African Journal of Pharmacy and Pharmacology

Volume 9 Number 47, 22 December, 2015
ISSN 1996-0816

Academic Journals

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African Journal of Pharmacy and Pharmacology

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Vol. 9(47), pp. 1080-1087, 22 December, 2015
DOI: 10.5897/AJPP2015.4447
Article Number: F98A40756767
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Hypolipidemic effect of ethanolic extract from whole plant of *Lactuca runcinata* (DC.) in atherogenic diet induced hyperlipidemic rats

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Received 18 September, 2015; Accepted 5 November, 2015

The present study was designed to investigate the hypolipidemic effect of ethanolic extract from whole plant of *Lactuca runcinata* (DC.) in rats fed with atherogenic diet (AD). The acute toxicity study shows that the ethanolic extract are safe up to 2000 mg/kg, thus one tenth of this dose was consider as evaluation dose. Ethanolic extract of *Lactuca runcinata* was administered in doses of 200 and 400 mg/kg/day to rats fed with atherogenic diet to assess its possible lipid-lowering potential. There was a recognize increment in the body weight in AD fed group ($p<0.001$), which was reduced by the administration of ethanolic extract of *L. runcinata* (400 mg/kg). The elevated levels of total cholesterol, triglycerides, phospholipids, low-density lipoprotein (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) all along with decrease in plasma HDL-C were observed in group II rats fed with atherogenic diet. After treatment of ethanolic extract of *L. runcinata,* (400 mg/kg/day) the result showed a significant ($p<0.001$) decrement in body weight, plasma and tissue total cholesterol, triglycerides, phospholipids, plasma LDL-C and VLDL-C although with an increase in plasma HDL-C when compared to group II AD rats. The ethanolic extract of *L. runcinata* could protect against atherosclerosis and decrease the atherogenic index and cardiac risk ratio. This finding provides some biochemical basis for the use of ethanolic extract of whole plant of *L. runcinata* as hypolipidemic agent having preventive and therapeutic effect against hyperlipidemia.

Key words: Atherogenic diet, hypolipidemia, *Lactuca runcinata,* wistar rats.

INTRODUCTION

Hyperlipidemia is a known risk factor for the advancement of cardiovascular diseases including atherosclerosis. The real risk components for the advancement of atherosclerosis are hypercholesterolemia and raised levels of low-density lipoprotein cholesterol (LDL-C). Moreover, free-radical-mediated peroxidative modification of polyunsaturated fatty acids of LDL and very low-density lipoprotein (VLDL) is thought to add to the development of atherosclerotic injuries. Oxidative anxiety is an early occasion in the advancement of hyperlipidemia, and it has been proposed that proper support for improving antioxidant supply in subjects with anomalous elevated lipid levels can lessen the course of the disease (Yang et al., 2008).

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Clinical trials have demonstrated that treatment of more seasoned high-risk subjects with lipid-lowering medications can lessen cardiovascular grimness and mortality (Aronow, 2008). The investigation for new agents fit for diminishing serum lipid levels has thusly turn into a vital examination center.

Plant items are by and large thought to be less harmful and less inclined to reactions than medications made by synthetic compounds. The potential therapeutic and preventive benefits of plant-based drugs have been the subject of broad studies, and numerous plant constituents have been revealed with significant pharmacologic activity (Kumari and Augusti, 2007; Son et al., 2007) incorporating agents with antiglycemic, hypolipidemic and antihypertensive properties (*Lactuca runcinata* DC; Synonyms, *Lactuca heyneana* DC.. Family: *Compositae; Asteraceae*). This occurs in many parts of India, as a common weed. It is considered as a valuable medicinal herb in traditional systems of medicine in India (Action diuretic, slightly aperients). It is used as a diuretic in calculous affections, also for chronic obstruction of liver and bowels (Khare, 2008). A smaller variance found in western Uttar Pradesh, Rajasthan, Saurashtra and the Deccan Penninsula, is equated with *Lactuca remotiflora* DC. However, the plant is reported to possess the activities like antibacterial activity in methanolic extract (Lakshmi et al., 2013), and *in vitro* cytotoxic activity in methanolic extract (Lakshmi et al., 2014). Literature survey revealed that there is no earlier scientific reports regarding hypolipidemic activity of this plant. Therefore, objective of the present investigation was to study the effect of ethanolic extract of whole plant of *Lactuca runcinata* (DC.) on hyperlipidemia elicited by atherogenic diet in rats.

