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EVALUATION OF THE ANTIHYPERLIPIDEMIC, CARDIOPROTECTIVE ACTIVITY OF A POLYHERBAL FORMULATION

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ABSTRACT

Back ground & objectives

Atherosclerosis is the leading cause of death in the developed and developing countries like India. It is associated with elevated lipid levels in the blood. Treatment of hyperlipidemia is one of the major approaches towards decelerating the atherogenic process. The objective of the study was to evaluate the antihyperlipidemic activity of Antichol, a polyherbal formulation in rats.

Method

Hyperlipidemia and myocardial infarction was induced by administration of Isoprenaline (85mg/kg, i.p. once) for 5 days. The degree of protection was determined by measuring levels of serum TG, TC, HDL-C, LDL-C, VLDL-C, ALP, ALAT, ASAT, cardiac LPO, GSH, SOD and CAT levels. The histopathological changes in the heart and in vivo antioxidant activities were also studied.

Results

Hyperlipidemia was evidenced by elevate levels of serum TG, TC, LDL-C, VLDL-C. Hyperlipidemic model rats also exhibited hyperglycemia and elevated levels of ALP, ALAT, and ASAT. Antichol pretreatment (126 and 630mg/kg, p.o.) significantly reduced the hyperlipidemia, hyperglycemia and elevated levels of serum ALP, ALAT, ASAT and lowered serum total protein. It also significantly reversed the histopathological changes of heart. Antichol also lowered LPO levels and elevated GSH, SOD and CAT levels in heart homogenate.

Interpretation & conclusion

Antichol exhibited antihyperlipidemic and antihyperglycemic activity, which would be attributed to its antioxidant activity.

Keywords: Arteriosclerosis; Antichol; Hyperlipidemia; Isoprenaline.

INTRODUCTION

The increasing morbidity and mortality from coronary heart disease is the biggest challenge to nutrionists and medical scientists all over the world. There is no single etiology to this multifaceted problem; certain risk factors have been identified. The elevated serum lipids are being the most important[1].

A recent survey, carried out by WHO indicates that coronary heart disease (CHD) alone accounts for more than half of the total mortalities associated with cardiovascular diseases. Atherosclerosis is the focal point of pathogenesis of these diseases. The American Heart Association identified the primary risk factors associated with atherosclerosis as elevated levels of cholesterol and triglycerides in the blood. The treatment of hyperlipidemia is to be one of the approaches towards decelerating the atherogenic process. Allopathic hypolipidemic drugs are available at large in the market but the side effects and contraindications of these drugs have marred their popularity. The herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs[2].

Catecholamine plays an important role in the pathogenesis of experimental and clinical atherosclerosis. Isoprenaline, a synthetic catecholamine and β adrenergic agonist has been reported to cause oxidative stress in the myocardium, resulting in infarct like necrosis of heart muscle at higher doses. Administration of ISO in rats produced myocardial necrosis and suggested this to be due to increased fatty acid mobilization and hypoxia. The pathophysiological changes following ISO administration are comparable to human myocardial infarction. Administration of ISO [85mg/kg., once daily for 5days] to rats causes marked cardiac necrosis, increased lipid levels and stimulation of lipid biosynthesis[,3]

The endothelial damage which ultimately generate atheroma and plaque formation, are characterized by high cholesterol and lipid concentrations along with free radical oxidative stress. The involvement of hydroxyl radicals (OH) is a major causative factor for the peroxidative modification in circulatory LDL i.e. responsible for initiation and progression of atherosclerosis[4.] Antichol a polyherbal formulation containing extracts of several medicinal plants including *Commiphora mukul, Curcuma longa, Emblica officinalis, Terminalia arjuna, Terminalia belerica, Terminalia chebula, Garcinia*

cambojia and Ptreocarpus marsupium. The plants have been reported to possess mainly hypolipdemic, cardioprotective, antidiabetic, antioxidant and digestive stimulant activity[5]

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats (150-200g) were purchased from Central Animal Research Facilities, NIMHANS, Bangalore. They were housed, three per poly propylene cage under standard laboratory conditions at room temperature (25^0 C$\pm$ 2^0 C) with 12 h light / dark cycle. The animals were provided with pellet chow and water ad libitum, except during experimentation. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of VIPS, Bangalore.

