Indian Journal of Experimental Biology
Vol. 46, April 2008, pp. 234-242

Beneficial effects of *Lagenaria siceraria* (Mol.) Standley fruit epicarp in animal models

J R Deshpande*, A A Choudhari, M R Mishra, V S Meghre, S G Wadodkar & A K Dorle**
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Amravati Road, Nagpur 440 010, India

Received 4 January 2007; revised 18 February 2008

Lagenaria siceraria (Mol.) Standley fruit (bottle gourd), a commonly used vegetable in India is described as cardiotonic and as a general tonic in Ayurveda. Keeping in view the presence of free radical scavenging activity in *L. siceraria* and involvement of free radicals in the development of various disorders, present studies were designed to evaluate the ethanolic extract of *L. siceraria* fruit against the disorders where free radicals play a major role in pathogenesis. The extract was found effective as hepatoprotective, antioxidant, antihyperglycemic, immunomodulatory, antihyperlipidemic and cardiotonic agent. The results showed that the radical scavenging capacity of *L. siceraria* fruit may be responsible for various biological activities studied.

Keywords: Antioxidant, Antihyperlipidemic, Antihyperglycemic, Cardiotonic, Hepatoprotective, Immunomodulatory, *Lagenaria siceraria*

Lagenaria siceraria (Mol.) Standley fruit (Syn. *L. vulgaris* Ser., *Cucurbita lagenaria* Linn., *L. leucantha* Rusbey, family Cucurbitaceae) commonly known as bottle gourd is used as a vegetable in India. The fruit is traditionally used as a cardiotonic, aphrodisiac and general tonic[1], liver tonic and against liver disorders and pain[2], anti-inflammatory, expectorant and diuretic agent[3]. Further, antihepatotoxic activity of fruit pulp[4], analgesic and anti-inflammatory activity of fruit juice[5] and hypolipidimic activity of the fruit have also been evaluated[6]. Recently, the antioxidant activity of ethanolic extract of epicarp and fresh juice of *L. siceraria* fruit have been reported[7].

Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of *L. siceraria* possesses immunoprotective, antitumor, antiHIV and antiproliferative properties[8]. Phytochemical screening of the fruit revealed the presence of fucosterol and compesterols[4] flavonoids, cucurbitacins, saponins and polyphenolics[5], triterpenoids and C-flavone glycosides [9-12] and ellagitannins[7].

**Correspondent author's address:
42, Ram Nagar
Nagpur 4400 33, India
Mobile: 09850396036 (AKD)
*Mobile: 09949189319 (JRD)
E-mail: jitendra_despande@rediffmail.com

Cardiovascular disorder is claimed to be relieved following regular intake of bottle gourd juice for about 4-6 months[13].

'Hriday Mitra Mandal'—a registered society, devoted to relief for cardiac patients, forcefully advocates daily use of the juice in order to prevent cardiovascular disorder in young as well as elderly persons[13]. The existence of interrelationship between production of free radicals and induction of cardiovascular disorders is well documented[14-16]. Similarly, the association of exogenous and endogenous generation of reactive oxygen species (ROS) with the pathogenesis of diseases like cancer[17], diabetes[18], arthrites[19] and aging[20] is also established. The present study has been undertaken to evaluate immunomodulatory hepatoprotective, antihyperglycemic, antihyperlipidemic and cardiotonic properties of ethanolic extract of *L. siceraria* (EELS).

Materials and Methods

Sprague-Dawley rats (150-200 g) of either sex and male Swiss albino mice (20-27 g) obtained from National Institute of Nutrition, Hyderabad were used. While rats were used for assessment of hepatoprotective, *in vivo* antioxidant, antihyperglycemic and antihyperlipidimic activities, albino mice were used for determination of immunomodulatory activity.

Male frogs (*Rana tigrina*, 20-30g) were used for evaluation of cardiotonic activity.

The fruits of *L. siceraria* (Mol.) Standley, purchased locally were authenticated from Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The fruit was separated into epicarp, mesocarp and pulp containing seeds. These were shade dried, coarsely powdered and soxhlet extracted successively with petroleum ether, benzene, chloroform and ethanol. The extracts were concentrated in vacuum to a syrupy consistency and evaporated to dryness at 45°C to a constant weight. Since preliminary *in vitro* experiments with 1-1 diphenyl-picryl-2 hydrazyl (DPPH) assay revealed that ethanolic extract of epicarp exhibits maximum free radicals scavenging activity[7], the same was employed for assessment of evaluation of biological activities. Protocols were approved from Institutional Animal Ethics Committee.

Acute toxicity studies were performed as per OECD-423 guide lines[21] on randomly selected rats of either sex (n=6). EELS (5 mg/kg) was administered orally after overnight fasting. Since mortality was not observed, the procedure was repeated with higher doses viz. 50, 300 and 2000 mg/kg body weight.

