

Modulatory effect of *Inula racemosa* Hook. f. (Asteraceae) on experimental atherosclerosis in guinea-pigs

Kalachaveedu Mangathayaru^a, Sarah Kuruvilla^b,
Kedike Balakrishna^d and Jayakothandaramaswamy Venkatesh^c

^aSri Ramachandra College of Pharmacy, ^bSri Ramachandra Medical College and Research Institute and ^cCentre for Toxicology and Developmental Research, Sri Ramachandra University, Porur and ^dCaptain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha, Arumbakkam, Chennai, India

Abstract

Objectives *Inula racemosa* Hook. f. is indicated for precordial chest pain in Ayurveda. In this study, the effects of a hexane (IrH) and an alcohol extract (IrA) of *Inula racemosa* on atherosclerosis induced by a high-fat diet in guinea-pigs were investigated.

Methods After 30 days on a high-fat diet (guinea-pig pellet diet + 0.2% w/w cholesterol) six animals were killed and evaluated for the onset of early atherosclerotic changes in coronary artery, aorta and major organs. The remaining animals were assigned to 5 groups of six animals each and fed for the following 90 days with a pellet diet + 0.15% w/w cholesterol (positive control) along with 100 mg/kg IrA, 100 mg/kg IrH or 10 mg/kg atorvastatin calcium. The normal control group received only the pellet diet. At the end of experimental period, serum lipid levels, heart and liver antioxidant status, area of lipophilic aortic lesions and histopathology of coronary artery were estimated.

Key findings IrA decreased total cholesterol, triglycerides, low-density lipoprotein cholesterol and the atherogenic index, and increased high-density lipoprotein cholesterol compared with the positive control. It scavenged thiobarbituric acid reactive substances and increased reduced glutathione in liver, and enhanced superoxide dismutase and glutathione peroxidase in heart. Aortic lesion area and % bodyweight increase was least in the IrA-treated group. Coronary artery changes due to the high-fat diet were reversed by the extracts. The observed effects are presumably mediated by phenolics in IrA and sesquiterpene lactones in IrH.

Conclusions The results demonstrate the anti-atherogenic effect of *I. racemosa*, thus validating the cardioprotective and anti-obesity claims in traditional medicine.

Keywords anti-obesity; antioxidant; atheroprotective; hypolipidaemic; *Inula racemosa*

Introduction

The multifactorial aetiopathology and progressive nature of atherosclerosis is well established.^[1] Despite lifestyle changes and the use of new pharmacologic approaches to lower plasma cholesterol concentration, cardiovascular disease continues to be the principal cause of death in the US, Europe and much of Asia.^[2] Apart from hyperlipidaemia, obesity, diabetes, hypertension and smoking, several emerging risk factors^[3] are being identified and the high mortality and morbidity associated with cardiovascular diseases makes it imperative to develop new approaches to their treatment. The polyvalent synergistic action of the different structurally complex phytochemicals from medicinal herbs in the amelioration of several chronic diseases is clinically established and gaining worldwide scientific acceptance.^[4]

Inula racemosa Hook. f. (Asteraceae), commonly known as ‘pushkarmool’ in India, was first recorded in the ‘*Charaka Samhita*’ written by the legendary Ayurvedic physician Charaka 2000 years ago.^[5] It is described as a medicine for precordial chest pain, cough and dyspnoea.^[6] Administered orally for rheumatic pains and inflammatory conditions of the liver, it is also applied externally as a paste or liniment to relieve pain. ‘Pushkara-guggulu’, a traditional remedy for ischaemic and coronary heart disease,^[7] is composed of *I. racemosa* and *Commiphora mukul*. *I. racemosa* is known to reverse exercise-induced ST–T changes in electrocardiographs of patients with ischaemic heart disease, and reportedly possesses beta-blocking activity.^[8] The root powder enhances prostaglandin-like

Correspondence: Kalachaveedu Mangathayaru, Sri Ramachandra College of Pharmacy, Sri Ramachandra University, Porur, Chennai-600 116, India.
E-mail: kvmanga@yahoo.com

activity in isoproterenol-induced myocardial ischaemia in rabbits.^[9] The aqueous extract is reportedly anti-anginal,^[10] hypoglycaemic^[11] and anti-asthmatic.^[12] Hepatoprotective,^[13] negative inotropic and chronotropic activity of the petroleum ether extract^[8] have also been reported.