Drugs and chemicals

Antichol	Gift from Sagar Pharmaceuticals, a division of BPRL Pvt Ltd.B'lore.
Isoprenaline Sulphate	Sigma Chemicals, USA.
Thiobarbituric acid	LOBA Chemic Pvt Ltd, Mumbai.
Ellman's reagent [DTNB]	ICN chemicals, Mumbai.
Nitro blue tetrazolium	ICN chemicals, Mumbai.
Serum glucose diagnostic kit	Span Diagnostics Ltd, Surat.
Serum total protein diagnostic kit	Span Diagnostics Ltd, Surat.
Serum ALP diagnostic kit	Span Diagnostics Ltd, Surat.
Serum ALAT diagnostic kit	Diasys Diagnostic Systems, Germany.
Serum ASAT diagnostic kit	Merck Ltd, Mumbai.
Serum triglyceride diagnostic kit	Span Diagnostics Ltd, Surat.
Serum total cholesterol diagnostic kit	Span Diagnostics Ltd, Surat.
Serum HDL cholesterol diagnostic kit	Span Diagnostics Ltd, Surat.

Other chemicals and reagents were of analytical grade.

Dose and Route of administration:

The proposed human dose of Antichol is 1400 mg, twice a day. According to the conversion table based on surface area given by Dr. M.N. Ghosh in 1984 [6] the human adult dose multiplied by 0.018 gave the dose for rat weighing 200g.

Therefore the rat dose = 1400x0.018 = 25.2 mg /200g or 126 mg/kg rat.

Antichol, being a polyherbal formulation of plant extracts a higher dose of five times i.e. 630mg/kg was selected for the study. The dried powder of the polyherbal formulation, Antichol was suspended in 2% v/v Tween 80 in distilled water and administered orally at doses of 126 mg/kg and 630mg/kg p.o., twice a day.

Methods

1. Isoprenaline (ISO) induced hyperlipidemia

Thirty six male Sprague-Dawley rats weighing 150-200 g were randomly divided into 6 groups of 6 each, marked to permit individual identification and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Group 1: Administered vehicle and served as normal control

Group 2: Administered isoprenaline and served as ISO control

Group 3: Administered Antichol 126mg/kg, p.o.

Group 4: Administered Antichol 126mg/kg, p.o and ISO 85 mg/kg, i.p.

Group 5: Administered Antichol 630mg/kg, p.o.

Group 6: Administered Antichol 630mg/kg, p.o and ISO 85 mg/kg, i.p.

At the end of the 60 days of pretreatment with test drug was done. Isoprenaline- sulphate was administered at the dose of 85mg/kg i.p. for 5 days, 24h after 5th administration of ISO the rats were sacrificed [2].Drugs were given at a constant volume of 1ml /100g body weight. The control group animals received the vehicle in the same volume and by the oral route.

On day 66, animals were anaesthetized with pentobarbitone sodium (30mg/kg i.p) and blood was collected from abdominal aorta by using a 2ml syringe. The blood was subjected to centrifugation to obtain serum. Serum was analysed for serum TGs, serum TC, serum HDL – C, serum LDL – C, serum VLDL – C. The heart homogenate was analyzed for LPO, GSH, SOD, CAT and histopathological analysis.

Statistical analysis: The results are expressed as mean $\pm$ SEM. Comparisons between the treatment groups and control were performed by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test.

RESULTS AND DISCUSSION

The antihyperlipidemic activity of Antichol was studied in isoprenaline induced hyperlipidemic model in rats.