MATERIALS AND METHODS

Plant materials

The fresh whole plants of *L. runcinata* DC were collected from the natural habitats of Kayathar, Thoothukkudi district, Tamil Nadu, India. Taxonomic distinguishing proof was produced using Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Chemicals

Atorvastatin free sample was received from Ranbaxy Laboratories Limited, Gurgaon, India. All other chemicals and solvents were analytical grade used in the experiments were purchased locally (Merck or SD fine Chemicals).

Preparation of extract

The earlier mentioned powdered materials were successively extracted with ethanol (40 to 60°C) by hot continuous percolation method in Soxhlet apparatus (Harborne, 1984) for 24 h. Then the extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animals

Thirty adult male wistar rats, weighing around 150 to 180 g were obtained from Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 animals per cage, with relative humidity (55%) in a 12 h light/dark cycle at 25°±2°C. They were offered access to water and a commercial diet *ad libitum*. The experiments were completed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and sanction by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/ CPCSEA/1083).

Animal diet

The compositions of the two diets were used as follows (Kottai et al., 2005):

Control diet: Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, saltblend with starch 4% and vitamin and choline blend 0.5%.

Atherogenic diet: Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, saltblend with starch 4% and vitamin & choline blend 0.5%, cholesterol 0.4%.

Acute toxicity studies

Oral acute toxicity studies were carried out with male wistar rats weighing 150 to 180 g as per (OECD) draft guidelines rules 423 adopted on 17 December, 2001 gotten from Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA). The rats were fed with ethanolic extract of *L. runcinata* suspended in 1% gum acacia at the dose of 2000 mg/kg body weight. The animals were observed independently at regular intervals subsequent to dosing the initial 24 h and from that point every day for an aggregate of 14 days. The time at which indications of toxicity appear and disappear was observed methodically and recorded for every animal.

Experimental design

A total number of 30 rats were divided into five groups of six rats each:

Group I: Standard chow pellet (Control).
Group II: Atherogenic diet (AD).
Group III: AD + Ethanolic extract of *L. runcinata* (200 mg/kg body weight)
Group IV: AD + Ethanolic extract of *L. runcinata* (400 mg/kg body weight)
Group V: AD + Standard drug atorvastatin (1.2 mg/kg body weight)

The ethanolic extract and atorvastatin were suspended in 2% tween 80 (Waynforth, 1980) individually and fed to the relevant rats by oral intubation. In the ethanolic extract at the dosage level of 200 and

Table 1. Body weight variations in control and experimental wistar rats.

Groups	Initial weight (gm)	Final weight (gm)	Average body weight gain (gm)
Group I	137.91±1.74^{bNS}	173.06±2.38 ^{b*}	35.26±4.39 ^{b*}
Group II	135.84±2.53^{aNS}	253.14±8.58^{a*}	117.44±6.05^{a*}
Group III	148.72±1.48^{aNS,bNS}	233.40±4.38$^{aNS, b*}$	84.83±5.32$^{aNS, b*}$
Group IV	152.33±2.84^{aNS,bNS}	200.30±3.74^{aNS,b*}	48.08±3.69$^{aNS, b*}$
Group V	76.83±2.29^{aNS,bNS}	217.47±3.78$^{aNS, b*}$	40.74±3.37$^{aNS, b*}$

Values are expressed as mean ± SE (n=6 rats) *P* values: *<0.001, **<0.05. NS: Non significant, a→group I compared with groups II, III, IV, V;b→group II compared with groups III, IV, V. Group I: Standard chow pellet (Control); Group II: Atherogenic diet (AD); Group III: AD + Ethanolic extract of *Lactuca runcinata* (200mg/kg body weight); Group IV: AD + Ethanolic extract of *Lactuca runcinata* (400mg/kg body weight); Group V: AD + Standard drug atorvastatin (1.2 mg/kg body weight).

400 mg/kg were fixed as per the Organisation for Economic Co-operation and Development (OECD) guidelines. At the end of 9 weeks, after overnight fasting all the rats were sacrificed by cervical dislocation. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anesthesia and blood sample withdrawn in heparinised tubes and plasma was separated. Liver, heart and aorta were cleared of adhering fat, estimated accurately and utilized for the preparation of homogenate. Animals were given enough care as per the Animal Ethical Committee's recommendations.

