

Antihyperlipidemic Activity of *Lens culinaris* Medikus Seeds in Triton WR-1339 Induced Hyperlipidemic Rats

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Abstract

Background: Hyperlipidemia involves abnormally elevated lipid levels. Its management using statins, fibrates, bile acid sequestrants and niacin lead to various adverse effects. *Lens culinaris* (Fabaceae) is a dhal with high nutritional and therapeutic value.

Objective: To evaluate antihyperlipidemic activity of *Lens culinaris* seeds in triton WR-1339 induced hyperlipidemic rats.

Materials and methods: Successive extracts, i.e., acetone (LCAE), methanol (LCME) and aqueous (LCWE) were prepared from *Lens culinaris* Medikus (Fabaceae) seeds. The extracts were evaluated for acute toxicity studies using OECD- 423 guidelines. Their antihyperlipidemic activity was assessed using Triton WR- 1339 induced hyperlipidemia model on Sprague dawley female rats. Animals were divided into 12 groups, i.e., normal, hyperlipidemic (triton), triton + atorvastatin, triton + LCAE, triton + LCME and triton + LCWE groups at a dose of 100, 200, 400 mg/Kg body weight, respectively. Total Cholesterol (TC), Tri Glyceride (TG), High (HDL), Low (LDL) and Very Low Density Lipoprotein (VLDL) levels were assessed after 1, 4, 8 and 24 hours of treatment. Total Phenol (TPC), Flavonoid (TFC) and Saponin content were also determined.

Results: None of the extracts showed toxicity or mortality to animals. Intra-gastric administration of extracts at various dose levels to the rats caused a significant decrease in plasma lipid levels. However, LCME (400 mg/Kg) was found to possess more antihyperlipidemic activity as compared to other extracts. It exhibited a decrease (%) of 57.51, 66.93, 66.95, 111.78 in TC, TG, VLDL, LDL levels, and an increase of 59.46% in HDL levels respectively. TPC (608 mg gallic acid equivalent/g of sample) and TFC (128 mg quercetin equivalent/g of sample) were also found highest in LCAE.

Conclusion: Results suggest that the extracts of *Lens culinaris* contain active phytoconstituents which might be responsible for antihyperlipidemic activity of the seeds.

Keywords: *Lens esculenta*; Lentils; Plasma lipid levels; Sprague dawley female rats

Introduction

Hyperlipidemia is considered as one of the most endemic risk factors associated with atherosclerosis and atherosclerosis-related conditions, such as coronary heart disease, ischemic cerebro-vascular disease and peripheral vascular disease. It is characterized by elevated level of serum Total Cholesterol (TC), Low Density (LDL), and Very Low-Density Lipoprotein (VLDL) and decreased High-Density Lipoprotein (HDL) levels in the bloodstream [1-3]. The pharmacological intervention for the treatment of hyperlipidemia involves the use of statins, fibrates, bile acid sequestrants and niacin. However, they cause various adverse effects such as osteoporosis, constipation, rhabdomyolysis, cardiomyopathy and myopathy, etc. [3,4]. Scientists have reported the role of medicinal plants in controlling as well as alleviating elevated serum lipid levels, which result in reduction of morbidity and mortality associated with it [5].

Pulses are an important component of a balanced diet due to their rich nutritional composition. Besides, they also possess therapeutic potential to prevent as well as treat various diseases [6]. *Lens culinaris* Medikus, (*Lens esculenta* Moench, Masoor and Lentil) is a well-known pulse, reported to have a higher protein content, carbohydrates and calories than other legumes. It is also a good source of dietary fibres, essential minerals such as calcium, iron and vitamin B [7]. The phytochemistry of its seeds has been well explored in many studies [7-9]. However, its pharmacological attributes are untouched. The

antihyperlipidemic activity of lentil seeds has been suggested, but, it is still unevaluated. The present study aimed at the assessment of the antihyperlipidemic activity of *Lens culinaris* seeds in Triton WR-1339 induced hyperlipidemic rats.

Materials and Methods

Plant material

Seeds of *Lens culinaris* Medikus were purchased in the month of February from local market, Yamuna Nagar, Haryana and authenticated by Mr. S. K Srivastava, Taxonomist, Botanical Survey of India, Dehradun, India. The plant has been retained as voucher specimen No. BSI/NRC/330 in Botanical Survey of India, Dehradun, India. The seeds were coarsely powdered and stored in a dry and clean container.

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Received February 08, 2016; **Accepted** February 24, 2016; **Published** March 01, 2016

Citation: Vohra K, Gupta VK, Dureja H, Garg V (2016) Antihyperlipidemic Activity of *Lens culinaris* Medikus Seeds in Triton WR-1339 Induced Hyperlipidemic Rats. J Pharmacogn Nat Prod 2: 117. doi:10.4172/2472-0992.1000117

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Preparation of lentil seeds extracts

The air dried, coarsely powdered *Lens culinaris* seeds were defatted using petroleum ether. The powder was then air dried and successively extracted with acetone (LCAE), methanol (LCME) and water (LCWE) using soxhlet extraction. Each time before extracting with the next solvent, the powdered material was dried in hot air oven below 50°C. Each extract was concentrated by distilling off the solvent under vacuum [10]. The phytochemical analysis of extracts has been reported in our previous paper [7].