An increased risk of Coronary Heart Disease and atherosclerosis is associated with high serum concentration of TC, LDL, TG and low serum concentration of HDL. HDLs inhibit the uptake of LDL by the arterial wall and facilitate the transport of cholesterol from peripheral tissue to the liver where they are catabolised.[7]

Table 1: Effect of pretreatment with Antichol (126/630mg/kg, p.o. twice weekly for 60 days) on serum lipid parameters levels in rats administered ISO (85mg/kg,i.p., once daily for 5 days)

S. No	Groups	Serum lipid parameters(mg/dl)				
		TG	TC	HDL-C	LDL-C	VLDL-C
I	Normal Control	91.3±1.19	83.05±1.07	35.3±1.35	29.38±1.80	18.38±0.33
II	ISO Control	188.47±3.57*	195±2.18*	24.16±0.79*	133.13±2.04*	37.69±0.71*
III	Antichol(126mg/kg) control	85.01±3.57	79.38±1.45	36.69±0.64	25.70±1.86	16.98±0.71
IV	Antichol(126mg/kg) and ISO treated group	170.86±6.95$	179.13±4.04$	40.33±1.74$	105.46±4.57$	34.16±1.39
V	Antichol(630mg/kg) control	86.02±1.69	76.97±3.33	37.49±4.16	20.62±4.16	17.2±0.34
VI	Antichol(630mg/kg) and ISO treated group	88.03±1.26$	88.03±0.79$	50.67±5.22$	21.69±4.93$	16.5±0.25$

Values expressed as mean ± SEM for six month, *P<0.001 considered statistically significant as compared to normal control group, $P<0.001 considered statistically significant as compared to ISOl control group

The decreased serum HDL level observed in rats treated with Antichol. The possible mechanism inhibition of hepatic TG –lipase on HDL [2]

Isoprenaline induced hyperlipidemia has been reported to cause ischemia in the myocardium and accumulation of cholesterol in the hepatocytes leading to fibrosis, proliferation of bile ducts.[8] Hypertriglyceridemia seen in ISO treated rats has been found to be due to decrease in the activity of lipoprotein lipase in the myocardium resulting in the decreased uptake of TGs from circulation and increased free fatty acid mobilization and hypoxia.[9]

High levels of portal free fatty acids have been associated with insulin resistance and therefore glucose intolerance.[10] this could explain the increased serum glucose levels observed in the model. Pretreatment with antichol has shown significant antihyperglycemic effect.

Table 2: Effect of pretreatment with Antichol (126/630mg/kg, p.o. twice weekly for 60 days) on cardiac oxidative stress parameters levels in rats administered ISO (85mg/kg,i.p., once daily for 5 days)

Sl. No	GROUP	Cardiac oxidative stress parameters			
		LPO(µmoles/mg tissue)	GSH(µmoles/mg tissue)	SOD(µmoles/mg tissue)	SOD(µmoles/mg tissue
I	Normal Control	0.504±0.028	4.23±0.270	11.84±0.428	9.47±0.272
II	ISO Control	0.896±0.035*	1.71±0.190*	7.55±0.335*	3.06±0.163*
III	Antichol(126mg/kg) control	0.473±0.016	4.40±0.161	12.05±0.530	9.35±0.282
IV	Antichol(126mg/kg) and ISO treated group	0.667±0.085$	2.06±0.310$	8.40±0.242$	4.31±0.174$
V	Antichol(630mg/kg) control	0.470±0.018	4.52±0.212	13.36±0.461	9.72±0.348
VI	Antichol(630mg/kg) and ISO treated group	0.521±0.056$	4.16±0.196$	10.89±0.072$	9.09±0.402$

Values expressed as mean ± SEM for six month, *P<0.01 considered statistically significant as compared to normal control group, $P<0.01 considered statistically significant as compared to ISO control group

Oxygen free radicals have been implicated in the development of hyperlipidemic atherosclerosis and liver fibrosis Isoprenaline has been reported to increase lipid peroxidation through free radicals formation [11] resulting in irreversible damage to heart and aorta in animals subjected to Isoprenaline stress. The increased levels of serum enzymes in myocardial infarction has been shown to be due to the leakage of enzymes into the blood [9] Antichol significantly reduced the lipid peroxides in the heart by significantly increasing SOD,GSH and CAT levels, which suggests its efficacy in preventing free radical induced damage. Treatment with Antichol also significantly decreased the serum enzyme levels, which may be by preventing the release of lysosomal enzymes through its membrane stabilizing activity64.Antichol not only corrected hyperlipidemia but also the associated hyperglycemia and liver function abnormalities thus the poly herbal formulation had additional benefits

The results obtained in the present investigation with Antichol thus indicate that, Antichol may offer protection by decreasing the serum lipid, enzyme levels and increasing the natural antioxidants like GSH, SOD and CAT, which in turn maintain the normal function of the heart.