Several sesquiterpene lactones have been isolated from the hexane soluble fraction of the root, of which alantolactone and isoalantolactone, occurring in a ratio of 4 : 6, are the major alantolides. Dihydroalantolactone, dihydroisoalantolactone, alloalantolactone, inunolide, dihydroinunolide, neoalantolactone and isoalantodiene are a few of the minor alantolides.^[14] The root yields 1.3–2.6% of steam volatile essential oil, principally composed of sesquiterpenes. Several simple phenolics such as phenyl acetonitrile and phenyl ethanol have been reported from the roots. Alantolactone and isoalantolactone are reported to have antioxidant,^[15] antibacterial, anthelmintic, anti-inflammatory and anticancer activities.^[16]

The ethnopharmacological usage of the root in ischaemic heart diseases, reported biological activities and the presence of sesquiterpene lactones, known for a wide variety of biological activities, led us to evaluate its possible hypolipidaemic and anti-atherogenic activity on atherosclerotic changes induced in male Hartley guinea-pigs. Guinea-pigs were used as the model because of its unique inducibility of atherosclerosis.^[17,18] It carries the majority of cholesterol in low-density lipoprotein (LDL) and has cholesterol ester transfer protein and lipoprotein lipase activities, which result in reverse cholesterol transport and delipidation cascades equivalent to the human situation.

Materials and Methods

Plant material

Authenticated roots of *I. racemosa* were obtained from Dr Padma Gurmet, Research Officer, Sowa Rigpa (Amchi) Research Centre, Leh, India, and a voucher sample was deposited in the herbarium of Sri Ramachandra University (no: IR/17/23.05.05).

Preparation of extracts

Roots (2.5 kg) were cut into small pieces, shade dried for 7 days and extracted with hexane by cold maceration. The filtered extract, upon concentration by vacuum distillation, yielded a yellowish oily mass (IrH) of 2.88% w/w yield. The air-dried hexane exhausted marc was subjected to cold maceration with alcohol and vacuum distilled to yield a syrupy brownish mass (IrA) of 5.2% w/w yield.

Determination of total phenolic content

The phenolic content of the extracts was determined by the Folin-Ciocalteu method^[19] and the result calculated in gallic acid equivalents (GAE). Whereas IrH had little phenolic content, that of IrA was 34 GAE/100 g of extract.

Chemicals

Cholesterol, bovine serum albumin, thiobarbituric acid, nitroblue tetrazolium, 5,5'-dithio bis (2-nitro benzoic acid) and Oil Red O were purchased from Sigma (St Louis, MO,

USA). Atorvastatin calcium was a gift from M/S Ordain Health Care (Pvt) Ltd, Chennai, India. Edible coconut oil was purchased from the local market and the guinea-pig pellet diet was from Vet Care, Bangalore, India. Commercial enzyme assay kits (Randox, Germany) were used for the determination of serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C). Other chemicals and reagents used in these experiments were of analytical grade and were purchased from reputed commercial sources.

Animals

Male Hartley guinea-pigs (10 months old, 750 g) were obtained from King's Institute of Preventive Medicine, Chennai, India. They were acclimatized to animal house conditions for 15 days and were fed guinea-pig pellet diet with free access to water. The study was performed in accordance with the protocol approved by the Institutional Animal Ethics Committee (ref: IAEC/SRMC and RI/41/2005).

Experimental design

Animals ($n = 36$) were fed 0.2% w/w cholesterol along with the normal pellet diet for 30 days. Six animals were killed and their aorta, coronary artery, heart, liver, gall bladder, lung, kidney and spleen harvested for histopathological evaluation of the onset of atherosclerotic changes. For the subsequent 90 days, the regression period, the remaining animals (except the normal control, which received the normal pellet diet) were given a high-fat diet (HFD) of 0.15% w/w cholesterol dispersed in coconut oil. While one group of six animals served as the positive control (group 2), the others received in addition a daily oral dose of 100 mg/kg IrA (group 3), 100 mg/kg IrH (group 4) and 10 mg/kg atorvastatin calcium (group 5), respectively. The animals were weighed weekly. At the end of the experimental period, they were fasted overnight, killed under ether anaesthesia and blood was collected by cardiac puncture. Blood was processed for serum lipid estimation. The aorta was accessed through the left ventricle and slit open longitudinally. The entire length of the aorta from the base of the aortic arch up to the diaphragmatic hiatus was resected out, washed in ice-cold saline, trimmed of adventitial fat and stored in formal calcium (10% formalin, 1% CaCl₂). The entire anterior descending left coronary artery was quickly identified and dissected out for histopathological examination. Heart and liver were harvested, washed with ice-cold saline, trimmed of adventitial fat, weighed and stored at -80° until needed for analysis.

Assessment of serum lipid levels

Serum TC, TG and HDL-C concentrations were determined by enzymatic methods using a Star 21 Plus semi-auto analyser (Bombay, India). LDL cholesterol (LDL-C) was calculated by Friedwal's formula^[20] and the atherogenic index (AI),^[21] a measure of the risk of atherogenicity, was calculated using the formula: $AI = TC - HDL-C / HDL-C$.

Preparation of tissue fractions for evaluation of oxidative stress biomarkers

Heart and liver tissue was homogenized in ice-cold 10% KCl using a polytron homogenizer, centrifuged at 224g, and the supernatant used for measurement of superoxide dismutase (SOD), glutathione peroxidase (GPX), thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH).