Immunomodulatory activity—Preparation of antigen: The blood withdrawn from the external jugular vein of the sheep (Sheep Farm, Nagpur Veterinary College, Nagpur) was mixed (1:1) with Alsever's solution, centrifuged at 2500 rpm for 20 min and washed twice with sterile phosphate buffer saline (PBS). A 2% sheep red blood cells (SRBC) suspension in PBS was prepared and stored at 4°C in refrigerator and used as required. A known amount (0.5×10^9cells/ml/100g body weight) was injected (ip) to the mice as the antigenic challenge.

The mice were divided into 6 groups of 8 animals each. Group I served as control and received normal saline only. The mice of group II-VI were injected with SRBCs on 14 and 21 day (0.5×10^9cells/ml/100g; ip). Gr II animals received only pyrogallol (50 mg/kg, ip) five days before the first antigen challenge. (Mice in Gr III were administered orally with vitamin E (50 mg/kg) and vitamin C (50 mg/kg) once daily. Gr IV, V and VI were administered with 4.6, 9.2 and 23 mg/kg of EELS respectively thrice daily, orally. Immunomodulatory activity was evaluated by the assessment of (a) humoral immune response (HIR) to SRBC by hemagglutination reaction (HR)[22], (b) cell mediated immune response (CMIR) by delayed type hypersensitivity reaction (DTH)[23] and (c) neutrophil adhesion to nylon fibres[24].

Hepatoprotective and antioxidant activity—Hepatoprotective activity was assessed by examining the influence of the EELS (in doses 100 and 200 mg/kg) on hepatotoxicity induced by administration of CCl_4 (30% in liquid paraffin)[25]. Sylimarin (100 mg/kg, po) was used as a standard. Blood was collected from retro-orbital plexus under light ether anaesthesia on 15th day for analysis of levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), acid phosphatase (ACP)[26] and bilirubin[27]. SGOT, SGPT and ALP kits were obtained from Erba Diagnostics, Mannheim, Germany, Merck Ltd, Mumbai and SPAN Diagnostic Ltd, Surat, respectively and methods prescribed by manufacturer of kits were followed. Histopathology of the hepatic cells was carried by using hematoxylin-eosin dye to stain the cells of rats sacrificed under light ether anaesthesia. Protein content[28], lipid peroxidation (LPO)[29], superoxide dismutase (SOD)[30], catalase (CAT)[31] and glutathione peroxidase (GPx)[32] were determined by homogenizing the remaining hepatic tissue in 0.1 M PBS (*p*H 7.4). Doses were based on preliminary experiments.

Antihyperglycemic activity —Hyperglycemia was induced in rats by alloxan monohydrate (150 mg/kg, ip single dose). Rats in which hyperglycemia (blood glucose level above 260 mg/dl) was induced after 48 hr of alloxan administration were divided into 5 groups of 6 rats each. Gr I served as the normal control and Gr II as a hyperglycemic control to which saline solution was administered. Animals in Gr III and IV were hyperglycemic and treated with EELS (100 and 200 mg/kg, po) for 14 days. Gr V was treated with glibenclamide (5 mg/kg po) for 14 days. Plasma levels of glucose were analysed on 0, 7 and 14th day of treatment[33], serum lipid levels were determined on 14th day which included total cholesterol (TC)[34], high density lipoprotein cholesterol (HDL-C)[35], triglycerides (TGL)[36], low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C)[37].

Antihyperlipidimic activity—Hyperlipidimia was induced in rats by administration of Triton WR 1339 (iso-octyl polyoxyethylene phenol) in phosphate buffer (*p*H 7.2, 0.05 *M*). The rats were divided into 5 groups of 6 animal each. Gr I served as a normal control and rats of group II to V were administered

with Triton WR 1399 (350 mg/kg/ip). Gr II served as hyperlipidimic control. Animals of Gr III and IV were administered with EELS (100 and 200 mg/kg, po respectively). The fifth group received simvastatin (0.57 mg/kg, po). The blood samples were withdrawn at 0, 24 and 48 hr after Triton injection, blood serum was separated by centrifugation and analysed for TC and TGL.

Action on frog heart—The action of EELS on frog heart was recorded by observing chronotropic and inotropic effect on heart preparation by using conventional method[38]. The frog heart was exposed and the venous cannula was inserted in the inferior vena cava. The universal lever was attached to heart by a thread and heart beats were measured on rotating drum. When normal rhythm of heart was attained, 1 μg $CaCl_2$ was injected in the perfusion tube very close to venous canula and effect was observed. This was followed by injecting two doses of EELS viz 0.1 and 1 μg separately and observing the effect. Same procedure was repeated using a modified ringer solution when concentration of Ca^{2+} was ¼ to that of original concentration used.

Statistical analysis—The results are expressed as mean±SE and the difference between the group were analysed by ANOVA followed by Dunnets test (P<0.05) and Student's t test.

Results and Discussion

Daily intake of homogenized bottle gourd (about 200 to 300 g) in the form of a juice serves as a prophylactic against cardiac disorders. Also, patients claim that their heart block as well as lipid profile came to normal following taking the bottle gourd juice regularly[13]. Perusal of their case papers amply support their claim. There is however no scientific data available to support the usefulness of bottle gourd against cardiac disorders. The results of the present work seem to support this practice.