Table 3: Effect of pretreatment with Antichol (126/630mg/kg, p.o. twice weekly for 60 days) on serum glucose and liver function parameters in rats administered ISO (85mg/kg,i.p., once daily for 5 days)

Sl. No	GROUP	Serum glucose and liver function parameters				
		Serum glucose(mg/dl)	Serum total protein(g/dl)	Serum ALP(IU/l)	Serum ALAT(IU/l)	Serum ASAT(IU/l)
I	Normal Control	79.54±2.21	5.76±0.092	243.65±3.74	45.63±1.74	111.24±4.43
II	ISO Control	188.90±4.43*	6.88±0.201*	310.63±12.5*	122.8±8.48*	235.07±8.69*
III	Antichol(126mg/kg) control	77.08±2.36	5.7±0.057	237.8±13.51	45.9±1.59	109.02±5.27
IV	Antichol(126mg/kg) and ISO treated group	155.66±1.69#	6.17±0.294#	283.66±8.49	115.71±6.94	221.69±7.29
V	Antichol(630mg/kg) control	79.45±1.46	5.75±0.159	232.9±10.19	50.12±4.58	112.41±3.71
VI	Antichol(630mg/kg) and ISO treated group	82.45±2.90$	5.76±0.127$	248.05±12.72$	52.9±2.28$	110.79±2.68$

Values expressed as mean ± SEM for six month,*P<0.01 considered statistically significant as compared to normal control group, $P<0.01 considered statistically significant as compared to ISO control group, #P<0.01 considered statistically significant as compared to ISO control group

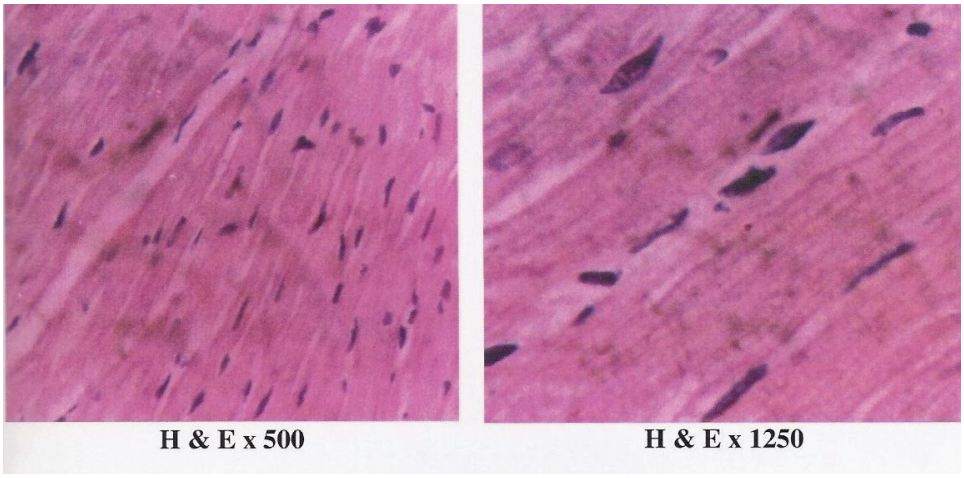

Fig. 1: Light microscopy of the cardiac tissue sections of normal control group showing normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils.