Measurement of TBARS and GSH

The degree of lipid peroxidation in the tissue homogenate was determined by estimating the accumulation of the peroxidative product, TBARS.^[22] Briefly, a mixture of 1 ml of homogenate and 0.8% w/v thiobarbituric acid was heated at 100° for 90 min. The solution was cooled to room temperature, centrifuged at 224g for 10 min, and the absorbance of the supernatant was measured at 532 nm using a Perkin Elmer Lambda 25 UV spectrophotometer (MA, USA). The amount of TBARS is expressed in nmol malondialdehyde/g tissue.

Reduced GSH content was estimated by measuring the absorbance of the complex formed with 5,5'-dithiobis (2-nitrobenzoic acid) reagent (DTNB).^[23] Tissue homogenate (0.25 ml) was treated with an equal volume of ice-cold trichloroacetic acid and centrifuged. Then, 0.25 ml phosphate buffer (0.2 M, pH 8.0) and 0.5 ml DTNB were added to the supernatant, mixed, and the absorbance measured at 412 nm. Values are expressed in $\mu\text{g/g}$ tissue.

Determination of antioxidant enzyme activity

SOD activity was measured by nitroblue tetrazolium reduction.^[24] The superoxide radical generated reduced nitroblue tetrazolium to a blue formazan dye. The colour intensity of the chromogen in butanol after inactivation of the reaction with acetic acid was measured at 560 nm. SOD activity in units was calculated based on % inhibition of absorbance/min. The 50% inhibition/min was taken as 1 unit of enzyme activity. Results are expressed as activity in units/mg protein

GSH consumed in a reaction in the presence of H_2O_2 is proportional to the GPX content. It was estimated^[25] and the results expressed as nmol GSH consumed/min per mg protein. Tissue total protein was estimated as per Lowry *et al.*^[26]

Histopathological analysis of the coronary artery

The formalin-fixed coronary artery was put in paraffin blocks, serially sectioned to 5 μm , processed, stained with haematoxylin and eosin, and examined microscopically for histopathological changes using a Nikon III Eclipse TE 2000 S (Tokyo, Japan).

Assessment of aortic atherosclerotic plaque

The area of atherosclerotic plaque in the aorta was assessed using Oil Red O staining.^[27] Briefly, formal calcium-fixed aorta was rinsed in distilled water and 60% isopropyl alcohol. The tissue was stained for 40 min by soaking in a saturated solution of Oil Red O in isopropyl alcohol. It was mounted in glycerin jelly to attach the stained tissue to the microscope slide for photography. The % luminal surface area covered

by lipid-filled Oil Red O stained deposits was measured using the Image ProPlus Image analysis system software version 6.0.

Statistical analysis

Results are expressed as mean \pm SEM. The data were analysed using one-way analysis of variance followed by Dunnett's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Histopathological assessment of the left coronary artery from animals killed after the initial HFD administration of 30 days discontinuous intima with foam cell appearance, confirming the initiation of atherogenic changes. While kidney, spleen and lung tissue were normal, liver, heart and gall bladder revealed degenerative histopathological changes characteristic of lipaemic assault (results not shown).

Effect of *I. racemosa* extracts on serum lipid levels

Serum lipid profiles of the experimental animals are summarised in Table 1. The HFD brought about a statistically significant rise ($P < 0.001$) in TC, LDL-C and AI compared with the normal control. Lipid profile results of the three treatment groups (3, 4 and 5) are in comparison with the positive control. IrA administration reduced TC, TG ($P < 0.001$), LDL-C ($P < 0.01$) and elevated HDL-C ($P < 0.001$). IrH enhanced HDL-C to remarkable levels (+340%, $P < 0.001$). As a result, LDL-C ($P < 0.01$) and AI ($P < 0.001$) decreased. Atorvastatin calcium brought about a typical hypolipidaemic response with respect to the tested parameters: TC ($P < 0.05$), TG, HDL-C, LDL-C and AI ($P < 0.001$). Statistical post-testing with Dunnett's revealed IrA to be more effective than atorvastatin calcium in reducing TC, while the latter was more effective with respect to other parameters. IrH, however, was more effective in enhancing HDL-C ($P < 0.001$). The results demonstrate the antihyperlipidaemic influence of IrA in dietary lipaemia.

Changes in bodyweight of the experimental animals on a monthly basis during the experimental period are presented in Figure 1. Group 2 recorded a sharp increase after the 30-day induction period. Any subsequent increase, however, was not possibly due to chronic lipaemia-associated emaciation. The percentage increase in bodyweight was least in the IrA treated group 3 followed by group 4, and they differed significantly ($P < 0.01$) compared with the positive control. This result is noteworthy in view of the anti-obesity claims for the drug in traditional medicine.