Biochemical analysis

Plasma samples were estimated for total cholesterol, HDL-cholesterol and triglycerides utilizing Boehringer Mannheim kits by Erba Smart Lab analyzer United States of America. Friedwald method (Freidewald et al., 1972) was used to determine the LDL-cholesterol and VLDL-cholesterol. Ester cholesterol (Sperry and Webb, 1950) and free cholesterol (Sperry and Webb, 1950) were estimated by utilizing digitonin. Segments of liver tissues, heart tissues and aorta tissues were blotted, measured and homogenized with 3 volumes of methanol and the lipid extracts were gotten by the method (Folch et al., 1957). Extract was utilized for the estimation of ester cholesterol and free cholesterol, triglycerides (Foster and Dunn, 1973), and phospholipids (Zilversmit and Davis, 1950). Free fatty acids were estimated by using method (Falholt et al., 1973). Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to access the atherogenic risk (Li et al., 2013), cardiac risk ratio (Huang et al., 2013) and Atherogenic coefficient (Mizuno et al., 2012).

Statistical analysis

The results were expressed as mean ± standard deviation of 6 rats in each group. The statistical significance between the groups was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

RESULTS

From the acute toxicity, it was found that the ethanolic extracts are safe up to 2000 mg/kg thus one tenth of this dosage (200mg/kg) was considered as the assessment dose. Table 1 shows the body weight of group II rats increased significantly (p<0.001) in comparison with normal control group I rats. The increment in the weight was reduced considerably (p<0.001) by the administration of ethanolic extract of *L. runcinata* (400 mg/kg), and standard drug atorvastatin 1.2mg/kg in comparison with the group II AD fed rats. The average food intake per rat per day was found to be 20.5±1.0 g. Food intake was the same in all the AD rats.

Table 2 reveals that there was a significant increase (p<0.001) in the level of plasma total cholesterol, ester cholesterol, free cholesterol, free fatty acid, phospholipids and triglycerides in the group II rats fed with AD in comparison with the normal untreated control rats (Group I). Treatment with ethanolic extract of *L. runcinata* at the dose 200 mg/kg body weight was found significantly reduced (p<0.001) in the level of plasma total cholesterol, ester cholesterol, free cholesterol, free fatty acid phospholipids and triglycerides in comparison with AD rats (group II). However, group IV (ethanolic extract of *Lactuca runcinata* with AD) showed that the plasma total cholesterol, ester cholesterol, free cholesterol, free fatty acid phospholipids and triglycerides level was restored to near normal as that of group V (atorvastatin 1.2mg/kg with AD).

Table 3 demonstrates in the AD group a significant increase in the value of atherogenic index of 4.39± 0.04 (p<0.001), while the group receiving ethanolic extract of *L. runcinata* along with atherogenic diet, showed a significant decrease in atherogenic index 1.86±0.03 (p<0.001), comparable to the normal control group 1.97±0.02 (p<0.001). Table 4 reveals the reduction in the HDL produced by the group of animals fed with AD was significant (p<0.001) in comparison with group I animals. However, the treatment with ethanolic extract of *L. runcinata* at the dose of 400 mg/kg considerably increased the HDL-cholesterol level when compared to group II atherogenic diet rats. The increased levels of LDL and VLDL-cholesterol in group II rats fed with atherogenic diet was significant (p<0.001) in comparison with group I control rats. Treating with ethanolic extract of *L. runcinata* (Group IV) showed noticeably reduction in the level of plasma LDL-cholesterol and VLDL-cholesterol when compared to group II AD rats.In comparison of the two dose of extract group (Group III and IV) with group II AD rats, the ethanolic extract of *L. Runcinata* at the

Table 2. Effect on plasma lipid profile by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Total cholesterol (TC) (mg/dl)	Free cholesterol (FC) (mg/dl)	Ester cholesterol (EC) mg/dl)	Free fatty acid (FFA) (mg/dl)	Phospholipids (PL) (mg/dl)	Triglycerids (TG) (mg/dl)
Group I	117.32±0.83^{b*}	26.54±0.18^{b*}	90.72±1.51^{b*}	40.60±0.27^{b*}	100.70±0.12^{b*}	70.88±0.95 ^{b*}
Group II	175.90±2.59^{a*}	45.02±0.18^{a*}	130.92±0.92^{a*}	59.66±0.13^{a*}	141.44±0.26^{a*}	111.44±0.67^{a*}
Group III	127.62±0.31$^{a**, b*}$	39.72±.0.23$^{a*, b**}$	87.49±0.51$^{a*, b*}$	48.18±0.22$^{a**, b*}$	121.23±0.35$^{a*, b**}$	88.10±0.47 $^{a*, b*}$
Group IV	107.12±2.05$^{a*, b*}$	29.18±0.23$^{a*, b*}$	78.08±1.43$^{a*, b*}$	41.15±0.26$^{a*, b*}$	108.89±0.35$^{a*, b*}$	69.28±0.30$^{a*, b*}$
Group V	102.55±2.58$^{a*, b*}$	26.10±0.21$^{a*, b*}$	76.46±1.21$^{a*, b*}$	39.48±0.16$^{a*, b*}$	99.62±0.15$^{a*, b*}$	57.46±0.32$^{a*, b*}$

