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LIPID LOWERING EFFECTS OF SOME PLANT SEEDS, BARK AND HONEY IN HYPERLIPIDAEMIC ALBINO MICE

Bilal Aslam*[1], Muzammal Sattar[1], Ijaz Javed[1], Tanweer Khaliq[1], Asghar Ali[1], Zia-ud-Din Sindhu[2], Muhammad Sarfraz[1]

[1]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan.

[2]Department of Parasitology, University of Agriculture, Faisalabad, Pakistan.

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***Correspondence for Author:**

Dr. Bilal Aslam
Assistant Professor,
Department of Physiology and Pharmacology,
University of Agriculture,
Faisalabad, Pakistan.
bilal.aslam@uaf.edu.pk

ABSTRACT

Effect of the mixture containing ajowain, kalonji, daarchini, apple vinegar and honey was investigated in hyperlipidaemic albino mice. Mice were grouped and maintained for 60 days as (I) untreated control on normal routine feed; (II) untreated control on atherogenic diet; (III) treated control on synthetic cholesterol lowering drug Tablets Survive® (Simvastatin 20mg) while (IV), (V) and (VI) served as treated control on three respective doses of experimental mixture. Results revealed that administration of atherogenic diet lead to an increase in serum lipid parameters like total cholesterol, triglycerides, total cholesterol and LDL-cholesterol while the administration of different doses of mixture significantly showed a graded reduction in all the serum lipid parameters with an increase in HDL-cholesterol levels, respectively. It was concluded that atherogenic diet resulted an increase in serum lipid levels, causing hyperlipidaemia, which could be reduced by the mixture at dose rate of 3.5 ml/Kg BW. Antihyperlipidaemic activity of simvastatin and mixture at dose rate of 3.5 ml/Kg BW was found to possess almost similar antihyperlipidaemic activity at post treatment day 60 in hyperlipidaemic mice.

Key words: Mixture, Simvastatin, Cholesterol, Triglycerides.

INTRODUCTION

Approximately 16.7 million deaths have been reported every year due to cardiovascular diseases and every fourth middle-aged person is suffering from coronary heart disease in

Pakistan [1]. A patent relationship is established between hypercholesterolemia and cardiovascular diseases by multiple clinical and epidemiological studies [2-3]. Hyperlipidaemia is characterized by elevated total cholesterol, serum triglycerides, low density lipoprotien, very low density lipoprotien, and a decreased high density lipoprotien cholesterol level [4]. Among lipids, cholesterol is an important biomolecules which is an integral component of cell membrane and is required for synthesis of bile acids as well as steroid hormones.

Hypertriglyceridemia is an independent risk factor of exaggeration and development of atherosclerosis while high density lipoprotien cholesterol (HDL-c) has protective role. Higher the HDL-c level, lower will be atherosclerotic events [5]. By decreasing serum total cholesterol, cardiovascular and related events may be reduced [6]. In order to reduce the risk of developing atherosclerosis, ischemic heart disease or the incidence of further cardiovascular or cerebrovascular diseases hyperlipidaemia should be treated effectively [7]. Among synthetic antihyperlipidaemic drugs, statins have been reported to be most effective. A significant reduction in cardiovascular mortality and morbidity have been reported by randomized, placebo controlled trials with statins but number of side effects like myopathy, asymptomatic rise in serum transaminases, nausea, dyspepsia, abdominal pain, flatulence and diarrhea have also been reported with their use [8-10].

About 2000 medicinal plants have imperative phytochemical ingredients against various cardiac problems such as ischemic heart disease and hypercholesterolemia and are safer as compared to synthetically derived pharmaceutical products [11].

Certain cholesterol lowering functional foods which are getting popularity these days and offer promising alternative therapy for treating the hypercholesterolemic patients whose blood cholesterol level is slightly raised rather, in treating the patients having very high cholesterol level [12]. Honey contains riboflavin, niacin, pantothenic acid, folate and various minerals. Besides this, honey is rich in antioxidants like chrycin, pinobanksin, vitamin C, catalases and pinocembrin [7].

Apple vinegar contains a variety of antioxidants which arrest the formation of oxidized LDL which is chemoattractant for monocytes and help in the proliferation of the macrophages and these changes could lead to vascular blockade or injury [13]. Ajowain, Daarchini and Kalonji has established role as antioxidant to arrest the lipid peroxidation.

