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THE EFFECT OF *CRATAEGUS* (HAWTHORN) EXTRACT ALONE AND IN COMBINATION WITH SIMVASTATIN ON SERUM LIPID PROFILE IN HYPERLIPIDEMIC ALBINO RATS

SAMINA KAUSAR,[1] ZUJAJA ZAHEER,[2] MUDASSARA SAQIB[3] AND BUSHRA ZIA[4]
Department of Pharmacology, [1]Post Graduate Medical Institute, [2]King Edward Medical University, [3]Sheikh Zayad Federal Post Graduate Medical Institute, Lahore [4]Aga Khan University, Karachi

ABSTRACT

Cardiovascular diseases are the most common causes of death in first world countries. Hyperlipidemia is one of the high risk factors for cardiovascular diseases. Crataegus monogyna has acquired an eminent position in remedies for hyperlipidemia.

Study Design: It is a case – controlled interventional study of eight weeks' duration.

Sample: 100 adult male albino rats weighing about 250 – 300g divided randomly into five groups A, B, C, D and E.

Result: Crataegus is as effective in lowering cholesterol as simvastatin. However, when compared with combination of Crataegus and simvastatin, Crataegus alone was found to yield better results. Both of the drugs are equally effective in lowering triglycerides. HDL level significantly increases in all groups whereas LDL level decreases in groups C, D and E. However only group E was close to normal control group A.

Conclusion: Crataegus shows tremendous potential as natural lipid lowering agent, devoid of side effects. In future there is scope in study and use of this 'miracle herb' as anti-hyperlipidemic agent.

Key words: Crataegus monogyna, hyperlipidemia, lipid profile.

INTRODUCTION

Cardiovascular diseases are the most common cause of death in industrial countries around the world.[1]

Hyperlipidemia is an important modifiable risk factor for cardiovascular disease. Particularly raised LDL is strongly associated with coronary heart disease risk. According to National Cholesterol Education Program, adult treatment guidelines 2001; desirable total cholesterol level is < 200 mg/dl, borderline to high level is 200 – 239 mg/dl, high level for cholesterol is > 240 mg/dl. For LDL cholesterol, desirable level is < 100 mg/dl. Borderline to high is 100 – 159 and high level is > 160 mg/dl. Desirable HDL cholesterol level is > 50 mg/dl.[2] Reducing LDL < 100 mg/dl will decrease the CHD event.[3] Low density lipoprotein oxidation is a primary event in atherosclerosis plaque formation and antioxidant such as polyphenols are shown to inhibit LDL oxidation and atherosclerosis development. An approach to reduce the incidence of morbidity and mortality is to take preventive measures to reduce risk factors, like diet intervention and anti hyperlipidemic agents. Many lipid lowering agents like HMG – CoA reductase inhibitors (Statins) are expensive and are associated with various adverse effects like myopathy, rhabdomyolysis, pancreatitis, increased level of creatine kinase, bone marrow depression, hepatotoxicity and muscle toxicity which can prove fatal.

Many natural products and herbs are also being used for this purpose like garlic, olive oil, fish oil, and gugulipid.[4]

Crataegus monogyna has acquired a prominent status in modern herbal literature as an important cardiac tonic. Its common name is Hawthorn, extract of both flowers and berries have been recommended to treat cardiac failure, atherosclerosis, hyperlipidemia, hypertension, angina and variety of geriatric conditions. *Crataegus* Plant contains mixtures of chlorogenic acid and flavonoids such as querin hyperoside, vitexin and vitexin 4rahmnoside. Other flavonoids identified are luteolin, apigenin-7-o-glucoside and rutin. Luteolin is an effective smooth muscle relaxant. It also contains amygdaline, other major constituents are triterpenoids e.g. oleanolic acid, ursolic acid, these two have anti inflammatory and anti-hyperlipidemic properties. Part of mechanism for anti-hyperlipidemic effects of hawthorn fruit might also involve the direct protection to human LDL from oxidation[5]. As oxidation is part of normal biological reactions, over loading the cells with free radicals could initiate the pathogenesis of many diseases.[6]

Crataegus has lipid lowering effect. It increases

LDL receptor binding capacity in the liver and enhances bile acid secretion. This indicates that *Crataegus* up regulates the receptors in liver and it enhances cholesterol influx and cholesterol degradation to bile, suppressing cholesterol biosynthesis.