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

Table 3. Effect on plasma lipid profile by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Atherogenic index	Cardiac risk ratio	Atherogenic coefficient
Group I	1.97± 0.02^{b*}	1.98± 0.02^{b*}	0.98±0.02^{b*}
Group II	4.39± 0.04^{a*}	4.38±0.56^{a*}	3.38± 0.04^{a*}
Group III	2.26± 0.05$^{a*, b*}$	2.79±0.36$^{a*, b**}$	1.79±0.02$^{a*, b*}$
Group IV	1.86± 0.03$^{a*, b*}$	1.90±0.03$^{a*, b*}$	0.90±0.02$^{a*, b*}$
Group V	1.75± 0.02$^{a*, b*}$	1.76±0.03$^{a*, b*}$	0.76±0.02$^{a*, b*}$

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

dosage of 400 mg/kg was revealed noteworthy reduction on both LDL-cholesterol and VLDL-cholesterol.

The atherogenic diet rats caused significant (p<0.001) increase in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. A significant (p<0.001) increase in the ratios of total cholesterol: HDL-cholesterol and LDL- cholesterol: HDL-cholesterol in the group II rats fed with AD in comparison with normal group I untreated rats. Administration of ethanolic extract of *L. runcinata* along with AD significantly reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol when compared to AD group (II).

Tables 5 and 6 shows the considerable (p<0.001) raise in levels of both free cholesterol and ester cholesterol, this were also observed in tissue of group II rats fed atherogenic diet when compared to group I control rats. On treating the atherogenic diet rats with ethanolic extract of *L. runcinata* at the dosage of 400 mg/kg, both the tissues cholesterol like ester and free cholesterol reduced remarkably.

Table 7 demonstrates the concentration of tissue triglyceride was elevated in rats fed with group II atherogenic diet rats as compared to group I control rats. The tissue triglyceride levels were considerably decreased in rats treated with ethanolic extracts of *Lactuca runcinata* at the dosage of 200 and 400 mg/kg and along standard drug atorvastatin all along with AD when compared with group II rats fed with atherogenic diet. Administration of ethanolic extract of *Lactuca runcinata* significantly reduced the triglyceride.

Table 8 illustrates the concentration tissue phospholipids which were significantly increased in group II rats fed with AD as compared to group I control animals. Treating with ethanolic extract of *L. runcinata* along with AD showed reduced the phospholipids levels when compared to group II AD fed rats.

Administration of ethanolic extract of *L. runcinata* significantly (p<0.001) reduced the phospholipids level.

Table 4. Effect on plasma lipoprotein by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	VLDL cholesterol (mg/dL)	LDL- c/HDL-c ratio	HDL-c/ TC ratio
Group I	59.23±0.36^{b*}	43.91±0.45^{b*}	14.18±0.15^{b*}	0.74±0.02^{b*}	0.50±0.03^{b*}
Group II	40.12±0.45^{a*}	113.06±0.23^{a*}	22.72±0.17^{a*}	2.82±0.09^{a*}	0.23±0.01^{a*}
Group III	45.68±0.38$^{a*, b*}$	64.31±0.25$^{a**, b*}$	17.63±0.11$^{a*, b*}$	1.41±0.03$^{a*, b**}$	0.36±0.01$^{a*, b*}$
Group IV	56.26±0.37$^{a*, b*}$	37.04± 0.36$^{a*, b*}$	13.82± 0.08$^{a*, b*}$	0.66±0.02$^{a*, b*}$	0.52±0.03$^{a*, b*}$
Group V	58.11±0.35$^{a*, b*}$	32.95± 0.36$^{a*, b*}$	11.49±0.06$^{a*, b*}$	0.57±0.01$^{a*, b*}$	0.57±0.01$^{a*, b*}$