MATERIALS AND METHODS

Study Design

150 mice were purchased and were kept in clean iron cages with a 12/12 hour period of light/dark at animal room of Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. Mice were randomly divided in to six groups each comprising of 25 animals. Mice were fed two times a day while, drinking water was available throughout 24 hours. Except normal control group all the groups were provided with atherogenic diet for 0-60 days. Lead-in period consisted of 0-15 days to induce hyperlipidaemia in mice. The atherogenic diet was comprised of cholesterol powder 0.5 %, coconut oil 20 % and cellulose 15 %, mixed in the normal routine mice feed. The mixture [14] was fed to hyperlipidaemic mice at the dose level of 1.5, 2.5 and 3.5 ml/Kg BW to treated group IV, treated group V and treated group VI, respectively, for 15-60 days, as replacement of cellulose in atherogenic diet. Synthetic lipid lowering drug at the dose rate of 0.6 mg/Kg BW was administered to the hyperlipidaemic mice of treated control groups for 15-60 days as cellulose replacement in atherogenic diet and has been mentioned in Table 1. Five mice from each group were sacrificed at each sampling time and blood 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 mice serum with reagent kits.

STATISTICAL ANALYSIS

Mean ± SE values for each pre and post-treatment lipid profile parameters were determined and respective percent reduction was computed. Significance of antihyperlipidemic effects of mixture was ascertained by Student's "t" test [15]. Level of significance was less than 0.05 and P >0.05 was considered as non-significant for all the groups.

RESULTS

Atherogenic diet alongwith normal feed was fed to mice in order to produce hyperlipidaemia from 0-60 days. The lipid lowering effect of the mixture at various doses rates like 1.5, 2.5 and 3.5 ml/Kg BW has been shown in Tables from 2-6.

Table: 1 Drugs and feeding administration schedule in mice during the experimental period of 0-60 days.

Group I: Normal control on normal routine feed	Routine normal feed 0 to 60 days
Group II : Untreated control on atherogenic diet	Atherogenic diet (Routine diet + cholesterol 0.5%, coconut oil 20 %, Cellulose 15 %) 0 to 60 days
Group III : Treated control on synthetic cholesterol lowering drug; Tablet survive® (Simvastatin, 20mg) 0.6mg/kg body weight	Atherogenic diet 0-15 days, atherogenic diet + Tablet survive® (Simvastatin, 20mg) 15 to 60 days as cellulose replacement
Group IV : Treated with mixture of *T. ammi, N. sativa, M. domestica, C. zeylanicum* and honey 1.5 ml / Kg body weight	Atherogenic diet 0-15 days, atherogenic diet + mixture for 15 to 60 days as cellulose replacement
Group V : Treated with mixture of *T. ammi, N. sativa, M. domestica, C. zeylanicum* and honey 2.5 ml / Kg body weight	Atherogenic diet 0-15 days, atherogenic diet + mixture for 15 to 60 days as cellulose replacement
Group VI : Treated with mixture of *T. ammi, N. sativa, M. domestica, C. zeylanicum* and honey 3.5 ml / Kg body weight	Atherogenic diet 0-15 days, atherogenic diet + mixture for 15 to 60 days as cellulose replacement