MATERIALS AND METHODS

Study Design

It is a case controlled interventional experimental study that took 8 weeks for completion.

Sample

100 adult, male albino rats weighing about 250 – 300 grams were purchased and kept in PGMI animal house.

Test Materials

200 g of good quality well dried berries of *Crataegus monogyna* were purchased and Ethanolic extract was prepared in PCSIR.

Drugs used

I Ethanolic extract of berries 0.5 ml /100 gm of body weight per day (standardized to contain 2.2% flavonoids).[7-9]

II Simvastatin 0.1 mg /100 gm of body weight per day dissolved in distilled water.[10]

Diet

Normal rat chow contains wheat starch, casein glucose, choline / methionine, mineral mixture, vitamin mixture and fat in quantities of 62.10, 20, 10, 00.50, 03.50 and 2.90 g to make total 100 g of the diet as basic constituents.[11]

Hyper lipidemic diet containing wheat starch 43.70 20%fat and 1% cholesterol bile salts 00.30 added to normal diet.[12]

Investigation Kits

Randox kits for lipid profile.

Methodology

100 albino rats were divided randomly in to 5 groups of twenty animals each. The groups were as follows; A, normal control, B experimental control, C, D, and E experimental groups. Group C was given *Crataegus* extract, group D simvastatin and E group was given combination of both *Crataegus* and simvastatin. Initially all the groups were fed on normal rat chow for the first two weeks for acclimatization. Then hyperlipidemic diet was started to all groups except A (normal control) which remained on normal diet for the whole study period of 8 weeks.

Blood samples were collected at zero, 4 and 8 weeks by cardiac puncture under light ether anesthesia, after fasting of 14 hours. Serum was separated by centrifugation and analyzed for lipid profile. After taking samples at fourth week drugs were started by flexible nasogastric tube of smallest gauge. Group C was given *Crataegus* extract, group D simvastatin and E group was given combination of both *Crataegus* and simvastatin. These three groups were fed on hyperlipidemic diet throughout study period.

Parameters

Lipid profile

- Total serum cholesterol.
- Triglycerides.
- High density lipoprotein HDL-C.
- Low density lipoprotein LDL-C.

Data Analysis

All numerical variables were represented as mean ± standard deviation. The individual comparison between any two groups was analyzed by t-test.

ANOVA test was used for comparison of all groups simultaneously.

P-values less than 0.05 were considered significant.

All analyses were done through the statistical package SPSS Version 12.

RESULTS

Lipid Profile was estimated at 0, 4 and 8 weeks.

Table 1 shows total cholesterol at 0, 4 and 8 weeks in all 5 groups. These results indicate that both drugs lowered cholesterol levels but not to the extent of original base line values Pair wise comparison showed decrease in cholesterol level in drug interventional groups i.e. C, D and E but remained higher than normal control. Group C versus D showed insignificant p value indicating that *Crataegus* is as effective as simvastatin in lowering cholesterol levels. Group C versus E showed significant P value indicating that *Crataegus* alone is significantly effective in lowering total cholesterol than combination. Group D versus E showed insignificant P value showing that simvastatin and its combination with *Crataegus* are of equal effectiveness.

Table 2 shows TGs (Triglycerides) in all groups at 0, 4 and 8 weeks.

At zero week mean TGs level in all groups was nearly same.