Association between oxidative stress, generation of free radicals and cardiovascular disorders is now well recognized[14-16]. It is also generally believed that scavanging the excessive free radicals could prevent occurrence of related disorders. EELS appears to exert significant free radical scavenging, antihyperlipidimic, immunomodulatory, hepatoprotective and antioxidant effect. These properties of EELS seem responsible for prevention of CVD symptoms and also alleviating the pathophysiological symptoms associated with CVD such as alteration in lipid profile and coronary block. Interestingly EELS seems to provide protection against stress hyperglycemia also.

Acute toxicity studies—No mortality occurred in the group of rats receiving upto 2000 mg/kg po of EELS. *L. siceraria* is being used since ancient time as a vegetable, its LD_{50} was not determined. Fresh juice obtained from 200-300 g *L. siceraria* fruit is recommended in 'Dudhi therapy'[13]. Therefore the dose was selected on the basis of EELS obtained from epicarp of 200 g of fruit and calculated for adult human as mg/kg body weight.

Immunomodulatory activity—Effects of EELS on humoral immune response (HIR), cellular immune response (CIR) and percent neutrophil adhesion (PNA) in mice in the presence of chemical stressor i.e. pyrogallol are presented in Table 1. EELS prevented to a significant extent the reduction of HIR, CIR and PNA induced by pyrogallol. In this respect 23 mg/kg of

Table 1—Immunomodulatory activity of *L. siceraria* in mice

[Values are mean ± SD of 8 mice in each group]

Group	Doses	Humoral immune response (HIR)		Cellular immune response (% increase in paw volume)	Neutrophil adhesion (%)
		Primary	Secondary		
I	Control	7.0 ± .92	10.25 ± 0.46	27.51 ± 1.92	23.09 ± 0.82
II	Pyrogallol (50 mg/kg)ip	4.12 ± 0.64	7.25 ± 0.46	16.36 ± 1.36	15.3057 ± 0.81
III	Pyrogallol (50 mg/kg) + Vitamin E (50 mg/kg) + Vitamin C (50 mg/kg) once daily	7.37 ± 0.51**	10.37 ± 0.51**	27.92 ± 2.13**	22.96 ± 0.72**
IV	Pyrogallol (50 mg/kg) + Extract (4.6 mg/kg) three times daily	5.25 ± 0.46**	7.75 ± 0.46	17.67 ± 0.98	16.49 ± 0.86*
V	Pyrogallol (50 mg/kg) + Extract (9.2 mg / kg) three times daily	6.5 ±0.53**	8.37 ± 0.51**	21.66 ± 0.96**	18.34 ± 0.88**
VI	Pyrogallol (50 mg/kg) + Extract (23 mg/kg) three times daily	6.87 ± 0.83**	9.37± 0.51**	26.96 ± 1.49**	22.95 ± 0.91**

ANOVA followed by Dunnett's test; P values: *<0.05; **<0.01 as compared to group II

EELS seems to be equivalent to 50 mg/kg of vit. C + 50 mg/kg of vit. E.

The cellular components involved in immune system are rich in polyunsaturated fatty acids, which on oxidative attack lead to highly cytotoxic products of peroxidation. Enhancement in prostaglandin level also results in immunomodulation. Enhanced endogenous generation of ROS, because of aging and pathophysiological conditions causes depression of lipoxygenase and cycloxygenase which adversely influence integral membrane function and cell mediated immune reaction. Such conditions may be effectively combated by enhancing cellular defence in the form of radical scavengers or antioxidants[39,40].

The increase in humoral immune response exhibited by EELS may be due to increase in antibody responsiveness to SRBC because of enhanced responsiveness of macrophages and β-lymphocytes involved in antibody synthesis[41]. The sensitized lymphocytes, when challenged with SRBC antigen, are converted to lymphoblasts, secrete lymphokines to attract more scavenger cells to site of reaction. The infiltrating cells become more defensive and cause inflammatory reaction[42]. This was evident when an increase in foot paw volume was observed with increased dose of EELS. The oral administration of EELS increased neutrophil adhesion of blood to nylon fibres indicating process of migration of cells to blood vessels.

Hepatoprotective and antioxidant activities—CCl_4 induced hepatotoxicity was evidenced by significant rise in marker enzyme levels in blood viz. SGOT, SGPT, ALP, ACP and bilirubin (Table 2). EELS (100 and 200 mg/kg, po) and silymarin (100 mg/kg, po) could prevent CCl_4 induced rise in the levels of these markers.

The histopathological examination revealed that CCl_4 treatment results in swollen hepatocytes loaded with fatty droplets and pushing of nuclei towards periphery of the hepatocytes (Fig. 1b). In a group which received 100 mg/kg of EELS there was mild degree of degenerative changes, which were perilobular and a mild degree of perivascular infiltration of leucocytes indicating partial hepatoprotective activity (Fig. 1d). EELS 200 mg/kg effectively prevented hepatic damage induced by CCl_4 as there was only mild fatty degeneration and focal necrosis was totally absent (Fig. 1e). The histopathological observations in the group treated with sylimarin (Fig. 1c) were almost comparable to that of the control group (Fig. 1a).

