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Effect of 'Abana' Pretreatment on Isoproterenol-induced Hyperlipidemia in Rats

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The cardioprotective effect of Abana, a polyherbal formulation on serum lipid and lipoprotein profile in isoproterenol-induced myocardial infarction was studied in rats. The levels of total cholesterol, ester cholesterol, free cholesterol, phospholipid, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and free fatty acids increased significantly while high density lipoprotein (HDL) was decreased in the serum of isoproterenol administered rats. Polyherbal formulation Abana, showed marked reversal of these metabolic changes induced by isoproterenol.

Cardiovascular diseases have become the number one killer disease in many parts of the world. An increased risk of coronary heart disease is associated with a high serum total cholesterol concentration[1] and low density lipoprotein (LDL)[2] and a decreased high density lipoprotein (HDL)[3].

Abana, an indigenous herbomineral formulation, is a cardiotonic drug of selected ingredients[4] which provide significant protection against ischemia[5] and hypertension[6]. The principal ingredients of Abana are *Terminalia arjuna, Terminalia chebula, Phyllanthus emblica, Nardostachys jatamansi, Zingiber officinale, Withania Somnifera* and *Tinosporia cordifolia*[7]. Abana has been reported to regulate the abnormal elevation of cholesterol either by decreasing the production or by increasing the clearance of lipoproteins[8].

Isoproterenol, 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[9]. The pathophysiological changes following isoproterenol administration are comparable to human myocardial infarction[10]. Mathew *et al.* reported an altered lipid metabolism in myocardial necrosis following isoproterenol administration[11]. The present communication embodies the beneficial effects of Abana in myocardial infarction induced by isoproterenol in rats.

MATERIALS AND METHODS

Adult male albino rats weighing 150-200 g were used for this study. They were acclimatised to the animal house conditions and fed with commercial pelleted rat chow (M/s. Hindustan Lever Ltd., Mumbai) and water was offered *ad libitum*. The rats were divided into two groups with 12 animals in each group. Group 1 control and group 2 Abana treated. To the animals of group 2, polyherbal formulation Abana (75 mg dissolved in water/100 g body weight) was administered orally by gavage for a period of 60 days. Control rats were given saline.

At the end of 60 days, rats were again grouped as follows (1) normal control group (2) control group administered with isoproterenol (3) Abana treated group (4) Abana treated group administered with isoproterenol. Each group contained 6 animals. Isoproterenol (Sigma, USA, 200 mg/kg, s.c) was administered twice at an interval of 24h after abana pretreatment.

After the experimental period (24 h after second administration of isoproterenol) the rats were sacrificed by decapitation and blood was collected in ice-cold containers without anticoagulant, the serum was separated and stored at 4° until analysed. Serum lipoprotein were sepa-

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TABLE 1: SERUM LIPID LEVELS OF CONTROL AND TREATED RATS

	Control	Isoproterenol	Abana	Abana + Isoproterenol
Total Cholesterol	86.35±6.64	125.74±8.46*	80.16±6.22	95.93±7.34#
Free Cholesterol	33.75±2.51	50.82±4.82*	31.8±2.93	38.39±4.2#
Ester Cholesterol	52.6±3.57	74.92±6.21*	48.36±3.79	57.54±4.56#
Phospholipids	88.94±6.6	99.26±6.2*	80.32±7.1	89.57±7.3###
Triglycerides	23.71±1.74	48.19±2.96*	20.36±1.58	28.36±1.8#
Free Fatty acids	23.55±1.24	32.87±2.21*	21.78±1.02	25.35±1.83#

Values are expressed as mean±SD for 6 animals in each group. The levels of lipid in serum are expressed in mg/dl *Significantly different from control group P<0.05 #Significantly different from Isoproterenol control group P<0.05.

rated by dual precipitation method[12] and cholesterol, triglycerides in each fraction were estimated by standard methods. Total cholesterol[13], free cholesterol[14], triglyceride[15], phospholipid[16] and free fatty acid[17] were estimated in serum. Data were analysed using students 't' test. The value of p less than 5% (P<0.05) was considered as significant.

RESULTS

Levels of various lipids in serum of control and experimental animals are presented in Table 1. Isoproterenol administered rats showed a significant increase in the level of total, free, ester cholesterol, triglyceride, phospholipid and free fatty acid in serum. But the elevation is significantly minimised in group 4 rats treated with abana and isoproterenol.

Table 2 presents the levels of cholesterol in HDL and LDL fraction. A significant increase was observed in serum LDL cholesterol in isoproterenol treated rats. The changes were minimum in rats pretreated with ayurvedic formulation abana.