At 4th week, TGs increased in all groups except A. At 8 weeks, after drug treatment, TGs decreased to the normal control level while it remained high in group B. Pair wise comparison at 0 week was insignificant showing TGs levels were almost same in all groups. At 4th week, TGs were significantly increased. At 8 weeks TGs of group B were significantly higher than those of group A and TGs of groups C, D and E were insignificant compared to those of group

Table 1: *Pair – wise comparison of total cholesterol (mg/dl) of different groups of animals at 0 week, 4 weeks and 8 weeks.*

(I) Groups	(J) Groups	0 week			4 Weeks			8 Weeks		
		Mean Difference (I-J)	P - value	Mean SD	Mean Difference (I-J)	P - value	Mean SD	Mean Difference (I-J)	P - value	Mean SD
"A"	B	-0.150	1.000	55.30 ± 3.71	-45.900(*)	<0.001	55.75 ± 3.34	-41.200(*)	<0.001	55.65 ± 3.79
	C	0.150	1.000		-49.800(*)	<0.001		-17.500(*)	<0.001	
	D	0.350	1.000		-48.000(*)	<0.001		-12.350(*)	<0.001	
	E	-4.350(*)	0.004		-49.400(*)	<0.001		-10.050(*)	<0.001	
"B"	C-	0.300	1.000	55.45 ± 3.58	-3.900	0.409	101.6 ± 9.42	23.700(*)	<0.001	96.85 ± 6.38
	D	0.500	1.000		-2.100	1.000		28.850(*)	<0.001	
	E	-4.200(*)	0.006		-3.500	0.660		31.150(*)	<0.001	
"C"	D	0.200	1.000	55.15 ± 3.20	1.800	1.000	105.55 ± 5.97	5.150	0.140	73.15 ± 9.39
	E	-4.500(*)	0.002		0.400	1.000		7.450(*)	0.005	
"D"	E	-4.700(*)	0.001	54.95 ± 3.27	-1.400	1.000	103.7 ± 5.24	2.300	1.000	68.00 ± 5.29

*The mean difference is significant at the .05 level
P ≤ 0.001 (Highly significant), P≤0.05 (Significant), P>0.05 (Non-significant)