CCl_4 induced hepatotoxic rats showed significant increase in TBARS and about 50% decrease in SOD, CAT and GPx levels. EELS effectively prevented the lipid peroxidation of the liver tissue. The values of TBARS, SOD, CAT and GPx in the group treated with EELS were also close to normal (Table 2).

Table 2—Effect of *L. siceraria* extract on CCl_4 induced hepatotoxicity and liver antioxidant defence system in rats

[Values are mean ± SE from 6 animals in each group]

Treatment	SGOT or ASAT (IU / L)	SGPT or ALAT (IU / L)	ALP (IU / L)	ACP (IU / L)	Total bilirubin (mg / 100 ml of blood)	TBARS (n mol Malondialdehyde / mg liver protein)	Superoxide Dismutase (U / mg liver protein)	Catalase (μM of H_2O_2 Decomposed / min / mg liver protein)	Glutathione Peroxidase (n mol of NADPH / mg liver protein)
Control	97.3 ± 1.18	35.08 ± 0.2	15.92 ± 0.72	10.5 ± 0.064	0.39 ± 0.04	1.29 ± 0.395	75.81 ± 1.94	296.83 ± 10.05	0.992 ± 0.05
CCl_4 (1 ml/kg ip)	186.7 ± 1.82	136.9 ± 1.94	98.3 ± 7.9	38.6 ± 2.9	0.89 ± 0.76	1.79 ± 0.14	47.84 ± 0.50	179.73 ± 5.78	0.61 ± 0.03
EELS (100 mg/kg)	132.64 ± 6.29*	78.1 ± 5.9*	61.3 ± 5.1*	23.2 ± 0.45*	0.7 1± 0.05	1.43 ± 0.16	65.74 ± 0.58*	261.25 ± 8.79*	0.82 ± 0.06*
EELS (200 mg/kg)	102.3 ± 7.85**	43.9 ± 3.7**	39.4± 2.7**	18.4 ± 0.12*	0.52 ± 0.04	1.31 ± 0.16*	84.25 ± 0.84*	283.38 ± 9.46*	0.96 ± 0.07*
Silymarin (100 mg/kg)	105.3 ± 4.3**	49.4 ± 3.6**	34.8 ± 2.9**	16.2± 1.2*	0.24 ± 0.03*	1.26 ± 0.14*	88.34 ± 2.54*	268.27 ± 6.46*	0.95 ± 0.03*