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

Table 5. Effect on tissues ester cholesterol profile by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Ester cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	1.90±0.05^{b*}	2.69±0.08^{b*}	1.98±0.06^{b*}
Group II	3.23±0.15^{a*}	7.02±0.11^{a*}	6.84±0.28^{a*}
Group III	2.26±0.10 ^{a*,b*}	3.42±0.10$^{a *,b*}$	3.50±0.09$^{aNS *,b*}$
Group IV	2.02±0.04 ^{a*,b*}	3.10±0.07 ^{a*,b*}	2.98±0.10 ^{a*,b*}
Group V	1.94±0.07$^{a*, b*}$	2.94±0.05 $^{a*, b*}$	2.82±0.15 ^{a*,b*}

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

Table 6. Effect on tissues free cholesterol profile by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	0.82±0.03^{b*}	0.72±0.03^{b*}	0.45±0.01^{b*}
Group II	1.30±0.07^{a*}	1.05±0.07^{a**}	2.37±0.10^{a*}
Group III	1.08±0.07 ^{a*,b**}	0.89±0.03 ^{a**,b*}	1.02±0.07 ^{a*,b*}
Group IV	0.95±0.04 ^{a*,b*}	0.69±0.02 ^{a*,b*}	0.75±0.03 ^{a*,b*}
Group V	0.86±0.04 ^{a*,b*}	0.63±0.03^{a*,b*}	0.63±0.03 ^{a*,b*}

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

DISCUSSION

The reduction of the plasma lipid and lipoprotein profile was due to the presence of total phenolic and flavonoids compounds. Atorvastatin has more side effects like headache, hoarseness, lower back or side pain, loss of appetite, heartburn and indigestion etc. So the study has selected herbal extract for medicine without any side effect compared with synthetic drugs. These days, hyperlipidemia, particularly hypercholesterolemia is related with a danger for the occurrence of coronary heart disease and fatty liver (Yadav et al., 2012). Numerous new synthetic oral antihyperlipidemic medications, for example, fibrates and bile acid sequestrants are accessible yet they have many adverse reactions, for example, myopathy, increment in hepatic amino transferases and rhabdomyolysis condition (Porez et al., 2012; Saito, 2012). Recently, numerous research work have concentrated on the therapeutic capability of plant constituents for treating numerous vital common diseases, particularly obesity and its complications (Heidarian et al., 2011a; Andersen et al., 2010; Heidarian et al., 2011b).

Plasma lipid profiles are elevated in the group receiving

Table 7. Effect on tissues triglyceride level by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Triglyceride (mg/g tissue)		
	Liver	Heart	Aorta
Group I	8.34±0.01^{b*}	10.98±0.01^{b*}	10.52±0.03^{b*}
Group II	29.14±0.19^{a*}	49.10±0.16^{a*}	22.86±0.28^{a*}
Group III	22.02±0.10 $^{a**, b*}$	29.98±0.12 ^{a*,b**}	18.08±0.12^{b*}
Group IV	14.08±0.09^{a*,b*}	22.72±0.16 ^{a*,b*}	14.18±0.09 ^{a*,b*}
Group V	13.02±0.27^{a*,b*}	21.90±0.13 ^{a*,b*}	13.48±0.09 ^{a*,b*}

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

Table 8. Effect on tissues phospholipids level by using ethanolic extract of *L. runcinata* in control and experimental wistar rats.

Groups	Phospholipids (mg/g tissue)		
	Liver	Heart	Aorta
Group I	19.52±0.15^{b*}	23.52 ±0.07^{b*}	9.72±0.05^{b*}
Group II	28.92±0.09^{a*}	36.78±0.12^{a*}	16.78± 0.09^{a*}
Group III	21.60±0.16^{a*,b**}	32.56±0.23^{b*}	12.88±0.12$^{a**, b*}$
Group IV	18.86±0.17^{a*,b*}	26.26±0.18^{a*,b*}	11.26± 0.10^{a*,b*}
Group V	19.35± 0.05 $^{a**, b*}$	27.57±0.15^{a*,b*}	11.61± 0.12$^{a*, b*}$

Values are expressed as mean±SE (n=6 rats), *P* values:* < 0.001,**< 0.05,NS: Non significant, a→group I compared with groups II, III, IV, V; b→group II compared with groups III, IV, V.

atherogenic diet; earlier studies reveal significant elevation of lipid parameters in plasma and tissue response to atherogenic diet or high fat diet (Prasad, 2005; Vijaimohan et al., 2006; Mehta et al., 2003). The reduction in the HDL produced by the group of animals fed with HFD, is highly significant in that low HDL-cholesterol is now considered as the most significant risk factor for atherosclerosis (Gordon and Rifkind, 1989; Brewer, 2004). After administration of ethanolic extract of *L. runcinata* showed significantly increased HDL-C concentration. It is well known that increased HDL-cholesterol levels have a protective role in coronary artery disease (Wilson et al., 1988). HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL (Parthasarathy et al., 1990).

