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EFFICACY OF *LAGENARIA SICERARIA* (MOL) ON LIPID PROFILE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Aim of the study: The present study was designed scientifically to evaluate the cardioprotective potential of ethanolic extract of ***Lagenaria siceraria*** fruit **(EELSF)** on the basis of lipid profile in isoproterenol induced myocardial infarction in albino rat models.
M*ethods:* Lipid profile namely phospholipids cholesterol, triglycerides, free fatty acids and lipoprotein were assayed in both serum and heart tissue.
Results: Significantly reduced the cholesterol, triglycerides, free fatty acids and phospholipids levels in heart of the cardiotoxicity myocardial infarcted rats. *Lagenaria siceraria* (Mol) fruits also increased the cholesterol, triglycerides, free fatty acids and phospholipids significantly in isoproterenol induced cardio toxic rats. In isoproterenol administered rats, LDL and VLDL fractions were increased significantly with a decrease in HDL cholesterol in serum. Oral administration of EELSF (125, 250 and 500 mg/kg body weight) significantly reverted the levels of almost all the selected parameters to near normal.
Conclusion: The experiment thus concludes that *Lagenaria siceraria* (Mol) fruits possess cardioprotective effect on experimentally induced cardiotoxic myocardial infarcted rats.

KEYWORDS

Isoproterenol, myocardial infarction, *Lagenararia siceraria,* Lipid profile.

1. INTRODUCTION

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. The world health organization (WHO) estimates that 17 million people die of cardiovascular disease annually[1]. WHO predicts that deaths due to circulatory system diseases are projected to double by 2015[2]. It is well known that CVD are directly or indirectly related to oxidative damage that shares a common mechanism of molecular and cellular damage.

The rat model of ISO-induced myocardial necrosis, out of many well-known models, has often been used to evaluate several cardiac dysfunctions[3]. ISO causes stress in the myocardium and causes severe increase in the levels of serum and myocardial lipids, which in

turn leads to coronary heart disease[4]. The current study is an attempt made to assess the protection of the heart, through pretreatment with ethanolic extract of *Lagenaria siceraria* (Mol) fruit, by reducing the excess lipids.

2. MATERIALS & METHODS

2.1 Collection of Lagenaria siceraria (Mol) fruits

The fresh fruits of *Lagenaria siceraria* (Mol) were collected in the month of August to December from the local market of Thiruchengodu, Tamilnadu, India, and authenticated by the authority of the botanical survey of India (BSI), Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. A voucher specimen (specimen No. BSI/SC/5/23/07-08/Tech-1579) was submitted at institute's herbarium department for further reference.

2.2 Preparation of fruit extract

The fresh *Lagenaria siceraria* (Mol) cut into pieces and dried in a shade to constant weight. The dried pieces (500g) were then ground into powder using an electrical blender. The dried coarsely powdered plant materials were extracted with 90% ethanol using soxhlet apparatus. The solvent was evaporated under vaccum which gave semisolid mass (23%w/w) with respect to the tried powder. The extracts were stored in tight containers in desiccators.

2.3 Source of chemicals

The chemicals used in the present study were of analytical reagent grade. It was purchased from SD fine chem., Himedia and Qualigens, India.

2.4 Experimental Model

Wistar albino rats, weighing 120 – 150g, procured from the small animal breeding centre, Agricultural University, Mannuthy, Kerala were used. The institutional animal ethics committee (IAEC) approved the research. Animals were acclimatized under standard laboratory conditions at 25°± 2°C. 50 ± 15% room humidity and normal photoperiod (12 h light: dark cycle) for seven days. The animals were fed with commercial rat pellet diet and water *ad libitum*.

2.5 Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected subcutaneously to rats (85 mg/kg) daily for 2 consecutive days to induce experimental myocardial infarction[5, 6].

2.6 Experimental design

The rats were divided into five groups of wistar albino rats, each comprising six animals. Group I served as a control, Group II rats were administered with isoproterenol (85mg/kg body weight administered subcutaneously twice at an interval of 24h) dissolved in normal saline. Groups III, IV, and V animals were pretreated with (ethanolic extract of *Lagenaria siceraria*) fruit ***EELSF*** (125mg/kg body weight, 250 mg/kg body weight and 500 mg/ kg body weight, respectively) for a period of 30 days and then administrated with isoproterenol (85 mg/kg body weight administered subcutaneously twice at an interval of 24 h) at the end of the treatment period on the 31st and 32nd day.