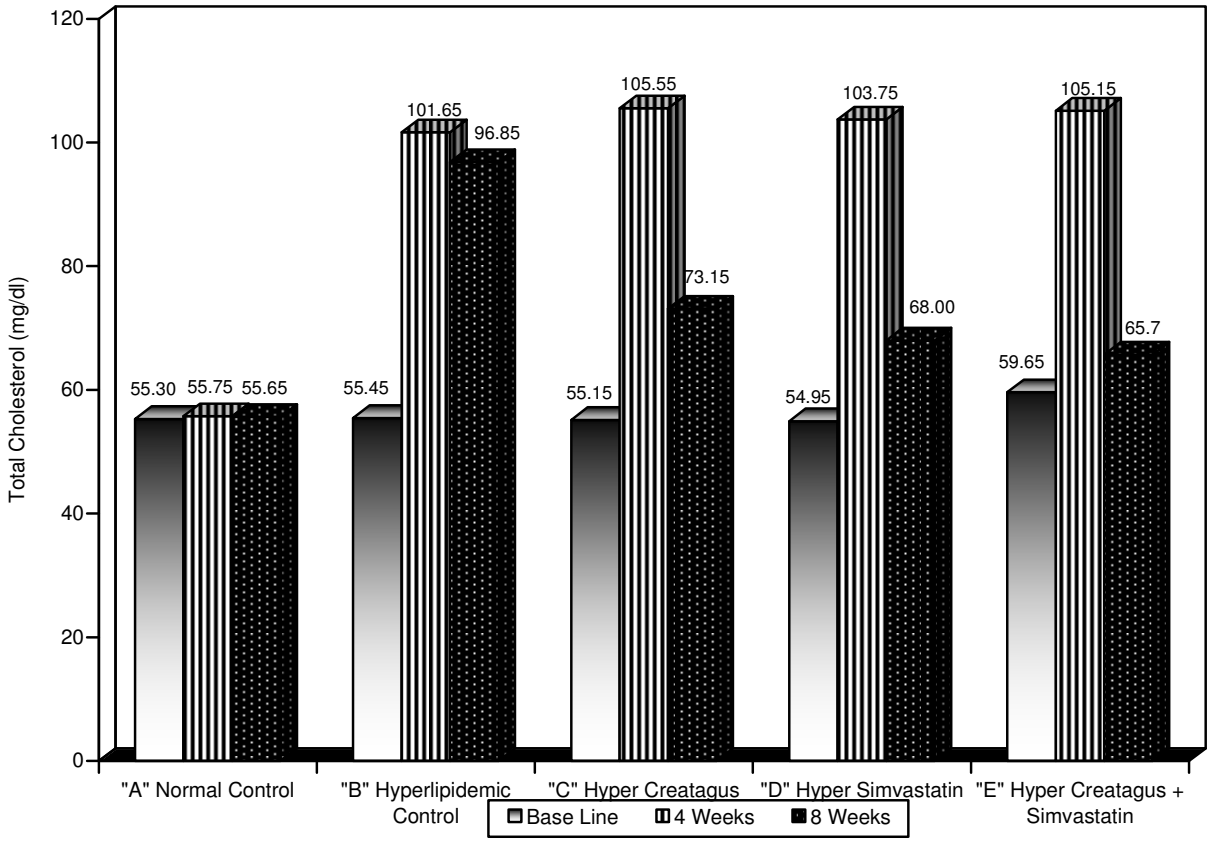

Fig. 1: *Comparison of Total Cholesterol of different times.*

Table 2: *Pair – wise comparison of triglyceride levels (mg/dl) of different groups of animals at 0 week, 4 weeks and 8 weeks.*

(I) Groups	(J) Groups	0 week			4 Weeks			8 Weeks		
		Mean Difference (I-J)	P - value	Mean SD	Mean Difference (I-J)	P - value	Mean SD	Mean Difference (I-J)	P - value	Mean SD
"A"	B	-0.800	1.000	104.25 ± 3.42	-38.650(*)	<0.001	105.85 ± 4.52	-48.800(*)	<0.001	105.25 ± 3.71
	C	-0.550	1.000		-39.800(*)	<0.001		-4.500	0.565	
	D	-1.150	1.000		-33.800(*)	<0.001		-3.900	0.975	
	E	-0.800	1.000		-38.700(*)	<0.001		-3.650	1.000	
"B"	C-	0.250	1.000	105.05 ± 3.75	-1.150	1.000	144.50 ± 3.86	44.300(*)	<0.001	154.05 ± 8.95
	D	-0.350	1.000		4.850	0.104		44.900(*)	<0.001	
	E	0.000	1.000		-0.050	1.000		45.150(*)	<0.001	
"C"	D	-0.600	1.000	104.80 ± 3.81	6.000(*)	0.017	139.65 ± 9.83	.600	1.000	109.75 ± 8.63
	E	-0.250	1.000		1.100	1.000		.850	1.000	
"D"	E	0.350	1.000	105.40 ± 3.63	-4.900	0.096	144.55 ± 4.32	.250	1.000	109.15 ± 6.93

P ≤ 0.001 (Highly significant), P ≤ 0.05 (Significant), P > 0.05 (Non-significant)