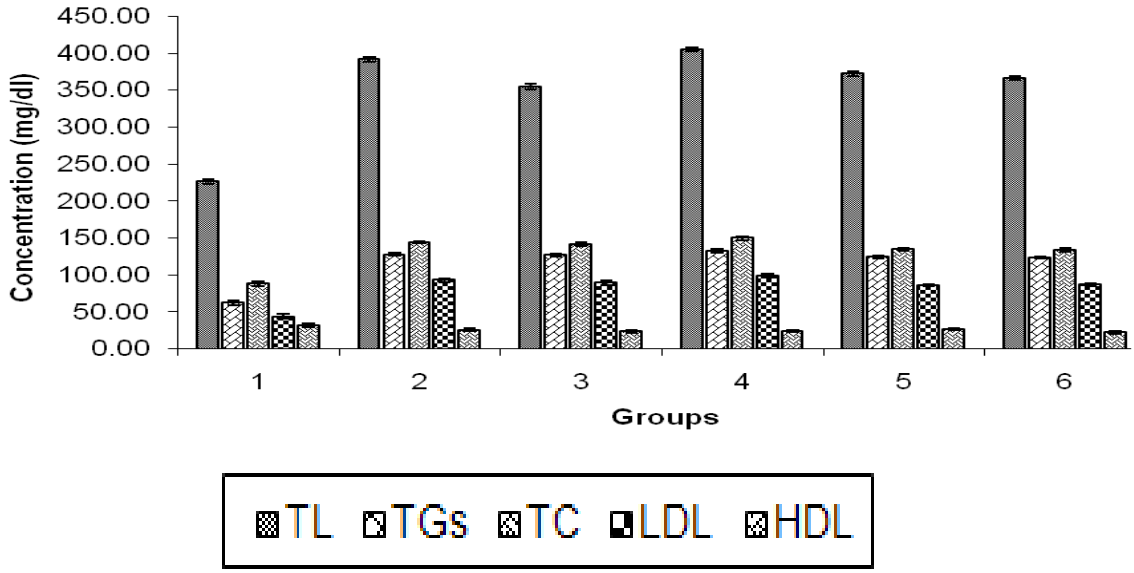

Fig A: Mean ± SE concentration of serum lipid profile parameters in albino mice fed with cholesterol powder from 0-15 day.

Table: 2 Mean± SE values of total lipids (mg/dl) and their percentage reductions in hypercholesterolemic albino mice after administration of mixture and simvastatin.

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
Group I	225.97 ±2.9	224.48 ±2.45	224.35 ±2.39	219.12 ±2.26	-	-	-
Group II	391.87 ± 3.1	439.16 ± 3.1	512.43 ± 2.6	579.43 ± 2.5	-	-	-
Group III	354.101 ± 3.9	301.28 ± 2.8a	231.71 ± 2.5a	207.17 ±2.2a	14.92 ± 3.10	34.72 ± 3.46	41.49 ± 3.96
Group IV	405.41 ± 2.7	385.62 ± 2.8	361.08 ± 2.6	344.73 ± 2.1	4.88 ± 3.2	10.93 ± 3.41	14.97 ± 4.12
Group V	372.64 ± 3.3	340.81 ± 2.9	310.12 ± 2.6	277.21 ± 1.9	8.54 ±1.67	16.78 ± 2.5	25.61 ± 3.51
Group VI	366.24 ± 2.4	316.55 ± 2.6	266.13 ± 2.8a	228.14 ± 1.7a	13.57 ± 3.27	27.33 ± 3.76	[b] 37.71 ± 3.93

n = Number of animals in each group

a = Significantly less (P≤0.05) than the pretreatment value at 15 days.

b = Non-significantly (P>0.5) different from respective value obtained with simvastatin.

Table: 3 Mean± SE values of triglycerides (mg/dl) and their percentage increases in hypercholesterolemic albino mice after administration of a mixture and simvastatin.

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
Group I	62.38 ±2.88	61.52 ±2.5	63.09 ±2.40	60.72 ±2.29	-	-	-
Group II	127.66 ±1.81	142.40 ± 1.9	163.52 ± 1.6	194.0 ±1.1	-	-	-
Group III	126.84 ±1.45	97.47 ± 2.08a	64.00 ± 1.84a	55.4 ± 1.52a	23.15 ± 2.1	49.54 ± 2.28	56.32 ± 2.73
Group IV	132.39 ± 2.69	121.12 ± 2.48	113.35 ± 2.41	103.5 ± 2.06	8.46 ± 1.47	14.38 ±	21.82 ± 2.47

						1.71	
Group V	124.51 ± 1.73	113.1 ± 2.3	103.51 ± 2.04	93.72 ± 1.3a	9.17 ±1.95	16.87 ± 2.07	24.73 ± 2.13
Group VI	123.42 ±1.20	105.18 ± 1.5	83.35 ± 1.35a	67.79 ±1.02a	14.78 ±2.68	32.46 ± 2.8	[b]45.07 ± 3.17

n = Number of animals in each group

a =Significantly less (P≤0.05) than the pretreatment value at 15 days.

b = Non-significantly (P>0.5) different from respective value obtained with simvastatin.