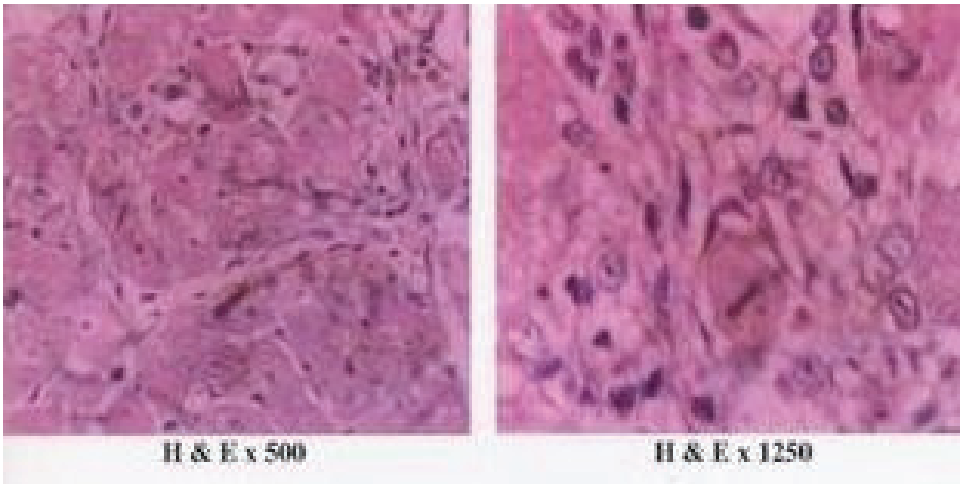

Fig. 2: Light microscopy of the cardiac tissue sections of ISO control group showing loss of normal architecture of cardiac muscle fibers, loss of cross striations and fragmentation of sarcoplasm, infiltration of mononuclear cells in between the muscle fibers and connective tissue proliferation in the area of necrosis.

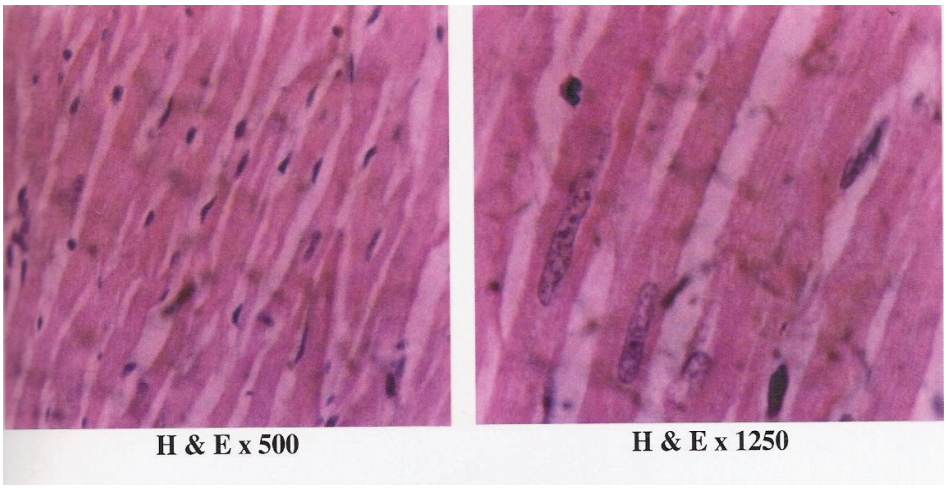

Fig. 3: Light microscopy of the cardiac tissue sections of Antichol (126mg/kg) treated group showing almost normal appearance of cardiac muscle fibers.

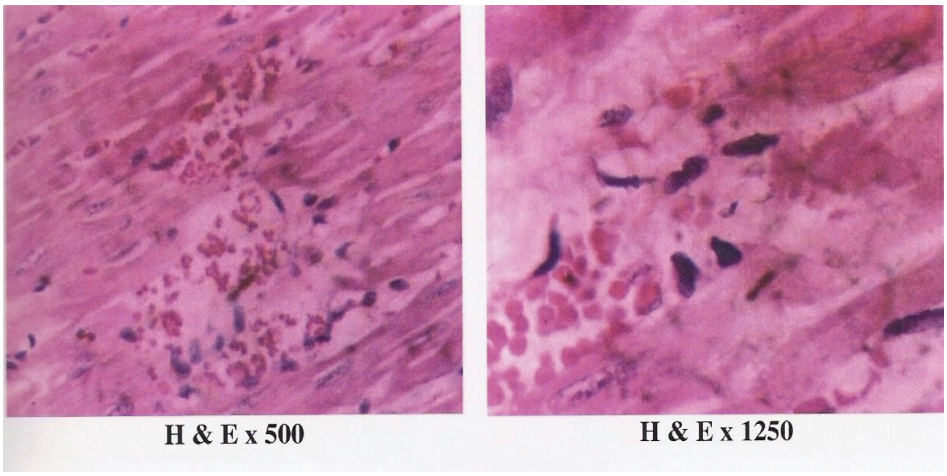

Fig. 4: Light microscopy of the cardiac tissue sections of Antichol (126mg/kg) and ISO treated group showing area of congestion & hemorrhage with fragmentation and loss of cross striation of muscle fibers in occasional areas.