Animals

Sprague dawley female rats weighing 180-225 g, bred in the animal house of the Institute (ASBASJSM College of Pharmacy) were caged in a controlled room with a 12 hours dark- light cycle, at room temperature of $22 \pm 02^\circ\text{C}$ and kept on standard pellet diet. The experimental protocol was subjected to the scrutinization of the Institutional Animal Ethical Committee (IAEC) and was duly approved by the same under the Protocol No. ASCB/IAEC/02/10/027 and care of animals was carried out according to the Guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (Regn. No.724/02/a/CPCSEA). The standard organistic canula and syringes were used for drug administration in experimental animals.

Acute toxicity studies

Sprague dawley female rats were subjected to acute toxicity as per OECD- 423 guidelines. The animals were fasted overnight. Subsequently, the animals were grouped and fed orally (by gastric intubation) with the extracts at the four fixed levels, i.e., 5, 50, 300 and 2000 mg/Kg Body Weight (BW). The groups were further observed for 14 days. Individual BWs of animals were determined prior to the administration of test substances and at least weekly thereafter [11,12]. Dose was selected on the basis of acute toxicity studies under OECD-423 Guidelines.

Experimental protocol

Overnight fasted female Sprague dawley rats were divided into twelve groups each consisting of six animals. Initially, all the rats were provided with the normal diet for 10 days. The first group served as control, received normal saline. All the groups received single dose of extracts / triton / atorvastatin. Animals of group II to XII received Triton WR 1339 (dissolved in sterile normal saline) intravenously at a dose of 250 mg/Kg BW. The group II served as hyperlipidemic group, whereas, the group III was the standard group (received Triton WR 1339 followed by atorvastatin at a dose of 2.70 mg/Kg BW). The group IV to VI received Triton WR 1339 followed by LCAE at a dose of 100, 200 and 400 mg/Kg BW, respectively. The group VII to IX received Triton WR 1339 followed by LCME at a dose of 100, 200 and 400 mg/Kg BW, respectively. The group X to XII received Triton WR 1339 followed by LCWE at a dose of 100, 200 and 400 mg/Kg BW, respectively. All the extracts were given orally to the rats [13,14].

Collection of blood

Blood was withdrawn after 1, 4, 8 and 24 hours of treatment with the standard drug or extract using heparinised capillaries from retro orbital sinus of the animals. The serum was obtained after centrifuging the blood for 10 minutes and was collected in clean micro centrifuge tubes [4].

Analysis of serum

The serum was used to estimate the concentration of biochemical parameters using the relevant profile kits and various calculations. The

diagnostic kit used for TC determination was purchased from Cogent Ltd. The kit used for the determination of HDL and TG was purchased from Reckon Ltd. The LDL and VLDL were determined using their respective formula [4,15,16].

Statistical analysis

The results were expressed as Mean \pm S.D. Statistical analysis was carried out by using 2 way ANOVA (Analysis of variance) followed by Bonferroni post-test using Graph pad PRISM software version 5.00 (2010). P values < 0.05 were considered as statistically significant.

Total phenolic content

The total phenolic content of seed extracts of *Lens culinaris* were determined by Folin- Ciocalteu reagent according to the method described by Slinkard and Singleton [17]. The calibration equation of gallic acid was $y = 0.010x + 0.035$ ($R^2 = 0.997$).

Total flavonoid content

The total flavonoid content in the extracts, i.e., LCAE, LCME and LCWE, was determined using Aluminum chloride colorimetric method [18]. The calibration equation of quercetin was $y = 0.015x + 0.002$ ($R^2 = 0.997$). Total flavonoid content in the seeds was also determined using method of Bohm and Kocipai- Abyazan [19].

Total saponin content

The saponin content was determined using the method followed by Obadoni et al. [20]. The powdered plant material weighing 20 g was used for analysis.

Results

Acute toxicity studies

As per acute toxicity studies performed for selection of dose of extracts for pharmacological activity in rats, none of the extracts produced any significant changes in the behavioural or neurological responses up to a dose of 2000 mg/Kg body weight. The extracts did not cause any death of the tested animals. The result obtained from the study indicated that LCAE, LCME and LCWE extracts of *Lens culinaris* Medikus seeds was safe to use in animals even at a dose of 200 mg/Kg per oral ($1/10^{\text{th}}$ of the non-toxic dose).

Experimental protocol

Induction of hyperlipidemia with Triton WR 1339: The change (in folds) in plasma TC, TG, HDL, VLDL and LDL in Triton treated groups after 24 hours from induction in comparison with normal group are reported in Table 1. In comparison with normal group, Triton WR 1339 caused a marked increase in TC, LDL, VLDL and TG levels and a decrease in HDL measured at 1, 4, 8 and 24 hours after induction (Figures 1-5).