P values : * < 0.01, ** < 0.001

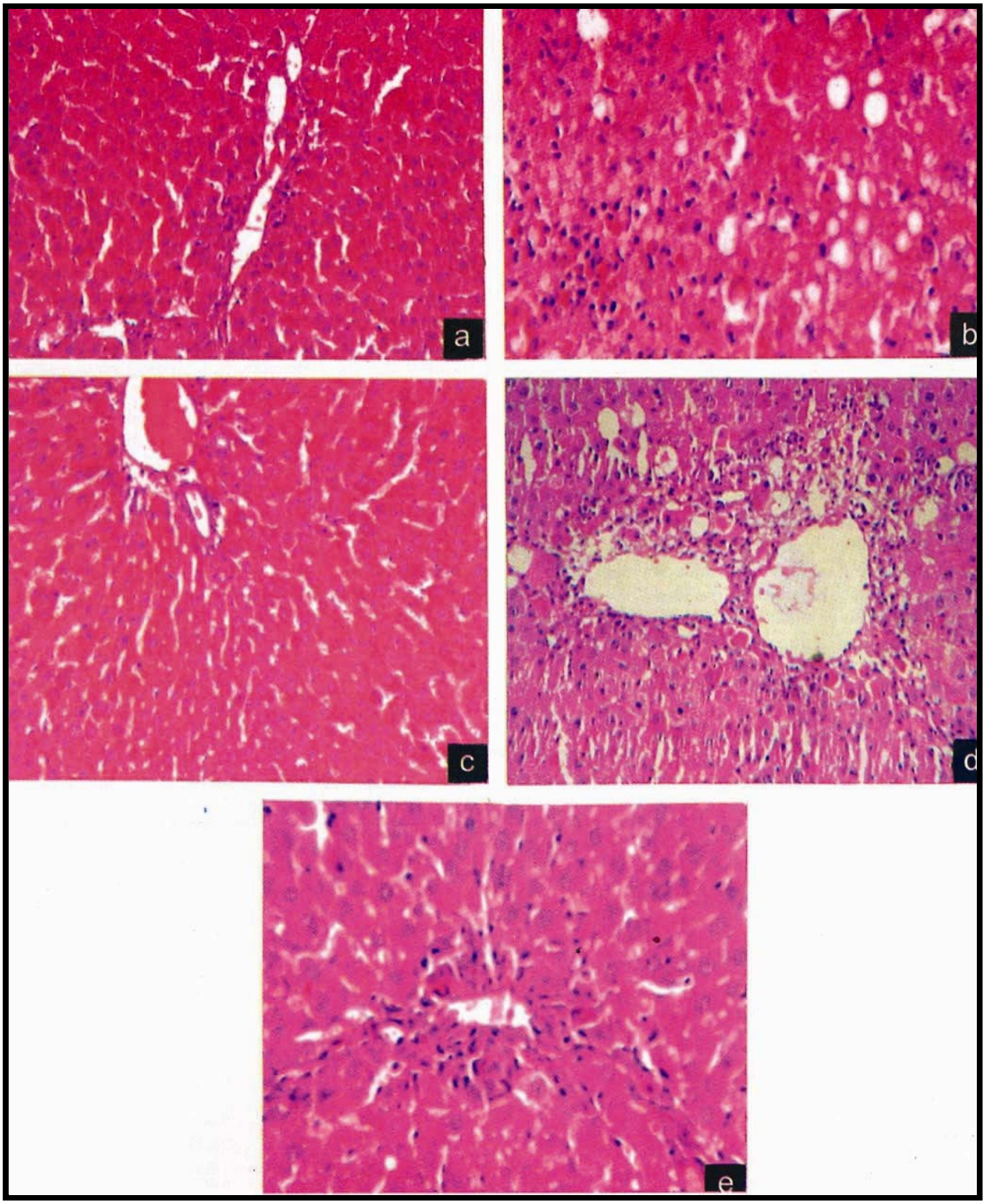

Fig. 1—Photomicrograph of (a) normal liver section of rat; (b) liver section of rat treated with CCl_4 ; liver section of rat treated with CCl_4 + (c) 100 mg/kg silymarin; (d) 100 mg/kg EELS; (e) 200 mg/kg EELS [a-e: 200×]

CCl_4 causes cell lysis with release of cytoplasmic hepatic enzyme in circulation[43] and is metabolized into trichloromethyl radical ($CCl_3^{\bullet}$) which reacts with molecular oxygen to form trichloromethyl peroxy radical ($CCl_3O_2^{\bullet}$)[44]. $CCl_3O_2^{\bullet}$ induce lipid peroxidation ultimately gives rise to various cytotoxins like molondialdehyde (MDA) and 4-hydroxynonenal[45]. In the present studies it appears that EELS detoxifies free radicals generated by CCl_4 administration.

Administration of EELS reduced the level of these enzymes significantly in dose dependent manner. Petroleum ether extract of whole fruit of *L. siceraria* has also been reported to exert antihepatotoxic effect and this was attributed to fucosterol and compesterol isolated from the fraction[4].

Antihyperglycemic activity—Alloxan (150 mg/kg, ip) induced hyperglycemic rats showed significant increase in blood sugar levels which were reduced to a significant extent by EELS (100 and 200 mg/kg, po) on 7th and 14th day of treatment, (Table 3). The percentage reduction by glibenclamide was 57.8 and 64.3% on 7th and 14th days respectively. Alloxan also elevated levels of TC, TG, VLDL-C and LDL-C and reduced that of HDL-C. Administration of EELS (100 and 200 mg/kg) effectively prevented these changes (Table 3). The effect of 200 mg/kg of EELS was almost comparable to that of glibenclamide (5 mg/kg).

EELS counteracted to a great extent, the effect of alloxan in experimental animals. Alloxan is reduced to diluric acid resulting in production of superoxide radicals which damage pancreatic β-cells[46]. The etiology of alloxan induced diabetes mellitus predominantly involves generation of free radicals. The antidiabetic, or more specifically antihyperglycemic, action of EELS may be attributed to its radical scavenging property.

Alloxan, by generating free radicals, cause damage to Islets of Langerhans resulting in insulin deficiency leading to diabetes. Accumulation of lipids in the form of total TC and TG is common symptom of insulin deficiency. The administration of alloxan caused rise in blood sugar, TG, LDL-C and VLDL-C and fall in HDL-C leading to 3-fold rise in TC. This effect was counteracted by simultaneous administration of EELS and may be mediated through its antioxidant activity.

Antihyperlipidemic activity—The blood levels of cholesterol and triglycerides were increased by 3.5 and 9.5-folds respectively 24 hr after the administration of Triton. EELS (100 and 200 mg/kg, po) administration effectively prevented this rise in cholesterol and triglycerides. The effect of EELS is almost comparable to that of simvastatin (Table 4).

It is presumed that triton enhances intra- and extra-mitochondrial synthesis of cholesterol by induction of related enzymes such as HMG-CoA reductase[47,48]. Antioxidants adversely influence the activity of reductase due to their free radical scavenging capability thus reducing the rate of cholesterol synthesis.