Effect of *I. racemosa* extracts on liver and heart tissue antioxidant status

Heart and liver tissue antioxidant status is reflective of hyperlipidaemia related to pro-oxidant damage (see Table 2). HFD caused a decrease in GSH ($P < 0.001$) and an elevation in TBARS ($P < 0.01$) in heart and liver tissues. Endogenous antioxidants GPX and SOD were decreased ($P < 0.05$) compared with the normal control in heart. Relative to the positive control, SOD and GPX were elevated ($P < 0.001$) in

Table 1 Serum lipid profile of experimental animals

Treatment	Total cholesterol (mg/dl)	% Change	Triglycerides (mg/dl)	% change	High-density lipoprotein (mg/dl)	% Change	Low-density lipoprotein (mg/dl)	% Change	Atherogenic index	% Change
Group 1 NC	27.67 ± 0.79		66.07 ± 4.12		5.32 ± 1.1		7.23 ± 1.09		3.24 ± 0.42	
Group 2 HFD	91.1 ± 10.33	+229.24	75.59 ± 2.28 (NS)	+14.41	6.35 ± 0.73 (NS)	+19.36	71.6 ± 11.43	+890	14.71 ± 2.56	+254
Group 3 HFD + IrA (100 mg/kg)	56.06 ± 6.73	-38.46	42.22 ± 5.04	-44.15	17.35 ± 4.31	+173.23	35.31 ± 7	-50.68	3.63 ± 1.33	-68.05
Group 4 HFD + IrH (100 mg/kg)	82.59 ± 6.05 (NS)	-9.34	79.15 ± 6.05 (NS)	+4.71	27.92 ± 0.66	+339.68	39.21 ± 5.38	-45.24	1.97 ± 0.24	-86.61
Group 5 HFD + At Ca (10 mg/kg)	65.52 ± 6.4	-28.08	31.28 ± 6.4	-58.62	27.35 ± 1.21	+330.71	31.75 ± 15.65	-55.66	1.58 ± 0.87	-89.26
One way analysis of variance	<i>F</i> 13.365 df (4,25) <i>P</i> < 0.001		<i>F</i> 14.868 df (4,25) <i>P</i> < 0.001		<i>F</i> 26.777 df (4,25) <i>P</i> < 0.001		<i>F</i> 10.54 df (4,25) <i>P</i> < 0.001		<i>F</i> 13.435 df (4,25) <i>P</i> < 0.001	

NC, vehicle-treated normal control group on normal pellet diet; HFD, high-fat diet; HFD + IrA, high-fat diet + alcohol extract of *Inula racemosa*; HFD + IrH, high-fat diet + hexane extract of *I. racemosa*; HFD + At Ca, high-fat diet + atorvastatin calcium. Values are mean ± SEM, *n* = 6 animals in each group. Group 2 compared with group 1. Groups 3, 4 and 5 compared with group 2. NS, not significant.

Table 2 Levels of oxidative stress markers in experimental animals

Treatment	Liver				Heart			
	SOD	GPX	TBARS	GSH	SOD	GPX	TBARS	GSH
Group 1 NC	21.7 ± 1.98	37.03 ± 2.67	11.39 ± 0.618	1.62 ± 0.2317	30.3 ± 2.07	57.17 ± 2.42	6.17 ± 0.174	1.698 ± 0.096
Group 2 HFD	18.37 ± 2.05 (NS)	29.22 ± 3.93 (NS)	50.44 ± 7.28	0.648 ± 0.01	22.53 ± 1.155	35.06 ± 4.74	8.06 ± 0.8	1.328 ± 0.137
Group 3 HFD + IrA (100 mg/kg)	16.76 ± 1.41 (NS)	21.6 ± 2.03 (NS)	21.9 ± 2.3	0.99 ± 0.1	52.71 ± 3.95	144.35 ± 13.45	11.95 ± 1.21 (NS)	1.262 ± 0.148 (NS)
Group 4 HFD + IrH (100 mg/kg)	10.77 ± 0.57	28.81 ± 3.79 (NS)	18.61 ± 3.66	0.69 ± 0.1 (NS)	19.44 ± 1.18 (NS)	59.16 ± 1.7	13.62 ± 1.15 (NS)	1.173 ± 0.08 (NS)
Group 5 HFD + At Ca (10 mg/kg)	14.47 ± 1.07 (NS)	22.76 ± 3.33 (NS)	10.42 ± 0.21	1.26 ± 0.166	31.86 ± 2	21.22 ± 0.79 (NS)	21.37 ± 4.76	1.133 ± 0.102 (NS)
One way analysis of variance	<i>F</i> 7.27 df (4,25) <i>P</i> < 0.001	<i>F</i> 3.64 df (4,25) <i>P</i> < 0.02	<i>F</i> 18.44 df (4,25) <i>P</i> < 0.001	<i>F</i> 8.22 df (4,25) <i>P</i> < 0.001	<i>F</i> 31.66 df (4,25) <i>P</i> < 0.001	<i>F</i> 53.98 df (4,25) <i>P</i> < 0.001	<i>F</i> 6.68 df (4,25) <i>P</i> < 0.001	<i>F</i> 3.82 df (4,25) <i>P</i> < 0.01

