	34.07±2.76	5.10±1.74	6.33±1.09	7.37±2.62
6	30.91±0.76	31.69±2.49	32.90±3.25	37.21±2.20*	2.52±2.73	6.44±1.98	20.38±2.37@

*=Significantly less (P<0.05) than the pretreatment value at 15 day; @=Non-significantly (P>0.05) different from percent reduction values of simvastatin.

relation between dietary lipids and hyperlipidemia (Javed *et al*., 2009). These results are in concurrence with other studies in which a fat rich diet resulted in elevated serum levels of cholesterol (Wang*et al*., 2009; Javed *et al*., 2012). The mixture at the dose level of 3.5 ml/kg BW significantly (P<0.05) reduced TL as 42.81 and 51.99%; TGs as 31.93 and 49.61%; TC as 47.38 and 59.54%; and LDL-c as 43.21 and 65.72% on post treatment days 45 and 60, respectively presented in Tables 1-5. Nevertheless, serum level of HDL-c increased significantly (P<0.05) as 20.38% on post treatment day 60. The results of present study also indicate that the values of percentage reduction 51.63 and 68.61 for TL, 49.61 and 60.26 for TGs, 59.54 and 64.72 for TC and 65.75 and 66.44 for LDL-c, induced after administration of mixture 3.5 ml/ kg and simvastatin 0.6 mg/ kg, respectively, at post treatment day 60, are non-significantly (P>0.05) different. Similarly, the respective percentage increase, 20.38 and 26.25 for HDL-c, produced by mixture and simvastatin are mutually non-significant (P>0.05). Thus results have indicated that mixture, 3.5 ml/kg and simvastatin, 0.6 mg/kg, are equieffective in treating hyperlipidemia in mice. Comparable results were attained when *T. ammi* seed powder extract in methanol equivalent to 2 g/kg seed powder and simvastatin (0.6 mg/kg BW) and *Cinnamomum zeylanicum* methanol extract equivalent to 0.75g/kg of its powder and simvastatin (0.6 mg/kg b. wt.) were administered in albino rabbits suffering from hyperlipidemia (Javed *et al*., 2006; Javed *et al*., 2012). A substantial 1% drop in serum cholesterol reduces cardiac illness and deaths up to 2% in the patients suffering from coronary heart diseases. Coronary heart diseases have been reported to cause deaths almost ten times as many as those caused by accidents and twice as many as those caused by cancer all over the world (Ghule *et al*., 2006).

Lipid and lipoprotein abnormalities are well known risk factors for heart disease. Elevated levels of TG, TC, and LDL-c are documented as risk factors for atherogenesis (Yokozawa *et al*., 2006; Adaramoye *et al*., 2008). The blood level of HDL-c in contrast bears antiartherogenic property, with an ability to inhibit LDL-oxidation (Assmann and Nofer, 2003). Genetic factors and diet both play a major role in regulating cholesterol and triglycerides level in the plasma. High levels of cholesterol particularly LDL-c are mainly responsible for hypercholesterolemia (Gardner*et al.,* 2000). Strong risk factor for coronary heart disease has been shown by increased lipid parameters (Makni *et al.,* 2008). In the present study the administration of mixture at 3.5 mg/kg BW reduced serum TC as well as LDL-c and increased HDL-c in hyperlipidemic mice. These findings indicate that the mixture could help in reducing the coronary incidents and thus proved to be advantageous clinically. Coronary artery index (CAI= LDL-C/HDL-C), being the ratio of low density lipoprotein cholesterol to high density lipoprotein cholesterol, is considered as indicator of whether or not cholesterol is deposited into tissue or metabolized and excreted (Harnafi *et al*., 2008). In present study, simvastatin 0.6 mg/kg reduced the pre-treatment 15 day index value 2.80 to 0.75 post treatment index value at 60 day. The same pattern of reduction in the pre-treatment index value 2.73 to post treatment index value 0.78 has been observed after the administration of 3.5 ml/kg mixture. Both the post treatment index values induced by simvastatin and mixture are non-significantly (P>0.05) different from each other suggesting that simvastatin and mixture possess same cardioprotective potential.

Keeping in view the contents of the ingredients of the mixture, its lipid lowering mechanism of action may be based on its antioxidant activity and/or enhanced lipid metabolism (Adel and Prakash, 2010; Alvarez-Suarez *et al*., 2010; Chai *et al*., 2011; Bhandari, 2012). Further extensive chemical characterization, phytochemical and pharmacological investigations are needed to be conducted for isolation and evaluation of newer active ingredients that could sufficiently help in reducing serum lipid profile in humans.

Conclusion: In the context of present study, it may safely be said that contents of ingredients of herbal mixturecould help in reducing the coronary incidents and thus proved to be advantageous clinically.

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