Original Article

Effects of *Lagenaria Sicessaria* Fruit Juice on Lipid Profile and Glycoprotein Contents in Cardiotoxicity Induced by Isoproterenol in Rats

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ABSTRACT

This study investigated antihyperlipidemic effects of *Lagenaria siceraria* fruit juice (LSFJ) in isoproterenol (ISO) induced cardiotoxicity in rats. Rats treated with ISO (200 mg/kg, s.c.) showed a significant increase in the levels of triglycerides, cholesterol, and free fatty acids, in both serum and heart tissue. An increase in the levels of phospholipids, low-density lipoprotein, and very low-density lipoprotein-cholesterol, and decrease in high-density lipoprotein-cholesterol in serum and phospholipid levels in the heart were observed. ISO intoxicated rats also showed a significant decrease in the activities of lecithin: cholesterol acyl transferase, whereas lipoprotein lipase was found to be increased. Administration of LSFJ (400 mg/kg, p.o.) for 30 consecutive days and challenged with ISO on day 29th and 30th significantly attenuated these alterations and restored the levels of serum and heart lipids along with lipid metabolizing enzymes. Histopathological observations were also in correlation with the biochemical parameters. These findings indicate the protective effect of LSFJ during ISO-induced cardiotoxicity in rats.

Key words: Glycoprotein, *Lagenaria siceraria*, lipid profile

INTRODUCTION

Lagenaria siceraria, commonly known as Bottle gourd, Syn. Doodhi, syn Lauki (Hindi), and Kadoo (Marathi), is official in Ayurvedic Pharmacopoeia. A modern pharmacological study shows that *L. siceraria* fruit possesses various favorable effects. Chloroform and alcoholic extract of *L. siceraria* showed significant lipid-lowering effects by maintaining total cholesterol (TC), triglyceride, and low-density lipoproteins (LDLs) along with an increase in high-density lipoprotein (HDL) level in triton-induced hyperlipidemia in rats.[1] The fruit of *L. siceraria* reported to shows analgesic, anti-inflammatory,[2] diuretic activity,[3] and *in vivo* and *in vitro* antioxidant activity.[4] Extract is also effective in CCl_4-induced liver damage and doxorubicin-induced cardiotoxicity where it maintained the level of endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and marker of lipid peroxidation to that of normal.[5] Isoproterenol (ISO), a □-adrenergic agonist and synthetic catecholamine, has been reported to produce stress in the myocardium due to its auto-oxidation. Some of the mechanisms proposed for its cardiotoxicity include hypoxia, coronary hypotension, calcium overload, ATPase depletion, and excessive production of free radicals.[6] In our previous study, we have reported the protective effects of *Lagenaria sicessaria* fruit juice (LSFJ) on electrocardiograph changes, blood pressure, endogenous antioxidants level along with serum, and tissue biochemical parameters in ISO-induced myocardial infarction.[7] Till date no study reports are available regarding the effect of LSFJ

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on changes in lipid profile, lipid metabolizing enzymes, and level of glycoproteins in ISO-induced cardiotoxicity in rats. So, an attempt was made to evaluate LSFJ for its cardioprotective activity in ISO-induced cardiotoxicity with reference to its anti-hyperlipidemic potential.

MATERIALS AND METHODS

Drugs and chemicals

(±)-ISO hydrochloride was procured from Sigma Chemicals (St Louis, MO, USA). Fresh fruits of *L. siceraria* were collected from nearby farm at Baroda, Gujarat. All the reagents and chemicals used in the entire study were of analytical grade

Experimental animals

Male adult albino rats (Wistar strain) weighing between 200 and 230 g were used in this study. All experiments were approved by the Institutional Animal Ethics Committee of the M. S. University of Baroda, India. The animals were housed in polyacredylin cages (38 × 23 × 10 cm) with not more than four rats per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (12 h light and 12 h dark) maintained at an ambient temperature 25 ± 2°C. The animals were fed standard pellet diet (Amrut feeds, Pranav Agro Industries Ltd., Sangali, India) and water *ad libitum*. The number for approval of ethical committee is 404 /01 /a/CPCSEA.