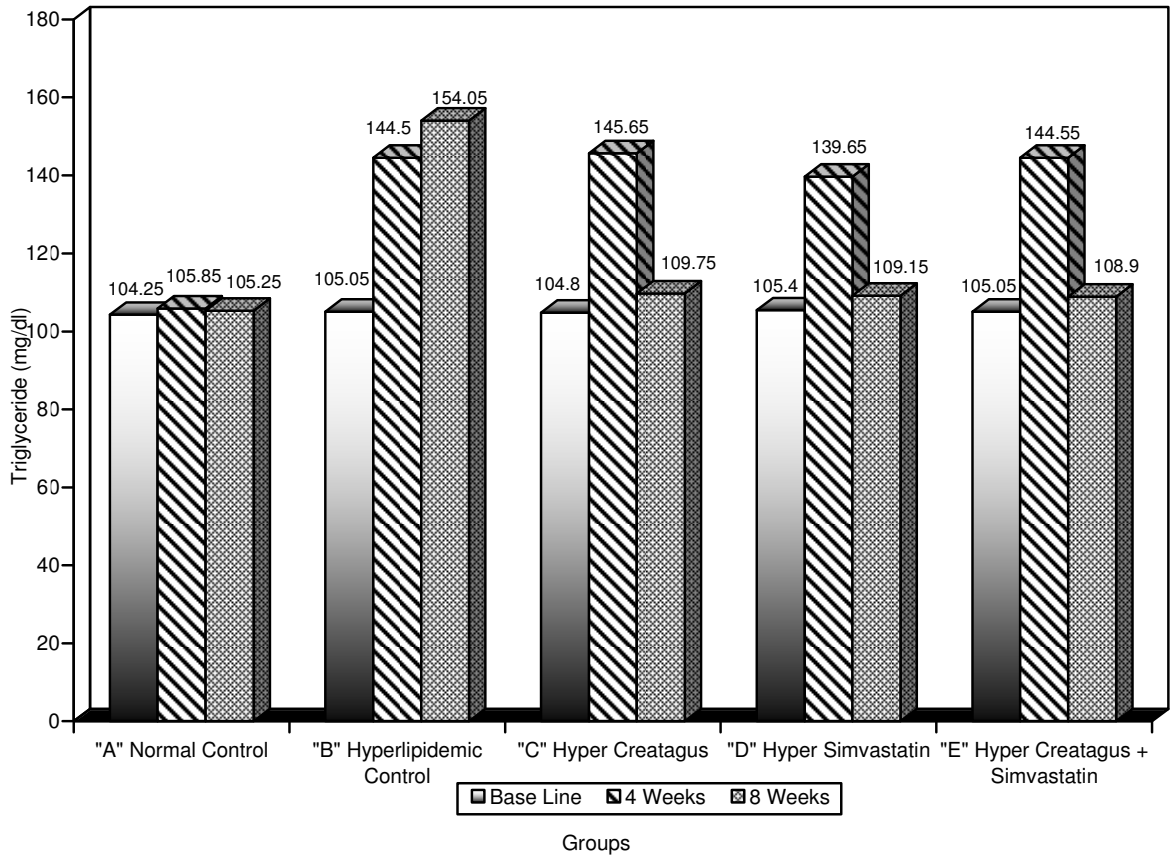

Fig. 2: *Comparison of Triglesride at different times.*

Table 3: *Pair – wise comparison of HDL – Cholesterol levels (mg/dl) of different groups of animals at 0 week, 4 weeks and 8 weeks*

(I) Groups	*(J) Groups*	*0 week*			*4 Weeks*			*8 Weeks*		
		Mean Difference (I-J)	*P - value*	*Mean SD*	*Mean Difference (I-J)*	*P - value*	*Mean SD*	*Mean Difference (I-J)*	*P - value*	*Mean SD*
"A"	B	0.300	1.000	18.65 ± 1.57	-3.650(*)	<0.001	18.25 ± 1.52	-5.300(*)	<0.001	18.70 ± 1.87
	C	0.350	1.000		-2.000	0.205		-7.250(*)	<0.001	
	D	0.200	1.000		-1.750	0.419		-7.650(*)	<0.001	
	E	-1.000	0.876		-2.900(*)	0.009		-9.050(*)	<0.001	
"B"	C-	0.050	1.000	18.35 ± 1.46	1.650	0.548	21.90 ± 3.67	-1.950	0.941	24.00 ± 3.64
	D	-0.100	1.000		1.900	0.275		-2.350	0.444	
	E	-1.300	0.271		0.750	1.000		-3.750(*)	0.016	
"C"	D	-0.150	1.000	18.30 ± 1.45	0.250	1.000	20.25 ± 2.10	-0.400	1.000	25.95 ± 5.31
	E	-1.350	0.219		-0.900	1.000		-1.800	1.000	
"D"	E	-1.200	0.410	18.40 ± 1.57	-1.150	1.000	20.00 ± 3.09	-1.400	1.000	26.35 ± 3.77

P ≤ 0.001 (Highly significant), P ≤ 0.05 (Significant), P > 0.05 (Non-significant)