The elevated levels of LDL and VLDL-cholesterol in rats fed with HFD, clinical and epidemiological studies have proved that individuals with elevated LDL show an increased risk for cardiovascular diseases (Keevil et al., 2007). Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl rich-VLDL by the liver and intestine (Damacker et al., 1991) and a reduced number as well as rate of cholesterol removal by the hepatic LDL receptors (Goldstein et al., 1983). In this study, the intake of the atherogenic or lipogenic diet led to the increase of the plasma levels of cholesterol, VLDL, LDL in group II (hyperlipidemic animals) while, in groups III and IV (rats treated with crude extract) the plasma levels of cholesterol, VLDL, LDL, significantly decreased compared to groups I and II, respectively. The level of LDL-C and VLDL-C were significantly reduced by administration of ethanolic extract of *L. runcinata* can be helpful to reduce the risk of atherosclerosis, cardiovascular diseases and fatty liver. There is strong evidence from several studies that the extent of reduction in the incidence of CHD is directly related to the magnitude of reduction in LDLc and VLDLc levels (Pekkanen et al., 1990).

The atherogenic diet rats significantly increased in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. A significant increased in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol indicate increased risk of atherosclerosis and coronary heart disease (Ram, 1996). Decline in the ratios of total cholesterol: HDL-cholesterol and LDL- cholesterol: HDL-cholesterol observed in the ethanolic extract of *L. runcinata* treated rats (group IV) might be consequence of higher proportion of HDL-cholesterol which reduced risk by virtue of increased reverse cholesterol transport from peripheral organs to liver (Kinosian et al., 1994; Hermansen et al., 2003).

The ester and free cholesterol levels were significantly increased in AD group (II) in comparison with control rats.

This high cholesterol concentration in blood circulation may injure the endothelial cells lining the large arteries and aorta, and this might be an initial event in the etiology of atherosclerosis (Hennig and Chow, 1998). Treatment with ethanolic extract of *L. runcinata* reduces the level of both ester and free cholesterol. This lipid lowering outcome may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of fecal sterol (Purohit and Vyas, 2006).

The concentration of plasma and tissue triglyceride was elevated in rats fed with atherogenic diet. Recent studies also show that triglycerides are independently related to coronary heart disease (Bainton et al., 1992; El-Hazmi and Warsy, 2001) and most of the anti-hypercholesterolemic drugs do not decrease triglycerides levels, but ethanolic extract of *L. runcinata* lowered it significantly ($p<0.001$) and this effect might be related to increase the endothelium bound lipoprotein lipase which hydrolyses the triglycerides into fatty acids. The concentration of plasma and tissue phospholipids were significantly increased in rats fed with AD, this may be due to decreased phospholipase activity (Mirhadi and Sudarshan Singh, 1991; Whereat and Robinowitz, 1975). The group receiving ethanolic extract of *L. runcinata* significantly ($p<0.001$) reduced the phospholipids. The plant extract may have stimulation of lipoprotein lipase activities resulting in decrease of plasma triglyceride and might increase the uptake of triglyceride from plasma by skeletal muscle and adipose tissues (El-Hazmi and Warsy, 2001).

CONCLUSION

The result of present study revealed that the ethanolic extract of whole plant of *L. runcinata* significantly reduced the plasma lipid and lipoprotein profile, thus reduced the atherogenic index and cardiac risk ratio. It also significantly reduced the tissues free cholesterol, ester cholesterol, triglycerides and phospholipids. This finding provides some biochemical basis for the use of ethanolic extract of whole plant of *L. runcinata* as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. This study plan to lead further studies is required to again more insight into the possible mechanism of action of this medicinal plant.

ACKNOWLEDGEMENT

The authors are grateful to UGC- BSR fellowship from University Grants Commission (UGC), New Delhi, India, for providing financial support for this study.

Conflict of interests

The authors have not declared any conflict of interest

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