Table 3—Effect of *L .siceraria* on blood sugar and lipid levels in alloxan induced hyperglycemic rats

[Values expressed in mg/dl are mean ± SE from 6 animals in each group]

Group	Groups and dose (mg/kg)	Blood sugar level			TC	TGL	HDL-C	VLDL-C	LDL-C
		1st day	7th day	14th day					
I	Control (Normal saline) (2)	90.3 ± 4.6	90.8 ± 3.6	90. 8 ± 6.6	82.53 ± 7.21	75.15 ± 5.03	25.6 ± 1.83	15.03 ± 1.006	41.9 ± 4.37
II	Diabetic control (Alloxan) (150)	263.3 ± 18.4	262.1 ± 19.2	262.4 ± 17.7	219.3 ± 10.49	116.48 ± 8.5	24.93 ± 6.52	23.30 ± 1.7	171.07 ± 2.27
III	EELS (100)	261.4 ± 16.0	167.3 ± 10.3*	110.2 ± 7.9**	128.4 ± 8.62	103.86 ± 7.79	36.84 ± 2.63*	20.77 ± 1.56	70.79 ± 4.44**
IV	EELS (200)	264.8 ± 18.9	112.4 ± 9.6**	93.3 ± 8.2**	107.94 ± 8.47	95.63 ± 5.83*	40.78 ± 3.52*	19.13* ± 1.17	48.04 ± 3.79**
V	Glibenclamide (5)	260.3 ± 14.3	110.5 ± 9.7**	93.5 ± 8.2**	84.64 ± 6.79	84.58 ± 6.7*	33.82 ± 2.79*	16.92 ± 1.34*	33.9 ± 2.66**

P values * < 0.01; ** < 0.001

TC = Total cholesterol TGL = Trigylcerides

HDL-C = High density lipoprotein cholesterol VLDL-C= Very low density lipoprotein cholesterol

LDL-L= Low density lipoprotein cholesterol

Table 4—Effect of *L. siceraria* on triton induced hyperlipidemia in rats

[Values expressed in mg/dl are mean ±SE from 6 animals in each group]

Group	Treatment and dose (mg/kg)	Cholesterol			Triglycerides		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
I	Normal	83.16 ± 2.414	85 ± 2.595	82.33 ± 1.726	85.915 ± 1.915	85.5 ± 1.839	83.33 ± 1.358
II	Triton (350)	83.5 ± 2.446	283.16 ± 8.44	144.83 ± 3.04	88.16 ± 2.21	748.16 ± 13.64	256.83 ± 5.61
III	EELS (100)	85.5 ± 2.029	149.16 ± 4.408*	111.5 ± 3.91*	84.16 ± 1.57	345.83 ± 9.84*	201.66 ± 6.73*
IV	EELS (200)	85.5 ± 2.604	91.5 ± 2.99*	78.83 ± 2.72*	86.33 ± 2.10	151.16 ± 6.20*	116.5 ± 5.57*
V	Simvastatin (0.57)	85.16 ± 1.95	75.16 ± 1.352*	72.16 ± 2.088*	86.5 ± 1.80	134.83 ± 4.30*	103.5 ± 3.11*

P values * < 0.05 ; ** < 0.001

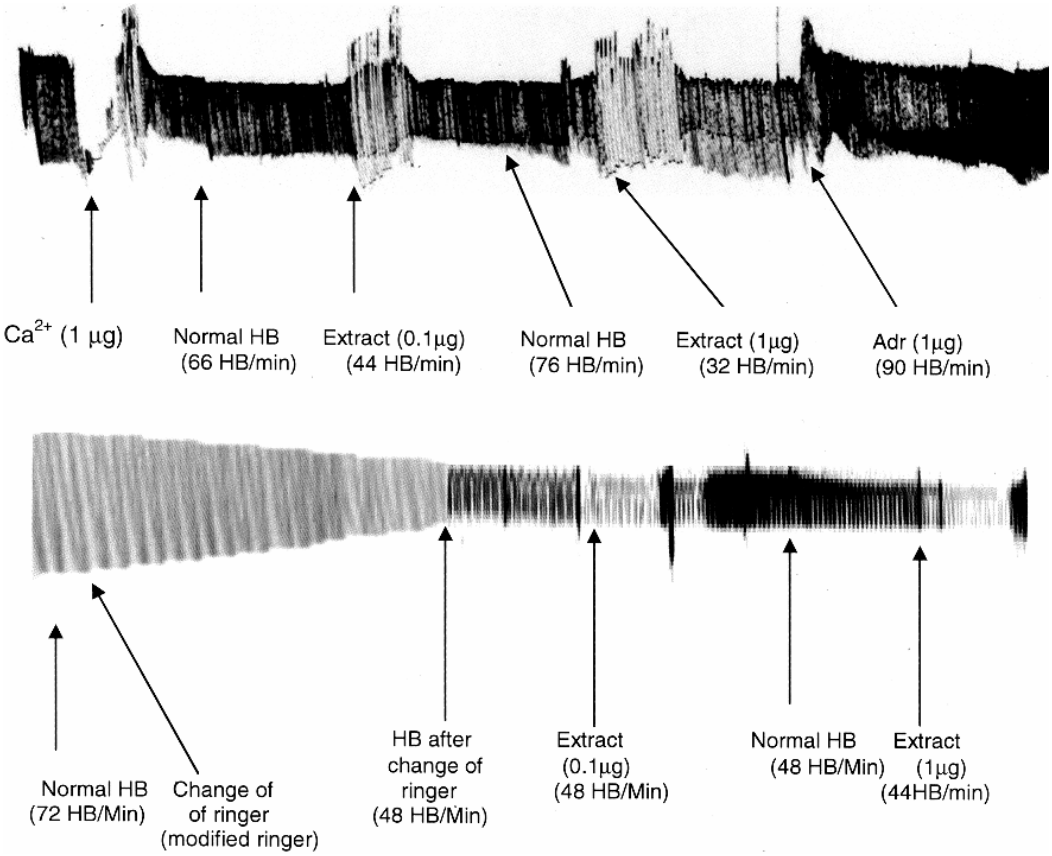

Fig. 2—Effect of *L. siceraria* extract on frog heart preparation by using normal ringer (upper panel) and modified ringer (lower panel) solution [HB = heart beat]