2.7 Extraction and estimation of heart tissue lipids

From the samples of heart tissue homogenate the lipids were extracted by the method of [7]. To a known volume of serum or tissue homogenate, 10 ml of chloroform–methanol (2:1 v/v) mixture was added and mixed well for 30 min and was filtered through Whatman filter paper (No. 42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing the lipid was drained off into preweighed beakers. The upper phase was re-extracted with more of chloroform–methanol mixture, the extracts were pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3 ml of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of serum and heart tissue lipids. Total cholesterol[8], triglycerides[9], free fatty acids[10], and phospholipids[11] and HDL[12] were assayed.

2.8 Statistical analysis

The data were expressed as Mean ± SD for six animals in each group. Total variation present in a set of data were estimated by one way analysis of variance (ANOVA) followed by the analysis of level of significance between different groups based on ANOVA using AGRES statistical package (Version 3.1). Difference among means was analyzed by DMRT at 5% level ($p<0.05$).

3. RESULTS AND DISCUSSION

Table 1 shows the levels of cholesterol, triglycerides, free fatty acids, and phospholipids in serum and the heart of normal and experimental rats. In the ISO administrated (Group II) rats, there was significant increase ($p<0.05$) in the level of total cholesterol, triglycerides, free fatty acids and phospholipids with a significant decrease ($p<0.05$) in phospholipids when compared with control group of rats. Pretreatment with ethanolic extract of *Lagenaria siceraria* (Mol) fruits (at doses of 125, 250 and 500mg / kg body weight) resulted in significant decrease in the cholesterol levels, triglycerides and free fatty acids levels with significant increase in the levels of phospholipids as compared with rats administrated with ISO group.

Table 1.

Effect of EELSF on the activities of cholesterol, triglycerides, free fatty acid and phospholipids in serum and heart tissue of control and ISO induced experimental animals

	TOTAL CHOLESTEROL		FREE FATTY ACID		TRIGLYCERIDES		PHOSPHOLIPIDS	
Groups	Serum (mg/ml)	Tissue (mg/g)	Serum (mg/ml)	Tissue (mg/g)	Serum (mg/ml)	Tissue (mg/g)	Serum (mg/ml)	Tissue (mg/g)
Group I	94.17±4.10	5.75± 0.06	28.67 ± 1.75	0.66 ± 0.04	144.52±4.37	3.95 ± 0.05	127.93±2.51	32.68±2.27
Group II	135.53±11.48[a]	7.22±0.09[a]	49.92± 1.77[a]	0.95±0.03[a]	184.94±4.36[a]	5.66±0.05[a]	161.03±2.76[a]	17.63±2.63[a]
Group III	125.25±13.62[b]	6.83±0.31[b]	41.42±1.24[b]	0.90±0.03[b]	173.12±4.61[b]	5.27±0.04[b]	154.40±3.41[b]	21.27±2.49[b]
Group IV	116.15 ± 7.47[b]	6.49 0.27[b]	36.46 ± 1.43[b]	0.82± 0.82[b]	165.45±4.25[b]	4.88± 0.04[b]	145.37±2.85[b]	24.80±2.67[b]
Group V	110.81 ± 7.63[b]	6.2 ±0.21[b]	33.72 ± 1.83[b]	0.75± 0.03[b]	158.60±3.68[b]	4.41± 0.04[b]	139.96±2.95[b]	27.30±3.19[b]

Values are expressed by mean ± SD of six animals in each group
Comparisons are made between: a- Group II and Group I; b – Group III, IV, V and Group II
Statistical significance: a, b significant at $p<0.05$

Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired[13]. Reactive oxygen species (ROS) may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids, which is the precursor of lipid peroxide formation[14]. Elevation of lipid peroxides in ISO-induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes.

Lipids play an important role in cardiovascular disease, not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure, and stability of cellular membranes. Excess lipids in the blood is considered to accelerate the development of arteriosclerosis and are the major risk factor in myocardial infarction. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage[15]. An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death that follows coronary artery occlusion[16]. The cardiac muscle generally utilizes fatty acid as the major source of energy of the total oxygen consumption; 60–90% is utilized to oxidize fatty

acid under aerobic condition. Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these long chain fatty acyl coA derivatives[17].