SOD, superoxide dismutase (units/mg protein); GPX, glutathione peroxidase (nmol GSH consumed/min per mg protein); TBARS, thiobarbituric acid reactive substances (nmol malondialdehyde/g tissue); GSH, reduced glutathione ($\mu\text{g/g}$ tissue); NC, vehicle-treated normal control group on normal pellet diet; HFD, high-fat diet; HFD + IrA, high-fat diet + alcohol extract of *Inula racemosa*; HFD + IrH, high-fat diet + hexane extract of *I. racemosa*; HFD + At Ca, high-fat diet + atorvastatin calcium. Values are mean ± SEM, *n* = 6 animals in each group. Group 2 compared with group 1. Groups 3, 4 and 5 compared with group 2. NS, not significant.

the heart tissue in IrA-treated group 3. In this group, tissue TBARS decreased ($P < 0.001$) and GSH content increased ($P < 0.001$) in liver. IrH administration returned GPX ($P < 0.02$) to normal control values in heart tissue, while in liver SOD was elevated ($P < 0.01$) and TBARS reduced ($P < 0.001$). Atorvastatin administration caused a decrease in

TBARS ($P < 0.001$) in both heart and liver, while GSH was increased in liver and SOD was decreased in heart ($P < 0.01$). Results suggest a beneficial alteration of redox status of the tested tissues during HFD administration by IrA and IrH.

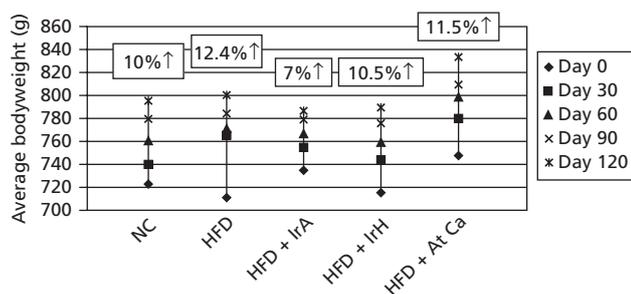


Figure 1 Bodyweight increase of experimental animals. NC, vehicle-treated normal control group on normal pellet diet; HFD, high-fat diet; HFD + IrA, high-fat diet + alcohol extract of *Inula racemosa*; HFD + IrH, high-fat diet + hexane extract of *I. racemosa*; HFD + At Ca, high-fat diet + atorvastatin calcium.

Effect of *I. racemosa* extracts on coronary artery histopathology

Representative photographs of histopathological sections of the coronary artery are presented in Figure 2. Normal coronary artery had an intact intima as seen in Figure 2. The HFD-treated group 2 (Figure 2) shows discontinuous endothelium with fatty changes in the surrounding cardiac tissue. Apart from intracellular lipid, extensive aggregates of foam cells are seen in the media (Figure 2). These have completely replaced its muscular pattern, typical of primary medial destruction in early atherosclerosis.^[28] IrA and IrH treatment appears to have reversed these changes. Sections from these groups in Figure 2 show a normal continuous endothelium. Also, the cardiac tissue is devoid of fatty degeneration. Sections of coronary artery from atorvastatin calcium-treated animals exhibit normal coronary histopathology.