Experimental design

The fresh juice of LSFJ was prepared and effective dose was determined by performing pilot study.[7] Animals were randomly allocated into four main groups comprising eight animals in each group. Six animals were used for lipid profile estimation and two animals for histopathological study. Group I: normal control rats received distilled water 2 ml/kg, s.c. for 30 days and normal saline on 29th and 30th day. Group II received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline. Group III received LSFJ (400 mg/kg, p.o.) for 30 days and challenged with ISO on 29th and 30th day. Groups IV received LSFJ (400 mg/kg, p.o.) for 30 days and challenged with ISO on 29th and 30th day.

At the end of experimental period (i.e., on the day 31), blood samples were collected and animals were killed. A heart tissue sample of each rat was collected and lipid extraction was carried out for further estimations.

Extraction of lipids from heart tissue

From the sample of heart tissue, homogenate lipids were extracted using the method of Folch, *et al* (1957).[8] To a known volume of tissue homogenate, 10 ml of chloroform methanol mixture was added and mixed well for 30 min using shaker 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 lipid was drained off into preweighted beaker. The upper phase was reextracted with more of chloroform–methanol mixture and the extract was pooled and evaporated under vacuum at room temperature. The lipid extract was redissolved in 3 ml of chloroform methanol (2:1) mixture, and the aliquots collected were used for the estimation of lipid levels.

Biochemical estimation

TC and triglyceride from heart lipid extracts were estimated using standard diagnostic kits (Reckon diagnostic Ltd., India). The content of free fatty acids[9] and phospholipids[10] from serum and heart lipid extract were estimated. Blood was collected from the retroorbital plexus under mild ether anesthesia. Serum was separated, and TC, triglyceride (TG), and HDL-c were determined by using standard diagnostic kits (Reckon Diagnostic Ltd.). LDL-c and very low-density lipoprotein cholesterol (VLDL-c) were determined by using Fridwald's formula.[11] The activities of lipid metabolizing enzymes such as lecithin: cholesterol acyl transferase (LCAT), and lipoprotein lipase (LPL) were determined from the heart sample as suggested by Hitz *et al* [12] and Slater *et al*.[13]

Histological examination

Periodic acid Schiff's staining was carried out as described in John 2008.[14] The stained sections were examined under Olympus (Magnus MLX series) India Pvt Ltd. Photomicroscope and photographed (10×). Result was considered as various glycoproteins and glycoconjugates show magenta color and Nuclei Blue color.

Statistical analysis

Results are presented as mean ± SEM. One-way analysis of variance followed by Bonferroni multiple comparisons using a computer-based fitting program (Prism, Graph Pad) were performed. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Level of various lipids in serum of control and experimental animals were recorded. ISO-injected rats showed a significant increase in the level of serum TC, TG, LDL, VLDL, FFA, and PL along with a significant decrease in serum HDL level when compared with control animal. Treatment with LSFJ for 30 days in ISO-injected rats (LSFJ + ISO) showed significant ($P < 0.01$, $P < 0.05$) decrease in the elevated levels of TC, TG, LDL, VLDL, FFA, and

PL and significantly ($P < 0.05$) increase in the level of HDL when compared with ISO-injected rats [Figures 1 and 2]. Table 1 shows the effect of LSFJ on heart tissue lipid in ISO ISO-injected rats. A significant increase in heart TC, TG, and FFA along with a significant reduction in phospholipids content was observed in ISO-injected rats. Administration of LSFJ for 30 days in rats followed by ISO injection did not show significant improvement in heart lipid profile except TG that was found to be significantly reduced when compared with ISO-injected rats.