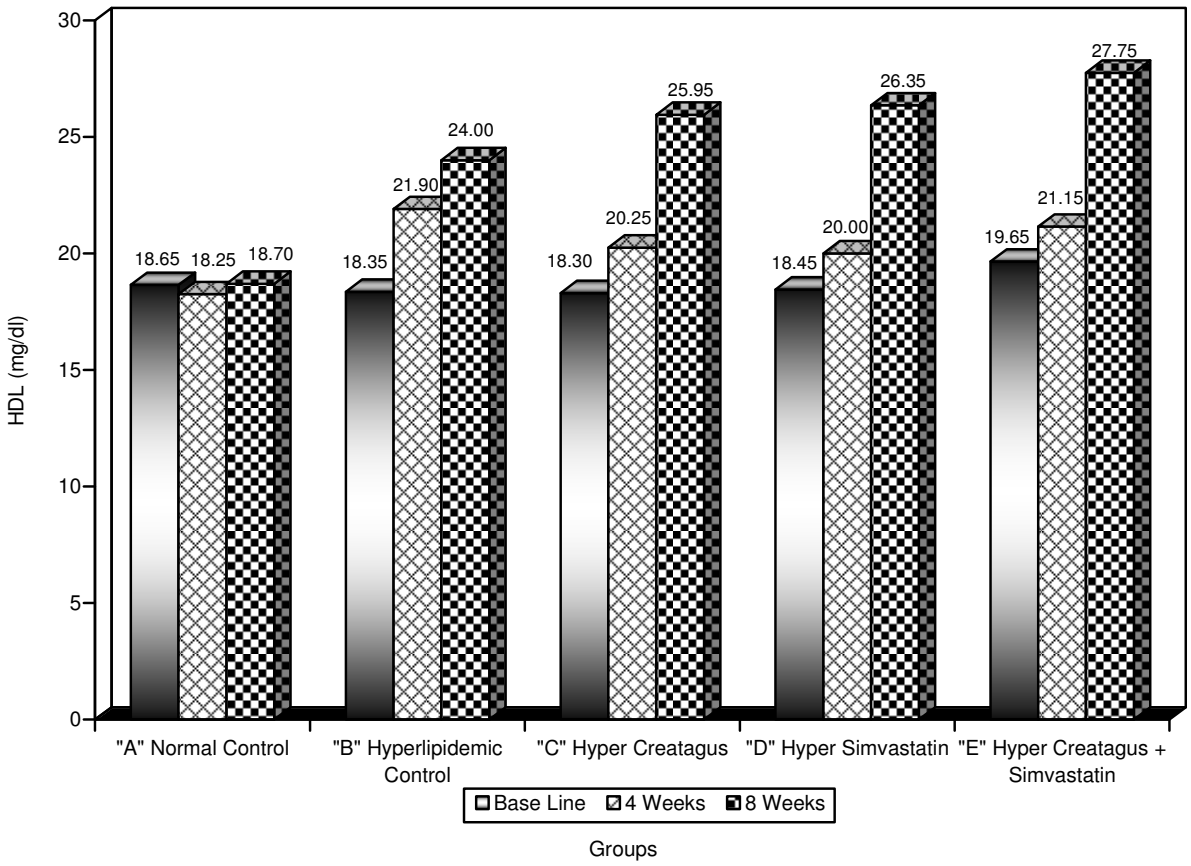

Fig. 3: *Comparison of HDL at different times.*

Table 4: *Pair – wise comparison of LDL – Cholesterol (mg/dl) of different groups of animals at 0 week, 4 weeks and 8 weeks.*