The levels of cholesterol and triglycerides in serum HDL, LDL and VLDL fractions were estimated (Table 3). In isoproterenol administered rats, cholesterol and triglycerides in LDL and VLDL fractions increased significantly with a decrease in HDL cholesterol. In group 4 rats (abana + isoproterenol-treated) the alterations in the levels of cholesterol and triglycerides in the LDL, HDL and VLDL fractions were minimum.

DISCUSSION

High level of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage[18]. Administration of isoproterenol mainly raised LDL cholesterol and decreased HDL cholesterol level in serum. High levels of LDL cholesterol have a positive relation and high levels of HDL cholesterol have negative relation with myocardial infarction[19]. Miller and

TABLE 2: LIPOPROTEIN LEVELS IN CONTROL AND TREATED RATS

	Control	Isoproterenol	Abana	Abana + Isoproterenol
HDL Cholesterol	18.16±1.42	14.74±1.17*	19.43±1.53	16.93±1.31#
LDL Cholesterol	36.28±2.81	48.75±4.24*	34.31±2.52	37.31±3.78#
Risk factor LDLc/HDLc	2.0±0.11	3.3±0.19*	1.8±0.08	2.2±0.12#

Values are expressed as mean±SD for 6 animals in each group. The levels of cholesterol present in each lipoprotein fraction is expressed mg/dl *Significantly different from control group P<0.05 #Significantly different from Isoproterenol control group P<0.05.

TABLE 3: LEVELS OF CHOLESTEROL AND TRIGLYCERIDE IN LIPOPROTEIN FRACTIONS IN SERUM OF CONTROL AND TREATED RATS

Lipid Fraction	Lipids	Control	Isoproterenol	Abana	Abana + Isoproterenol
HDl	Cholesterol	18.16±1.42	14.74±1.17*	19.43±1.53	16.93±1.31##
	Triglyceride	13.12±1.12	18.93±1.32*	11.9±0.96	14.34±1.2#
LDL	Cholesterol	36.28±2.81	48.75±4.24*	34.31±2.52	40.18±3.78###
	Triglyceride	26.63±2.24	38.42±3.51*	24.32±2.24	30.42±3.12#
VLDL	Cholesterol	22.25±2.2	36.79±3.64*	20.81±1.82	27.54±2.63#
	Triglyceride	32.67±3.24	44.58±4.12*	30.74±2.85	37.46±3.52##

Values are expressed as mean±SD for 6 animals in each group Cholesterol and Triglyceride concentration in each lipoprotein fraction are estimated by Standard methods and the values are expressed as mg/dl serum. *Significantly different from control group P<0.05 #Significantly different from Isoproterenol control group P<0.05.

Miller presented that HDL is inversely related to body cholesterol[20]. They have also reported HDL inhibits the uptake of LDL by arterial wall and also facilitates the transport of cholesterol from peripheral tissue to the liver where it is catabolised and excreted out of the body.

Treatment with the polyherbal preparation Abana, elevated HDL cholesterol levels. HDL alters the balance of unesterified cholesterol between plasma and cells by increasing its utilization in lecithin cholesterol acyl transferase system to form cholesterol ester which moves rapidly back into the cells[21]. Abana also maintained a favourable risk factor (LDLc/HDLc ratio).

Isoproterenol administration resulted in significant increase in the free fatty acid level. Increased peroxidation of membrane phospholipid released free fatty acid by the action of the enzyme phospholipase A_2[22] and Ca^{2+} ions have been reported to be one of the inducers of phospholipase A_2. So the observed increase in free fatty acid concentration could have been due to the indirect effect of calcium level which was reported to be altered in isoproterenol-treatment[23]. Accelerated membrane phospholipid degradation results in cell injury and cell death[24] Pretreatment with abana maintains the level of phospholipid and free fatty acid to near normal values.

Hypertriglyceridemia seen in isoproterenol treated rats, a condition observed in ischemic heart disease, is due to a decrease in the activity of lipoprotein lipase in the myocardium resulting in decreased uptake of triglycerides from circulation. In rats pretreated with abana, there is a reduction in triglyceride level in serum. Abana, is a combination of many important medicinal plants used in Indian system of traditional medicine. The selected ingredients present in this formulation contribute to the hypolipidaemic property of abana.

Terminalia arjuna, the major constituent of polyherbal formulation Abana, has been found to increase HDLc and decrease total cholesterol and triglyceride in circulation[25]. *Phyllanthus emblica* is reported to reduce the total cholesterol level in circulation[26]. *Zingiber officinale*[27] and *Piper longum*[28] are found to be potent inhibitors of HMG CoA reductase and thus reduce the synthesis of cholesterol in the system.

The results obtained in the present investigation with Abana thus indicate that this polyherbal formulation may offer protection by decreasing the level of myocardial lipid preventing overloading of the myocardium with lipids, which inturn maintain the normal function of the myocardium.

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