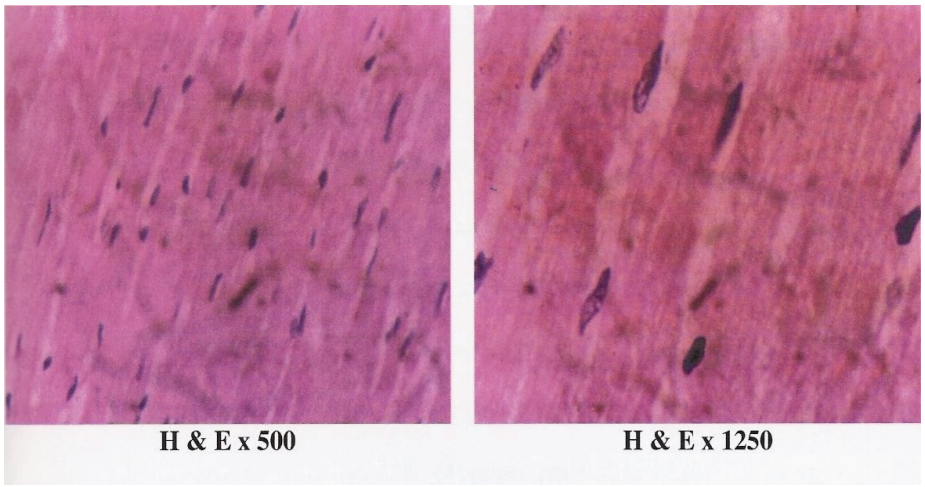

Fig. 5: Light microscopy of the cardiac tissue sections of Antichol (630mg/kg) treated group showing almost normal appearance of cardiac muscle fibers.

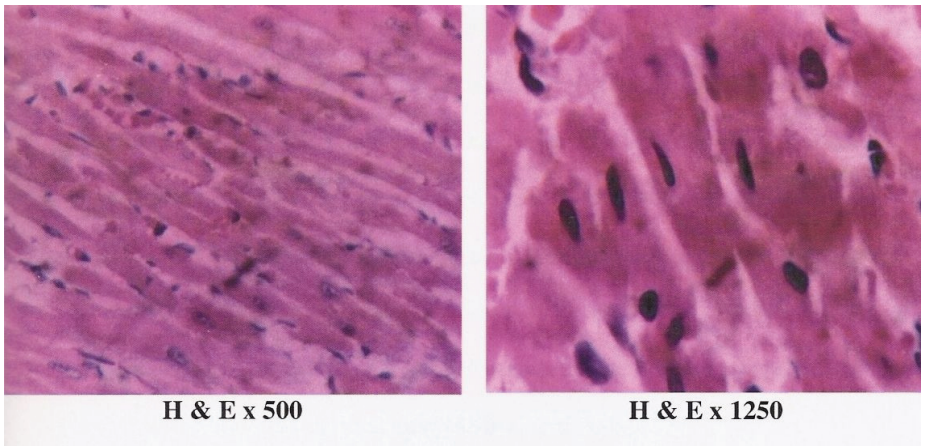

Fig. 6: Light microscopy of the cardiac tissue sections of Antichol (630mg/kg) and ISO treated group showing occasional areas of mild hemorrhage focal necrosis of muscle fibers.

CONCLUSION

In conclusion, the finding in this study suggests that the Antichol posses cardio protective, antihyperglycemic activity in addition to anti hyperlipidemic activity. The mechanism of anti hyperlipidemic, antihyperglycemic, cardio protective activity may attributed to its antioxidant activity.

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