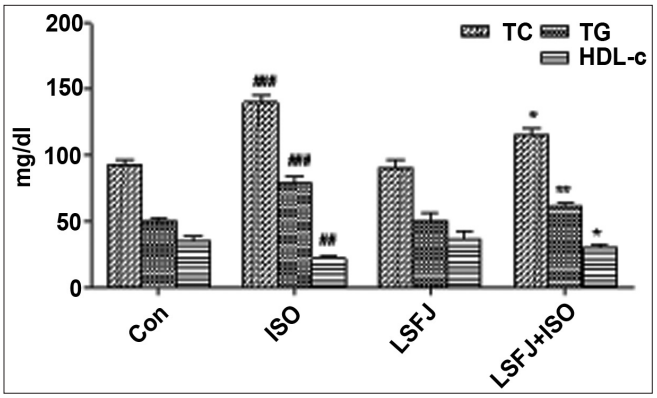

Figure 1: Effect of LSFJ (400mg/kg/day, p.o) for 30 days on serum TC, TG and HDL levels in normal and ISO injected rats, Values are expressed as mean ± SEM (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ values compared with control groups; $^{*}P < 0.05$, $^{**}P < 0.01$ values compared with ISO-injected groups.

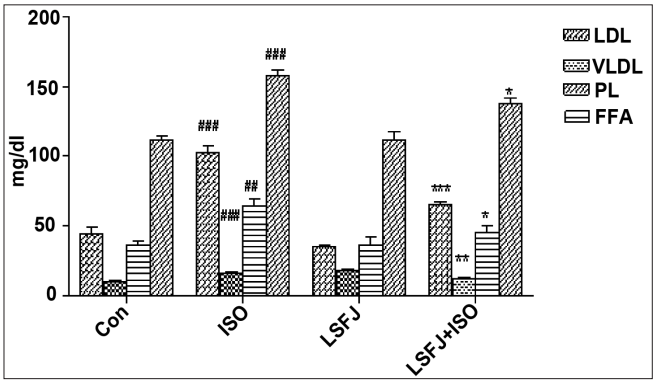

Figure 2: Effect of LSFJ (400 mg/kg/day, p.o.) for 30 days on LDL, VLDL, FFA, and PL levels in normal and ISO-injected rats, Values are expressed as mean ± SEM ($n = 6$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ values compared with control groups; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared with ISO-injected groups.

The activities of lipid metabolizing enzymes such as LCAT and LPL were carried out in heart tissue. ISO-injected rats show a significant ($P < 0.01$, $P < 0.001$) decrease in the activities of LCAT and LPL when compared with control group. Treatment of LSFJ in ISO-treated rats (LSFJ + ISO) showed a significant ($P < 0.05$) increase in LCAT and LPL activities compared with ISO-injected rats [Figure 3]. PAS staining of ISO-injected rats showed an arbitrary increase in the amount of glycoproteins or glycoconjugates [Figure 4b] when compared with normal control rats [Figure 4a]. Administration of LSFJ in ISO-injected rats (LSFJ + ISO) for 30 days showed decrease in membrane-bound glycoconjugates [Figure 4c] when compared with control animals. LSFJ-alone-treated groups did not show any alteration in above parameters suggesting the non-toxic nature of the fruit juice.

DISCUSSION

High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage.[13] ISO, a synthetic catecholamine, induces free radicals, which may cause cellular cholesterol accumulation, by increasing cholesterol biosynthesis and its esterification, decreasing cholesterol ester hydrolysis, and reducing cholesterol efflux.[15] ISO increases the LDL cholesterol concentration in the blood, which in turn leads to the buildup of harmful deposits in the arteries, thus favoring coronary heart disease.[16] High levels of serum triglyceride have been strongly linked with low serum HDL cholesterol, and these low HDL levels may contribute to increased risk for CVD.[17]

In this study, ISO-injected rats showed alteration in lipid profile which agree with the previous reports.[18,19] Yeagle[20] reported that changes in membrane cholesterol content affect its fluidity, permeability to ions, activities of membrane-bound enzymes, and increased degradation of phospholipids. The decline in the cardiac phospholipid content with a concomitant increase in the serum can be due to ISO- mediated peroxidation of unsaturated membrane lipids in biomembranes and tissues causing the leakage of these lipids into circulation. In addition, the increased peroxidation of membrane phospholipids releases free fatty acids by the action of phospholipase A2.[21]