Lipid hydroperoxides give rise to a large variety of products, many of which react with TBA and with serum lipids. Oxidation of LDL from hydroperoxide products may have substantial importance on dyslipidemic profile. Oxidative modification of LDL is the key step in the sequence of events leading to atherosclerosis. Since serum TBA and LDL-cholesterol concentrations were increased in sesame oil fed animals, we can assume that increased lipoperoxidation was associated to LDL cholesterol. The HDL-cholesterol is extensively degraded due oxidative processes. The most significant risk indicators for cardiovascular alterations, which are considered to be parameters of oxidative stress, are increased serum cholesterol, triacylglycerol, LDL-cholesterol and decreased HDL-cholesterol. Various theories have suggested that cardiovascular damage was the result of an oxidative stress process.

Cholesterol homeostasis is achieved when the body balances the rates of synthesis and dietary intake with rates of elimination. Excessive cholesterol may be released from cells and travelled in the blood as a HDL, which is removed by the liver. Indeed, a high proportion of cholesterol in HDL as compared to LDL is beneficial, since it indicates that cholesterol may be travelling away from the blood vessels to the liver. The weight reduction and decrease in serum LDL cholesterol are important for lowering the risk of cardiovascular attack[18].

The free fatty acids liberated from adipose tissue also enter into the myocardium, and the process is proportional to the free fatty acid concentration in the coronary sinus. Though the heart can utilize free fatty acids for its energy requirements, the excess free fatty acid may be used for the synthesis of triglycerides, resulting in hypertriglyceridemia.

Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD). Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound enzymes. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with mediated atherosclerosis[19].

Phospholipids are essential components for the integrity of cellular membrane and subcellular organelles. Many fatty acids are substrates for the biosysthesis of phospholipids[20]. Tumor cells are known to differ from normal cells in structure and functioning of the membranes and action of various carcinogenic factors leading to alterations in membrane system accompanied by changes in phospholipids composition of tumor cell membranes. The decreased phospholipids content may be due to greater degradation, the event in hepatomas during liver cell injury[21]. The phospholipids content was increased to near normal levels in ethanolic extract of *Lagenaria siceraria* (Mol) fruits treated animals. Due to its membrane stabilizing activity, ethanolic extract of *Lagenaria siceraria* (Mol) fruits may induce myocytes capacity to regenerate new phospholipids which are necessary to repair the damaged membrane.

Isoproterenol administration has been reported to increase adenylate cyclase activity resulting in enhanced cAMP formation. The significant increase observed in the lipid profile in rats treated with isoproterenol alone could be due to enhanced lipid biosynthesis by cardiac cAMP on isoproterenol administration. HDL is known to be involved in the transport of cholesterol from the tissues to the liver for catabolism. Increase in the myocardial cholesterol on isoproterenol administration could be due to increased direct uptake of LDL from the blood by the tissues.

The abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and cell membranes. The high level of VLDL on isoproterenol

administration could be due to the decreased activity of extrahepatic lipoprotein lipase[22].

The result of this study implies that the cardioprotective effect of *Lagenaria siceraria* (Mol) fruits in ISO induced myocardial infarction rats by preserving the membrane integrity and restoring the activities of enzymes to near normal levels. This might be due to the antioxidant effect of *Lagenaria siceraria* (Mol) fruits, and hence *Lagenaria siceraria* (Mol) fruits seem to be promising tools to explore as therapeutic agent in cardiovascular diseases.

Table 2
Levels of Lipoproteins in serum of control and experimental rats

Groups	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)
Group I	65.11 ± 1.06	58.30±1.53	27.50±1.98
Group II	30.33±0.96[a]	142.64±1.62[a]	35.22±2.50[a]
Group III	41.69±1.23[b]	121.87±3.97[b]	33.52±1.56[b]
Group IV	46.80±3.07[c]	107.09±3.51[c]	32.09±1.41[c]
Group V	59.24±0.64[d]	86.76±3.25[d]	30.60±1.58[d]
Group VI	62.32±2.69[e]	73.29±2.91[e]	29.50±1.05[e]

Values are expressed by mean ± SD of six animals in each group
Units: µmoles of NADH oxidized /min / mg protein
Comparisons are made between: a- Group II and Group I; b – Group III, IV, V and Group II
Statistical significance: a, b significant at $p<0.05$

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