(I) Groups	*(J) Groups*	*0 week*			*4 Weeks*			*8 Weeks*		
		Mean Difference (I-J)	*P - value*	*Mean SD*	*Mean Difference (I-J)*	*P - value*	*Mean SD*	*Mean Difference (I-J)*	*P - value*	*Mean SD*
"A"	B	-0.2900	1.000	15.74 ± 4.09	-34.4400(*)	<0.001	16.41 ± 3.57	-25.9700(*)	<0.001	15.90 ± 3.81
	C	-0.0900	1.000		-35.0100(*)	<0.001		-9.2400(*)	0.001	
	D	0.3800	1.000		-39.4100(*)	<0.001		-3.9200	0.795	
	E	-3.1900	0.087		-34.3800(*)	<0.001		-.0200	1.000	
"B"	C-	0.2000	1.000	16.03 ± 3.74	-.5700	1.000	50.85 ± 10.85	16.7300(*)	<0.001	41.87 ± 7.85
	D	0.6700	1.000		-4.9700	0.168		22.0500(*)	<0.001	
	E	-2.9000	0.167		.0600	1.000		25.9500(*)	<0.001	
"C"	D	0.4700	1.000	15.83 ± 3.19	-4.4000	0.337	51.42 ± 5.45	5.3200	0.181	25.14 ± 8.42
	E	-3.1000	0.107		.6300	1.000		9.2200(*)	0.001	
"D"	E	-3.5700(*)	0.035	15.36 ± 3.05	5.0300	0.155	55.82 ± 5.25	3.9000	0.811	19.82 ± 6.70

P ≤ 0.001 (Highly significant), P ≤ 0.05 (Significant), P > 0.05 (Non-significant)