Table 1: Effect of LSFJ (400 mg/kg/day, p.o.) for 30 days on tissue lipid profile in normal and ISO-injected rats

Groups	TC (mg/g wt tissue)	TG (mg/g wt tissue)	FFA (mg/g wt tissue)	PL (mg/g wt tissue)
Con.	7.922 ± 0.777	6.832 ± 0.637	0.792 ± 0.123	23.178 ± 2.098
ISO	12.653 ± 2.142$^{\#\#}$	10.982 ± 0.872$^{\#\#\#}$	1.562 ± 0.971$^{\#\#}$	17.024 ± 1.403$^{\#\#}$
LSFJ	8.116 ± 2.087	6.001 ± 1.302	0.884 ± 0.208	24.891 ± 3.211
LSFJ+ISO	11.181 ± 1.004^{ns}	8.825 ±1.221*	1.302 ± 0.321^{ns}	18.112 ± 2.088^{ns}

Values are expressed as mean ± SEM ($n = 6$)., $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ values compared with control groups; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared with ISO-injected groups.

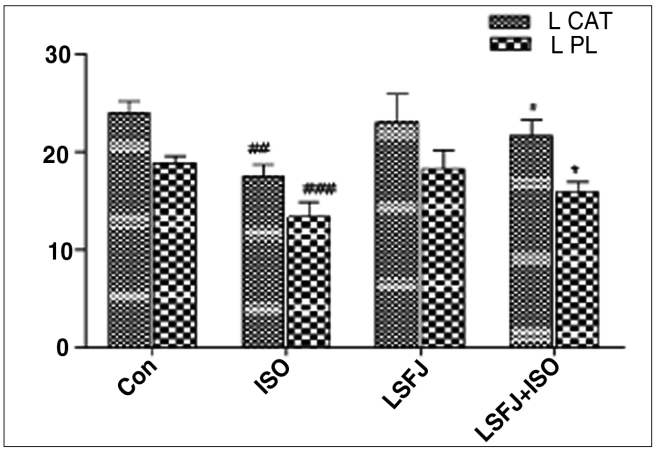

Figure 3: Effect of LSFJ (400 mg/kg/day, p.o.) for 30 days on LCAT and LPL levels in normal and ISO-injected rats, Units: LCAT (μmoles of cholesterol esterified/hr/100 mg tissue), LPL (μmoles of fee fatty acids liberated/100 mg tissue), Values are expressed as mean ± SEM (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ values compared with control groups; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ values compared ISO-injected groups.

Alteration in lipid profile is controlled by those enzymes that are responsible for lipid metabolism. In this study, ISO-injected rats showed a significant decrease in cardiac LCAT and LPL activities. LPL is the enzyme that is involved in the metabolism of triglyceride-rich lipoproteins. Elevated triglyceride-rich lipoprotein levels may not only promote a more rapid progression of atherosclerosis but also lead to myocardial ischemia.[22] LCAT is the serum enzyme that esterifies free cholesterol, primarily at the surface of the HDL particle after which the cholesteryl ester molecules migrate to the inner core of this lipoprotein.[23] Through this action, LCAT plays a key role in the maturation of HDL particles. LSFE shows significant lipid-lowering activity in this study which might be due to the presence of plant sterols and saponins. Fixed oil in the fruit is considered as a good source of mono, polyunsaturated fatty acids and cardiac aglycones. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids resulting in decreased body lipids.[24] Saponins are also reported to bind in intestinal lumen with cholesterol, decrease its absorption, increase its fecal excretion, and increase the LPL activity.[24] Ghule *et al*[1] reported that LSFE improved lipid profile in triton and high fat diet induced hyperlipidemic rats. Further, treatment of LSFJ decreased lipid peroxidation in this study, which supports the lipid-lowering activity of LSFJ.