Effect on frog heart—EELS (0.1 mg) caused increase in force of contraction with decrease in rate of contraction (from 66 to 44) in isolated frog heart perfused with normal Ringer solution. The contraction of heart could not be recovered to normalcy when depleted Ringer solution was used (Fig. 2).

The cardiotonic action of EELS may be considered as a contributory factor for its overall beneficiary effect on prevention of disorders related to cardiovascular system in general. The possibility of cardiac stimulation action of EELS due to presence of calcium salts was carefully examined by using perfusion fluid with reduced concentration of calcium. The position of cessation of cardiac contraction by higher doses of EELS was also different from that of calcium indicating that it does not resemble to that of Digitalis glycosides. The lack of any action on isolated blood vessels as indicated by hind limb perfusion rules out any adrenergic involvement.

It is interesting to note that the radical scavenging antioxidant activity of the fruit is located largely in its epicarp which may be used for extraction of the active principal.

Of further interest is the influence of EELS on cardiac function in frogs. The mechanism and significance of this aspect needs further investigation. It should also be interesting to isolate and characterize the active principle in the fruit.

References

1 Vaidya L S, *Shaligram Nighantu Bhushanam*, (Khemraj Srikrishnadas, Steam Press, Mumbai) 1953, 891.

2 Kirtikar K R & Basu B D, *Indian medicinal plants* (International Book Distributors Dehradun) 1998, 1116.

3 Warrier P K, Nambiar V P K & Ramankutty C, *Lagenaria siceraria* (Mol.) Standley, in *Indian medicinal plants*, Vol.3 (Orient Longman Limited, Madras) 1995, 292.

4 Shirwaikar A & Sreenivasan K K, Chemical investigation and antihepatotoxic activity of the fruits of *Lagenaria siceraria*, *Indian J Pharm Sci*, 58 (1996)197.

5 Ghule B V, Ghante M H, Upganlawar A B & Yeole P G, Analgesic and antiinflammatory activities of *L. siceraria* (Mol.) Stand. fruit juice extract in rats and mice, *Pharmacognosy Magazine*, 2 (2006) 232.

6 Ghule B V, Ghante M H, Saoji A N & Yeole P G, Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol) fruit extracts, *Indian J Exp Biol*, 44 (2006) 905.

7 Deshpande J R, Mishra M R, Meghre V S, Wadodkar S G & Dorle A K, Antioxidant activity of *Lagenaria siceraria* (Mol.) Standl. fruit, *Natural Prod Rad,* 6 (2007) 127.

8 Wang H X & Ng T B, Lagenin, a novel ribosome-inactivating protein with ribonucleolytic activity from bottle gourd (*Lagenaria siceraria*) seeds, *Life Sci*, 67 (2000) 2631.

9 Duke J A, *Handbook of phytochemical and constituents of GRASS herbs and economic plants* (CRC Press, Florida) 1999, 98.

10 Evans W C, *Trease and Evan's Pharmacognosy* 14th edition (W.B. Saunders, London), 1996, 338.

11 Sonja S & Herman S, Analysis of cucurbitacins in medicinal plants by HPLC-MS, *Phytochem Anal*, 11 (2000) 121.

12 Baranawska M K & Cisowaski W, High performance chromatographic determination of flavone C- glycosides in some species of cucurbitaceae family, *J Chromatography*, A675 (1994) 240.

13 Kothari M, in *Hridaya rakshak lauki ras* (Hridaya Mitra Mandal, Nagpur, India) 2005, 43.

14 Ross R, The pathogenesis of atherosclerosis: A perspective for the 1990s, *Nature*, 362 (1993) 801.

15 Steinberg D, Parthasarathy S, Carew T E, Khoo J C & Witztum J L, Beyond cholesterol: Modifications of low-density lipoprotein cholesterol that increase its atherogenicity, *N Engl J Med,* 320(1989)915.

16 Steinberg D, Low density lipoprotein oxidation and its pathobiological significance, *J Biol Chem*, 272 (1997) 20963.

17 Ray G & Husain S A, Oxidants, antioxidants and carcinogenesis, *Indian J Exp Biol*, 40 (2002)1213.

18 West I C, Radicals and oxidative stress in diabetes, *Diabet Med*, 17 (2000) 171.

19 Tak P P, Zvaifler, N J, Green D R, Firestein G S, Rheumatoid arthritis and p53: How oxidative stress might alter the course of inflammatory diseases, *Immunol Today*, 21 (2000) 78.