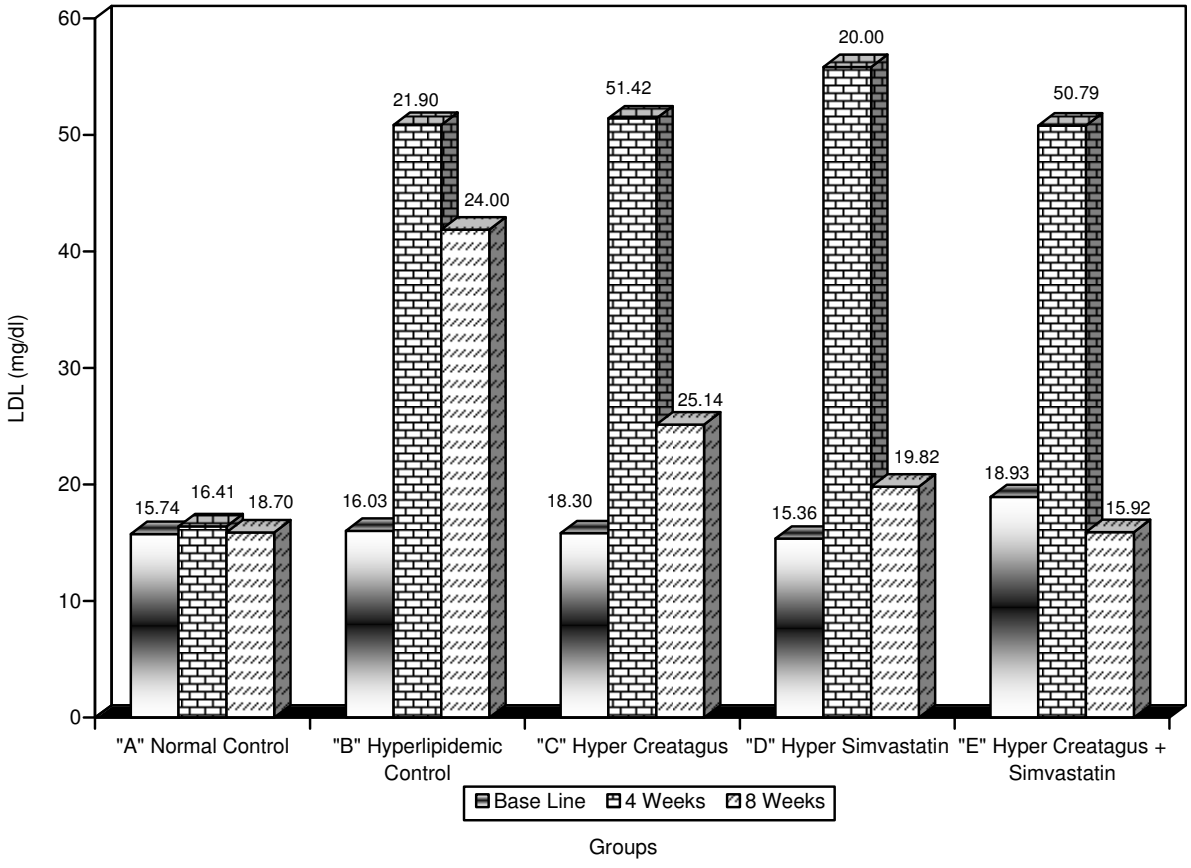

Fig. 4: *Comparison of LDL at different times.*

A. Whereas TG levels of groups C, D and E compared to group B, was significant. Inter comparison of groups C, D and E was insignificant showing all drugs were equally effective in lowering TGs.

Table 3 shows HDL levels of all groups at 0, 4 and 8 weeks. At 0 and 4 weeks the levels were nearly same while at 8 weeks these levels were increased. Pair wise comparison between groups at 0 and 4 weeks was insignificant showing same HDL level in all groups. At 8th week group A versus other groups was significant indicating increase in HDL level in experimental groups. Inter-comparison between B, C, D, and E groups was also significant.

Table 4 shows LDL-c (low density lipoprotein cholesterol) level in all groups at 0, 4 and 8 weeks. LDL-c value of group A remained same throughout the study period. At the 4th week LDL-c value was increased in the remaining four groups. At 8th week the LDL-c value decreased in groups C, D and E but did not decrease enough to reach the normal control levels, whereas it remained high in group B. The combination of drugs in group E was effective enough to bring LDL-c levels back to the level of group A.

DISCUSSION

Physicians are looking for complete control of hyperlipidemia while minimizing side effects. Research for more effective, safer and cheaper lipid lowering agents from natural sources is taking place globally. Many natural products and herbs used for this purpose are garlic, gugulipid, olive oil, fish oil and many others. *Crataegus monogyna* has acquired a prominent status in modern herbal literature as an important cardiac tonic. An alcoholic extract prepared from the berries of *Crataegus* causes significant reduction in cholesterol, triglyceride, LDL and VLDL levels. Studies further reveal that it increases LDL receptor binding capacity in the liver and prevents elevation of total cholesterol and up-regulates hepatic LDL receptors resulting in greater influx of plasma LDL cholesterol in the liver. By enhancing cholesterol degradation to bile acids promoting bile flow and suppressing cholesterol biosynthesis[13]. It also lowers atherogenic component beta lipoprotein. Part of mechanism for antihyperlipidemic effects of hawthorn fruit might also involve the direct protection to human LDL from oxidation or indirect protection via maintaining the concentration of alpha-tocopherol in human LDL.[14] So upon reviewing the accumulated data especially pertaining to *Crataegus* lipid lowering action, the current study was designed to establish antihyperlipidemic effect of *Crataegus* on hyperlipidemic albino rats. The available lipid lowering agents are expensive and may need to be given for decades. Some other agents are also under investigations and may soon be available. In group comparisons, when *Crataegus* (group C) was compared with simvastatin (Group D), it was found that both were equally effective in lowering cholesterol, TGs and LDL-c levels. While combination of both *Crataegus* and simvastatin in group E, was significantly effective in lowering LDL-c as compared to *Crataegus* or simvastatin alone (P value 0.001).

So far, no study has been reported on combined effect of *Crataegus* with simvastatin. Research has been carried out on the combination of ezitimibe and simvastatin showing that this combination results in dual inhibition of absorption and synthesis of cholesterol.[15] The results of our present study show that alcoholic extract *Crataegus* berries possess significant anti-hyperlipidemic properties. Animal data is valuable for developing cost effective and efficacious anti-hyperlipidemic agents. *Crataegus* being an antioxidant and anti-hyperlipidemic herb is worthwhile to use alone and in combination with other anti-hyperlipidemic agents clinically.

It is *concluded* that *crataegus monogyna* is a multifaceted drug which effectively lowers cholesterol, is relatively cheap, convenient to administer and has virtually no side effects. But further work is required to ascertain the pharmacokinetic and pharmakodynamic properties of this herb.

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