Glycoproteins/glycoconjugates are important components of intracellular matrix, cell membrane, and membranes of the subcellular organelles.[25] Glycoconjugates are specific markers for oxidative injury of membrane components. Significant increase in the levels of glycoprotein or glycoconjugates in serum and the heart of ISO-induced rats have already been reported.[26,27] Report shows that increase in protein bound carbohydrate complexes

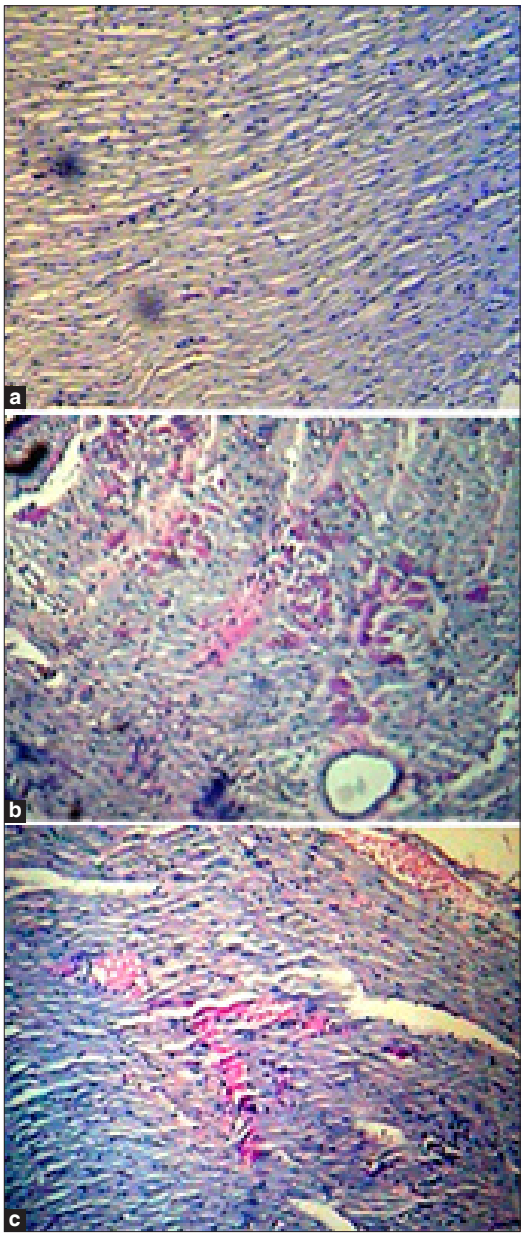

Figure 4 (a-c): Periodic acid Schiff's staining of normal and LSFJ-treated rats. (a) Control showing normal architecture, (b) ISO-injected rat shows increase in expression of glycoconjugates, (c) LSFJ-treated rats showed decrease in glycoconjugation contents. Nucleus stain with blue color and glycoconjugates with magenta color

may be the reason for myocardial dysfunction.[28] The observed increase in the levels of glycoproteins in ISO-injected rats may also be due to increased deposition of macromolecular components, which is a physiological adjustment to the pathological process. Lysosomal membranes contain large amounts of glycoproteins that play an important role in maintaining lysosomal structure and function. These glycoproteins are considered to be the susceptible target for reactive oxygen radicals produced during hypoxic conditions.[29] In this study, periodic acid Schiff's staining of control and experimental groups of rats was carried out. ISO-injected rats show increased

expression of glycoproteins which may be due to ISO-induced lipid peroxidation and diminished antioxidant status. The membrane deterioration of lysosomes by ISO-induced LPO could result in the release of more glycoproteins.[29] Treatment with LSFJ in ISO-injected rats showed near-normal architecture of membrane and decrease of membrane bound glycoconjugates compared with ISO-injected rats. ISO injection causes generation of free radicals as a result of its autooxidation and thereby formation of metabolites. Free radical injury can increase the expression of glycoconjugates which is reported to prevent by the administration of antioxidant. Pre-co-administration of LSFJ in this study reduced the expression of glycoconjugates which might be due to the presence of polyphenols in the juice. The observed effects may be due to membrane stabilizing effects of LSFJ through the termination of lipid peroxidation reaction.

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