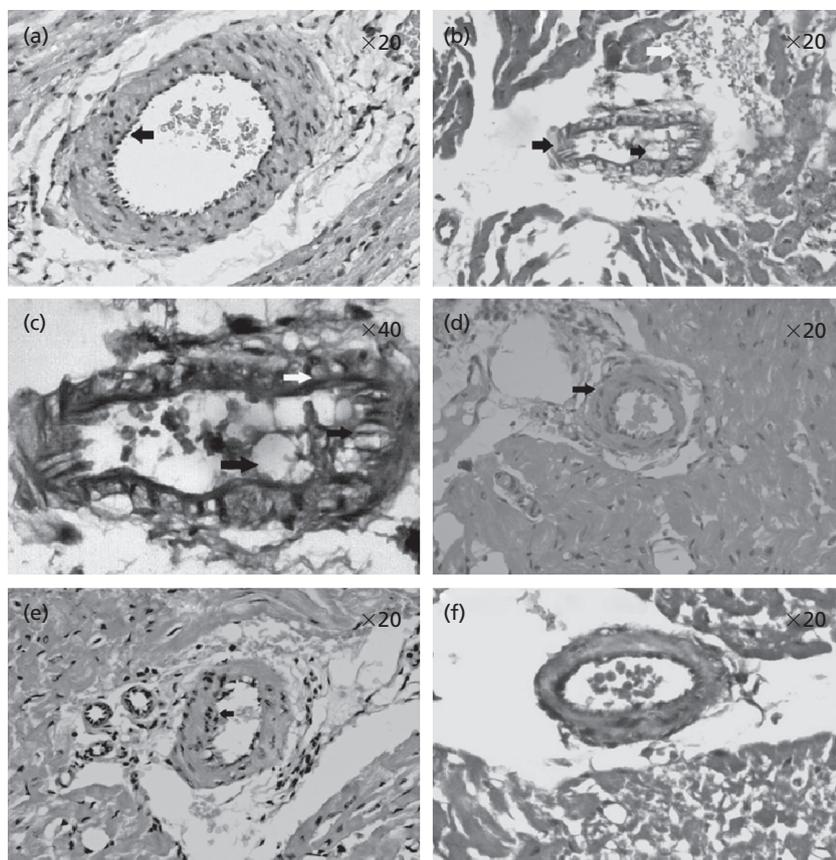


Figure 2 Histopathological examination of coronary artery from experimental animals. (a) Vehicle-treated normal control group on normal pellet diet. (b) Positive control; black arrows show coronary artery with damaged intima and luminal fat; white arrow shows fatty degeneration in cardiac tissue. (c) Positive control group showing discontinuous intima with large foam cells. Note the foam cells in the media (white arrow). Sections treated with the alcohol extract of *Inula racemosa* (d), hexane extract of *I. racemosa* (e) and atorvastatin calcium (f) show normal coronary artery. Foam cells are less evident in both intima and media.

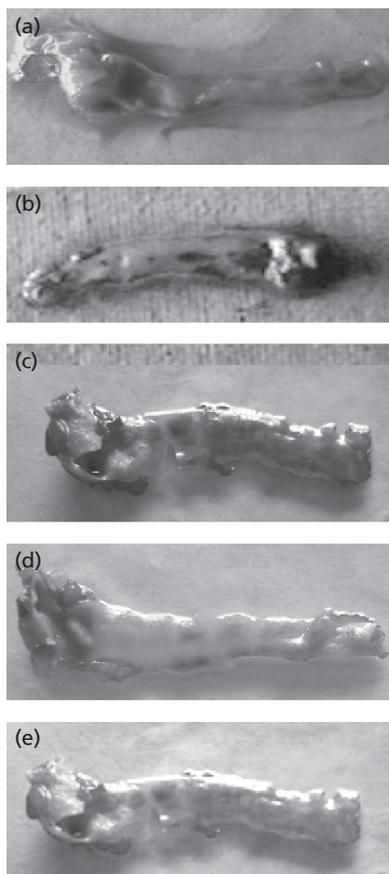


Figure 3 Whole mount en face visualization of Oil Red O stained intimal surface of aorta from experimental animals. (a) Normal control group. (b) Positive control; note marked lipid deposits with their long axes parallel to the longitudinal axes of aorta. Aorta from animals treated with the alcohol extract of *Inula racemosa* (c) and the hexane extract of *I. racemosa* (d) show minimal lipid-laden areas. (e) Atorvastatin calcium-treated group.

En face visualization of aorta

Representative photographs of the Oil Red O stained intimal surface of aorta from experimental animals is shown in Figure 3. The lipid deposits appear brick red with Oil Red O staining (Figure 3b). Aorta from the untreated controls are devoid of plaque areas (Figure 3a). The percentage of lipid-laden areas relative to the total intimal surface is represented in Figure 4. The HFD-treated group shows visible lesions that measured ~15% of the total intimal surface. The percentage lesion area was found to be ~6.5, 10 and 11% for IrA-, IrH- and atorvastatin-treated animals, respectively.

Discussion

Atherosclerosis is an inflammatory disease and atherosclerotic lesions represent different stages in the chronic inflammatory process in the artery.^[29] According to recent versions of the response-to-injury hypothesis of atherosclerosis, endothelial dysfunction and denudation is the primary event leading to atherosclerosis. Possible causes of endothelial dysfunction include, among others, elevated and

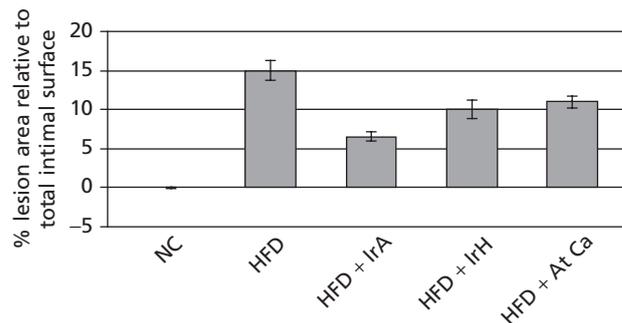


Figure 4 Extent of aortic atherosclerosis in experimental animals. NC, vehicle-treated normal control group on normal pellet diet. HFD, high-fat diet; HFD + IrA, high-fat diet + alcohol extract of *Inula racemosa*; HFD + IrH, high-fat diet + hexane extract of *I. racemosa*; HFD + At Ca, high-fat diet + atorvastatin calcium.

modified LDL and free radicals. LDL modified by oxidation, glycation, aggregation and association with proteoglycans or incorporation into immune complexes^[30] is a major cause of injury to the endothelium and underlying smooth muscle.^[31] In addition to its ability to injure these cells, modified LDL triggers and expands the initial inflammatory response, which is primarily protective, by inducing the removal and sequestration of modified LDL by macrophages, and initiates a series of intracellular events leading to formation of lipid peroxides. This facilitates accumulation of cholesterol esters, resulting in the formation of foam cells. In animals with hypercholesterolaemia, antioxidants are known to reduce the size of lesions^[32] and they reduce fatty streaks in non-human primates.^[33] The latter observation suggests that antioxidants have anti-inflammatory effects.^[34] Antioxidants are known to increase the resistance of human LDL to oxidation *ex vivo*^[35] in proportion to antioxidants of the plasma.