Table: 4 Mean± SE values of total cholesterol (mg/dl) and their percentage increases in hypercholesterolemic albino mice after administration of mixture and simvastatin

Groups	**Lead – in – Period Day 15**	**Post treatment days**			**Percentage reduction on post treatment days**		
		30	**45**	**60**	**30**	**45**	**60**
Group I	88.04 ±3.10	87.63 ±2.76	88.20 ±2.51	86.47 ±2.36	-	-	-
Group II	144.44 ± 1.3	162.62 ± 2.87	190.81 ± 2.60	212.05 ±2.40	-	-	-
Group III	141.40 ± 2.79	112.30 ±2 .47a	85.66 ±1.48a	75.73 ± 1.37a	20.58 ±2.08	39.42 ± 2.45	46.44 ±3.01
Group IV	149.89 ± 2.63	144.27 ± 2.16	137.7 ± 2.06	131.95 ±1.80	3.75 ± 1.47	8.13 ± 1.76	11.97 ± 2.22
Group V	135.05 ± 1.97	123.46 ± 1.9	112.47 ± 1.7	100.22 ±1.6a	8.58 ± 1.67	16.72 ± 2.02	25.79 ± 3.25
Group VI	133.75 ± 2.58	116.20 ±2.10	99.17 ± 1.8a	85.51 ±1.24a	13.12 ± 3.15	25.82 ± 3.68	[b]36.07 ± 4.10

n = Number of animals in each group.

a = Significantly less (P≤0.05) than the pretreatment value at 15 days.

b = Non-significantly (P>0.5) different from respective value obtained with simvastatin.

Table: 5 Mean± SE values of HDL- cholesterol (mg/dl) and their percentage increases in hypercholesterolemic albino mice after administration of mixture and simvastatin.

Groups	Lead – in – Period Day 15	Post treatment days			Percentage increase on post treatment days		
		30	45	60	30	45	60
Group I	31.95 ±2.98	31.58 ±2.69	31.79 ±2.45	31.37 ±2.31	-	-	-
Group II	25.74 ± 1.93	22.90 ± 1.71	21.58 ± 1.30	19.09 ± 1.10	-	-	-
Group III	23.265 ± 1.79	30.45 ± 1.89a	31.75 ±2.24a	34.51 ± 2.91a	30.87 ±3.38	36.45 ±2.91	47.57 ±2.80
Group IV	23.99 ± 1.60	24.75 ± 1.69	26.10 ± 1.99	26.75 ± 2.20	3.16 ± 3.30	8.79 ±2.92	11.49 ± 2.25
Group V	26.91 ± 1.61	28.20 ± 1.91	29.23 ± 2.56	30.39 ±2.80	4.78 ± 2.02	8.62 ± 1.80	12.95 ± 1.60
Group VI	22.2 ± 2.4	26.55 ± 1.98a	29.73 ±2.81a	32.12 ± 3.14a	19.58 ±1.75	33.94 ± 1.56	[b]44.69 ± 1.07

n = Number of animals in each group.

a = Significantly higher (P≤0.05) than the pretreatment value at 15 days.

b = Non-significantly (P>0.5) different from respective value obtained with Simvastatin.

Table: 6 Mean± SE values of LDL- cholesterol (mg/dl) and their percentage reductions in hypercholesterolemic albino mice after administration of mixture and simvastatin.

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
Group I	43.61 ±3.16	43.75 ±2.65	41.47 ±2.53	40.56 ±2.46	-	-	-
Group II	93.10 ± 2.57	111.24 ± 2.40	136.52 ± 2.50	154.28 ± 3.30	-	-	-
Group III	89.27 ± 3.52	61.06 ± 1.67a	50.3 ±1.51a	41.55 ± 1.38a	31.60 ± 2.14	43.65 ±2.25	53.45 ± 3.30

Group IV	99.26 ±2.68	95.48 ± 3.22	88.60 ± 2.86	82.53 ±2.65	3.8 ±1.75	10.74 ± 2.19	16.85 ±3.01
Group V	86.19 ± 1.21	75.97 ± 2.32	64.95 ± 2.02	52.88 ± 1.8a	11.86 ± 2.19	24.64 ± 2.80	38.65 ± 3.78
Group VI	86.87 ± 2.05	68.62 ±1.86	53.88 ±1.24a	42.72 ±1.05a	21.01 ± 2.09	37.98 ± 2.98	[b]50.82 ± 3.12

n = Number of animals in each group.

a = Significantly less ($P \leq 0.05$) than the pretreatment value at 15 days.

b = Non-significantly ($P > 0.5$) different from respective value obtained with simvastatin.