20 De La Fuente M, Hernanz A & Vallejo M C, The immune system in the oxidative stress conditions of aging and hypertension: Favorable effects of antioxidants and physical exercise, *Antioxidants & Redox Signaling*, 7 (2005) 1356.

21 Ecobichon D J, *The basis of toxicology testing*. (CRC Press New York) 1997.

22 Talwar G P & Gupta S K, *A handbook of practical and clinical immunology*, Vol.1, 2nd edition (CBS Publishers and Distributors, New Delhi) 2002, 194.

23 Papadimitriou C, Hahn H, Naher H & Kaufmann S H, Cellular immune response to sheep erythrocytes: interrelationship between proliferation of popliteal lymph node cells and footpad swelling, *Immunobiology*, 164 (1983) 361.

24 Prasad V, Jain V & Dorle A K, Evaluation of *Momordica charantia* ghrita for immunomodulatory activity, *J Plant Sci*, 1 (2006) 80.

25 Rao G M M, Rao C V, Pushpangadan P & Shirwaikar A, Hepatoprotective effect of rubiadin, a major constituent of *Rubia cordifolia* Linn, *J Ethnopharmacol*, 103 (2006) 484.

26 Hillman G Z, Fortlaufende photometrische messung der sauren phosphatase- aktivitat, *Z Klin Chem Klin Biochem*, 9 (1971) 273.

27 Jendrassik L & Grof P, Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins, *Biochem Z*, 297 (1938) 81.

28 Lowry O H, Rosenbrough N J, Farr A L & Randall R J, Protein measurement with the folin phenol reagent, *J Biol Chem*, 193 (1951) 265.

29 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95 (1979) 351.

30 Misra H P & Fridovich I, The role of superoxide anion in the auto-oxidation epinephrine and a simple assay for superoxide dismutase, *J Biol Chem*, 247 (1972) 3170.

31 Aebi H U, Catalase: in *Methods in enzymatic analysis,* edited by H U Bergmeyer, (Academic Press, New York) 1983, 276.

32 Paglia D E & Valentine W N, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J Lab Clin Med*, 70 (1967)158.

33 Trinder P, Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, *Ann Clinical Biochem,* 6 (1969) 24.
34 Pesce M A & Boudourian S H, Enzymatic rate method for determining cholesterol in the serum, *Clin Chem*, 23(1977)757.
35 Izzo C G F & Murador E, Improved method for determination of high density lipoproteins by using polyethylene glycol 6000, *Clin Chem*, 27 (1981) 371.
36 Fossati P & Prencipe L, Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, *Clin Chem*, 28 (1982) 2077.
37 Bairaktari E T, Seferiadis K I & Elisaf M S, Evaluation methods for the measurement of low density lipoprotein cholesterol, *J Cardiovasc Pharmacol Therapeut*, 10 (2005) 45.
38 Kulkarni S K, *Handbook of experimental pharmacology* IIIrd edition (Vallabh Prakashan, Delhi) India, 1999, 155.
39 Devasagayam T P A, Molecular damage by reactive oxygen species: Its possible role in health and disease, in *Biological concepts in radiotherapy,* edited by BB Singh & D Bhattacharjee (Narosa Publishing House, New Delhi) 1995, 102.
40 Devasagayam T P A & Kamat J P, Free radicals and antioxidants and human disease, *EMSI Newslett,* 23 (2000) 3.
41 Mungantiwar A A**,** Nair A M, Shinde U A, Dixshit V J, Saraf M N, Thakur V S & Sainis K B, Studies on the immunomodulatory effects of *Boerhaavia diffusa* alkaloidal fraction, *J Ethnopharmacol*, 65 (1999)125.
42 Sen P, Mendiratta P K & Ray A, Effects of *Azadirachta indica* on some biochemical, immunological and visceral parameters in normal and stressed rats, *Indian J Exp Biol*, 30 (1992) 1170.
43 Tesehke C B, Vierke W & Goldermann L, Carbon tetrachloride levels and serum activities of liver enzymes following acute carbon tetrachloride intoxication, *Toxicol Lett,*17 (1983) 175.
44 McCay P B, Lai E K, Poyer J L, DuBoss C M & Janzen E G, Oxygen and Carbon-centered free radical formation during carbon tetrachloride metabolism. Observations of lipid radicals *in vivo* and *in vitro, J Biol Chem,* 259 (1984) 2135.
45 Shukla S, Bhadauria M & Jadon A, Effect of propolis extract on acute carbon tetrachloride induced hepatotoxicity, *Indian J Exp Biol* , 42 (2004) 993.
46 Rerup C C, Drugs producing diabetes through damage of insulin secreting cells, *Pharmacol Rev*, 22 (1970) 485.
47 Burton S D, Robbins E D, Feigenbaum L Z, Friendman M, Byers S O & Ishida T, Discharge of cholesterol into the perfusate of isolated rat livers, *Am J Physiol,* 182 (1955) 89.
48 McPherson (Jr) J C, Schuster G S & Dirksen T R, The *in vitro* effects of triton WR-1339 on lipid synthesis by bone cells, *Proc Soc Exp Biol Med*, 149 (1975) 172.