In the HFD-fed group 2, hyperlipidaemia associated oxidative stress triggered lipid peroxidation, marked by elevated TBARS, reduced GSH in both the tested tissues, and reduced levels of endogenous antioxidants SOD and GPX in heart. Peroxidized lipids unable to maintain cellular integrity damage surrounding membrane proteins resulting in cellular damage, as evidenced by coronary intimal and cardiac tissue changes. Pro-oxidant and lipid peroxidation associated proinflammatory changes trigger atherosclerotic changes, as evidenced by the lipid-laden lesion areas in the aorta.

The antihyperlipidaemic influence of IrA, accompanying increase in levels of cell protectants SOD and GPX in heart tissue akin to atorvastatin calcium and reduced TBARS in liver are all indicative of improved antioxidant reserves in these tissues. Subsequent inhibition of lipid peroxidation, and reduced lipid uptake, resulting in a reduction of fatty streak formation, via decreased foam cell formation, are presumably a result of antioxidant, anti-inflammatory changes exerted by IrA. It also modulated the HFD-induced bodyweight increase, suggestive of an anti-obesity effect. Attention is drawn to the phenolic content, as phenols are antioxidant due to the resonance stabilized phenoxyl radicals they form with oxidants.^[36] By inhibiting LDL oxidation, phenolic antioxidants prevent uptake and degradation of oxidized LDL by macrophages.^[37]

IrH on the other hand elevated HDL-C remarkably and there was a 33% reduction in the lesion area in the aorta. We do not have an explanation for this effect. It is to be noted that sesquiterpene lactones alantolactone and isoalantolactone isolated from the hexane fraction are reported to have antioxidant and anti-inflammatory properties. These lactones have structural similarity to enterolactone and other lignans, which as with isoflavones are categorised as phytoestrogens on account of their weak estrogenic activity.^[38] These groups of compounds lower cholesterol and their influence on tyrosine kinases have a role in suppression of cellular processes leading to atherosclerosis.^[39]

Conclusions

Diet-induced hypercholesterolemic atherosclerosis is associated with an increase in oxidative stress. HFD-related bodyweight increase was least in the group treated with the alcohol extract of *I. racemosa*, which also reduced the extent of atherosclerotic changes. The hexane extract elevated plasma HDL-C levels. The high phenolic content of IrA and presence of lactones alantolactone and isoalantolactone in IrH may be contributing to the observed effects by preventing oxidative damage and associated inflammation, by free radical scavenging and possibly other/newer mechanisms involving upregulation of the molecular processes leading to generation of endogenous antioxidants.^[40] Results of this study therefore demonstrate the anti-atherogenic activity of *I. racemosa*, validating traditional medicine claims of its anti-obesity and cardioprotective properties. Work ahead is focused on major compound isolation from the extracts and assessment of their biopotency.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

- Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340: 115–124.
- Ling WH *et al.* Red and black rice decrease atherosclerotic plaque formation and increased antioxidant status in rabbits. *J Nutr* 2001; 131: 1421–1426.
- Mohammed H, Moghadasian D. Experimental atherosclerosis – a historical overview. *Life Sci* 2002; 70: 855–856.
- Williamson EM. Synergy in relation to the pharmacological action of phytomedicinals. In: Evans WC, Daphne TE, eds. *Trease and Evans Pharmacognosy*, 15th edn. London: Harcourt, 2002: 49–54.
- Charak. *Charak Samhita*, 3rd edn. Mumbai: Nirnaya Sagar Press, 1941.
- Arora RK *et al.* Mano (*Inula racemosa*): little known aromatic plant of Lahaul valley, India. *Econ Botany* 1980; 34: 175–180.
- Singh R *et al.* Pushkara-guggulu an anti anginal and hypolipidaemic agent in coronary heart disease. *J Res Ayur Siddha* 1990; 12: 1–18.
- Tripathy YB *et al.* Assessment of adrenergic beta blocking activity of *Inula racemosa*. *J Ethnopharmacol* 1988; 23: 3–9.
- Dwivedi S *et al.* Influence of certain indigenous drugs on prostaglandin E2 like activity in the ischaemic rabbit aorta. *Ind Drugs* 1987; 24: 8–10.
- Tripathy SN *et al.* Beneficial effect of *Inula racemosa* (Pushkarmoola) in angina pectoris: a preliminary report. *Ind J Physiol Pharmacol* 1984; 28: 73–75.
- Gholaps S, Kar A. Effects of *Inula racemosa* and *Gymnema sylvestre* leaf extracts in the regulation of corticosteroid induced diabetes mellitus: involvement of thyroid hormones. *Pharmazie* 2003; 58: 413–415.
- Sekhar AV *et al.* An experimental and clinical evaluation of anti asthmatic potential of Devadaru compound (DC). *Ind J Physiol Pharmacol* 2003; 47: 101–107.
- Rao KS, Mishra SH. Hepatoprotective activity of *Inula racemosa* root. *Fitoterapia* 1997; 68: 510–514.
- NISCAIR. *Wealth of India, Raw Materials*. New Delhi: National Institute of Science Communication and Information Resources, 2002.
- Milman IA. Alanto and isoalantolactones. *Khimiya Prirodnykh Soedinenii* 1990; 3: 307–320.
- Hall IH *et al.* Mode of action of sesquiterpene lactones as anti inflammatory agents. *J Pharm Sci* 1980; 69: 537–543.
- Rath M, Pauling L. Immunological evidence for the accumulation of lipoprotein (a) in the atherosclerotic lesion of the hypoascorbic guinea pig. *Proc Natl Acad Sci USA* 1990; 87: 9388–9390.
- Fernandez ML. Guinea pigs as models for cholesterol and lipoprotein metabolism. *J Nutr* 2001; 131: 10–20.
- Slinkard K, Singleton VL. Total phenol analysis: automaton and comparison with manual methods. *Am J Enol Vitic* 1977; 28: 49.
- Freidwal WT *et al.* Estimation of the concentration of low density lipoprotein-C without the use of ultracentrifuge. *Clin Chem* 1992; 18: 499–502.
- Yoon NY *et al.* Anti-hyperlipidaemic effect of an edible brown algae, *Ecklonia stolonifera*, and its constituents on poloxamer 407-induced hyperlipidaemic and cholesterol fed rabbits. *Arch Pharm Res* 2008; 31: 1564–1571.
- Okhawa H *et al.* Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351–358.
- Moran MS *et al.* Levels of glutathione reductase and glutathione S transferase activities in rats' lung and liver. *Biochim Biophys Acta* 1979; 582: 67–78.
- Poonam K *et al.* A modified spectrophotometric assay of super oxide dismutase. *Ind J Biochem Biophys* 1984; 21: 130–132.
- Rotruck JT *et al.* Selenium: biochemical roles as a component of glutathione peroxidase. *Science* 1973; 179: 588–590.
- Lowry OH *et al.* Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
- Efendy JL *et al.* The effect of aged garlic extract 'kyolic' on the development of experimental atherosclerosis. *Atherosclerosis* 1997; 132: 37–42.
- Malinow MW *et al.* Effect of alfalfa meal on shrinkage of atherosclerotic plaque during cholesterol feeding in monkeys. *Atherosclerosis* 1978; 30: 27–43.
- Russel R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340: 115–125.
- Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997; 272: 20 963–20 966.
- Morel DW *et al.* Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. *J Lipid Res* 1983; 24: 1070–1076.

32. Navab M *et al.* The yin and yang of oxidation in the development of the fatty streak: a review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol* 1996; 16: 831–842.
33. Chang MY *et al.* Inhibition of hypercholesterolemia induced atherosclerosis in the non human primate by probucol II. Cellular composition and proliferation. *Arterioscler Thromb Vasc Biol* 1995; 15: 1631–1640.
34. Fruebis J *et al.* Effect of probucol treatment on gene expression of VCAM- 1, MCP-1 and M-CSF in the aortic wall of LDL receptor deficient rabbits during early atherogenesis. *Arterioscler Thromb Vasc Biol* 1997; 17: 1289–1302.
35. Reaven PD *et al.* Effect of dietary antioxidant combinations in humans: protection of LDL by vitamin E but not by beta carotene. *Arterioscler Thromb Vasc Biol* 1993; 13: 590–600.
36. Baum BO, Perun AL. Anti oxidant efficiency versus structure. *Soc Plast Eng Trans* 1962; 2: 250–257.
37. Mangiapane H *et al.* The inhibition of the oxidation of low density lipoprotein by (+) catechin – a naturally occurring flavonoid. *Biochem Pharmacol* 1992; 43: 445–450.
38. Setchell KDR *et al.* Lignan formation in man. *Lancet* 1981; i: 4–7.
39. Hodgson JM *et al.* Soybean isoflavonoids and their metabolic products inhibit *in vitro* lipoprotein metabolism. *J Nutr Biochem* 1996; 7: 664–669.
40. Arnal JF *et al.* Ethinyl estradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting super oxide anion production. *Proc Natl Acad Sci USA* 1996; 93: 4108–4113.