DISCUSSION

Serum levels of total lipids, triglycerides, total cholesterol and low density lipoprotein cholesterol levels were increased up to two fold as compared to the results obtained at day 0, whereas HDL levels remained nearly similar at day 15 as demonstrated in Fig: A.

Lipid lowering trends after the administration of the mixture in hyperlipidaemic mice have been organized in Tables 2 to 6. The mixture (1.5 ml/Kg BW) does not showed any significant lipid lowering activity (total lipids 15%, triglycerides 22% and total cholesterol 12%) in hyperlipidaemic mice up to post treatment day 60. However, the mixture (2.5 ml/kg Kg BW) significantly reduced the (total lipids 26%, triglycerides 25% and total cholesterol 26%) in hyperlipidaemic mice at post treatment day 60. LDL-cholesterol concentrations were non significantly decreased at the dose rate of 1.5 ml/Kg BW up to 17% and significantly decreased at dose rate of 2.5 ml/Kg BW to 39% at the post treatment day 60, respectively. HDL-cholesterol concentrations were increased non significantly by both of the doses (1.5, 2.5 ml/ Kg BW) at the post treatment day 30, 45 and 60 in hyperlipidaemic mice.

The mixture at the dose of 3.5 ml/kg body weight showed a significant lipid lowering activity in hyperlipidaemic mice as (total lipids 38%, triglycerides 45%, total cholesterol 36% and LDL-cholesterol 51%) more significant at day 60 as compared to the mixture (2.5 ml/kg body weight) these were (total lipids 26%, triglycerides 25%, total cholesterol 26% and LDL-cholesterol 39%). HDL-cholesterol concentrations increased significantly as 45% by mixture (3.5 ml/kg body weight) at day 60 as compared to a non-significant increase of 13% by mixture (2.5 ml/kg body weight) in hyperlipidaemic mice.

Percent reductions in the serum levels of total lipids, triglycerides, total cholesterol and LDL-cholesterol produced by simvastatin at day 60 were 42%, 57%, 47% and 53% respectively in hyperlipidaemic mice. Lipid lowering activity of simvastatin was similar to that of the mixture (3.5 ml/Kg BW) at day 60 in hyperlipidaemic mice. However, simvastatin and mixture (3.5 ml/Kg BW) significantly increased the HDL cholesterol levels as 48% and 45% respectively in hyperlipidaemic mice at day 60.

The same dose of simvastatin produced the comparable lipid reduction as 15 % when *T. ammi* seed powder extract in methanol equivalent to 2 g/kg seed powder and simvastatin (0.6 mg/kg b. wt.) was administered in albino rabbits suffering from hyperlipidaemia [16]. A substantial drop in serum cholesterol levels even 1 % can put off cardiac illness and deaths up to 2 % due to CHD. It causes deaths almost ten times as many as those caused by accidents and twice as many as those caused by cancer all over the world [17].

High levels of TC and most notably, LDL-C are principal coronary risk factors. Increased level of LDL-C may cause deposition of cholesterol in the arteries and aorta and is therefore bad for health and a direct risk factor for coronary heart disease [18]. With a few differences, these results are in concurrence with other studies in which a fat rich diet resulted in the elevation of serum levels of cholesterol [19]. The administration of mixture at 3.5 mg/kg body weight reduced both types of lipids in hyperlipidaemic mice which indicated that the mixture could help in reducing the incidence of coronary incidents possibly due to the restraining of the formation of lipid peroxides in the hyperlipidaemic mice. The suppression of formation of lipid peroxide ultimately leads to reduction of atherogenic plaques [20-21]. Several studies have shown that flavonoids, steroids and saponins containing plants have exhibited several pharmacological activities including antiatheogenesis [22]. Atherogenic index and coronary artery index are strong and trustworthy indicators of whether or not cholesterol is deposited into tissue or metabolized or excreted [11]. In this study, treatment with mixture at the dose rate of 3.5 ml/Kg BW caused profound reduction in the ratio of LDL-C/HDL-C, which suggested that the mixture possesses cardioprotective potential as well [23].

Hence the present study confirms that this herbal mixture have antihyperlipidaemic potential and can be helpful for designing a refined dosage form in order to treat lipid disorders. Moreover, extensive chemical characterization and pharmacological investigations should be

accomplished to isolate and evaluate the newer active constituents which could suitably help in reducing serum lipid levels in humans.

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