Effect of various Lentil seed extracts and atorvastatin on plasma lipid profile in rats: The normal group receiving only vehicle showed normal lipid levels. The administration of various extracts in a dose dependent manner lowered the lipid levels (Table 1). Most of the test extract groups showed significant ($p < 0.05$) decrease in the lipid levels, dose dependently. An improvement in lipid levels was observed after 8 and 24 hours of treatment with various extracts. After 24 hour, LCME (400 mg/Kg) exhibited a decrease in TC, TG, VLDL and LDL by 57.51%, 66.93%, 66.95% and 111.98% respectively as compared to the hyperlipidemic group. The LCAE at 200 mg/Kg of dose showed 44.71% decrease in TC level, but it was less than that shown by LCME (51.52%) and LCWE (50.04%) at 100 mg/Kg (Figure 1). LCME (400

mg/Kg) exhibited a decrease of 66.93% in TG levels (Figure 2). After 24 hours of treatment, the percentage increase in HDL level observed in atorvastatin was 62.94% (Figure 3). The standard drug exhibited the maximum decrease in VLDL level by 18.42%, 47.80%, 71.59% and 75.22% after 1, 4, 8 and 24 hours of treatment (Figure 4). The LCAE at 200 mg/Kg

of dose showed a decrease of 83.18% in LDL levels after 24 hours and it was comparable to the activity shown by LCWE at 100 mg/Kg (Figure 5).

Total phytochemical content: Total phenolic, flavonoid and saponin content in lentil seeds and extracts are exhibited in Table 2.

Extracts	Lipid levels (mg/dL) after 24 hours of treatment				
	TC	TG	HDL	VLDL	LDL
Normal	52.11 ± 2.82 ^a	50.13 ± 3.69 ^a	35.76 ± 4.08 ^a	10.02 ± 0.73 ^a	6.28 ± 5.24 ^a
Triton	127.25 ± 4.45 ^{ab} (2.44)	162.67 ± 21.08 ^{ab} (3.24)	21.08 ± 3.11 ^{ab} (0.58)	32.53 ± 4.21 ^{ab} (3.24)	73.63 ± 8.7 ^{ab} (11.72)
Std	38.98 ± 3.93 ^{ab} (69.36)	40.32 ± 4.71 ^{ab} (75.21)	56.89 ± 3.35 ^{ab} (62.94)	8.06 ± 0.94 ^{ab} (75.22)	-25.97 ± 4.3 ^{ab} (135.27)
LCAE 100	78.29 ± 3.56 ^{ab} (38.47)	83.37 ± 3.10 ^{ab} (48.74)	38.46 ± 2.62 ^{ab} (45.18)	16.67 ± 0.60 ^{ab} (48.75)	23.16 ± 4.73 ^{ab} (68.54)
LCAE 200	70.35 ± 3.62 ^{ab} (44.71)	75.72 ± 2.70 ^{ab} (53.45)	42.81 ± 2.17 ^{ab} (50.75)	15.14 ± 0.54 ^{ab} (53.45)	12.38 ± 4.09 ^{ab} (83.18)
LCAE 400	74.41 ± 5.34 ^{ab} (41.52)	76.37 ± 3.38 ^{ab} (53.05)	34.53 ± 1.99 ^{ab} (38.95)	15.27 ± 0.65 ^{ab} (53.05)	24.60 ± 3.83 ^{ab} (66.58)
LCME 100	61.69 ± 1.29 ^{ab} (51.52)	62.95 ± 0.68 ^{ab} (61.30)	41.68 ± 4.81 ^{ab} (49.42)	12.59 ± 2.13 ^{ab} (61.29)	7.41 ± 5.69 ^{ab} (89.93)
LCME 200	58.98 ± 4.51 ^{ab} (53.65)	58.77 ± 9.14 ^{ab} (63.87)	47.97 ± 7.28 ^{ab} (56.05)	11.75 ± 1.82 ^{ab} (63.87)	-0.74 ± 1.95 ^{ab} (101.0)
LCME 400	54.06 ± 5.60 ^{ab} (57.51)	53.78 ± 2.73 ^{ab} (66.93)	52.00 ± 2.04 ^{ab} (59.46)	10.75 ± 0.54 ^{ab} (66.95)	-8.68 ± 6.63 ^{ab} (111.78)
LCWE 100	63.57 ± 2.83 ^{ab} (50.04)	63.51 ± 8.81 ^{ab} (60.95)	37.48 ± 4.60 ^{ab} (43.75)	12.70 ± 1.76 ^{ab} (60.95)	13.38 ± 6.61 ^{ab} (81.82)
LCWE 200	62.46 ± 7.44 ^{ab} (50.91)	62.01 ± 7.16 ^{ab} (61.87)	44.55 ± 3.30 ^{ab} (52.68)	12.40 ± 1.43 ^{ab} (61.88)	5.50 ± 9.95 ^{ab} (92.53)
LCWE 400	57.23 ± 4.59 ^{ab} (55.02)	59.09 ± 4.67 ^{ab} (63.67)	47.46 ± 3.40 ^{ab} (55.58)	11.81 ± 0.93 ^{ab} (63.69)	-2.04 ± 5.09 ^{ab} (102.77)

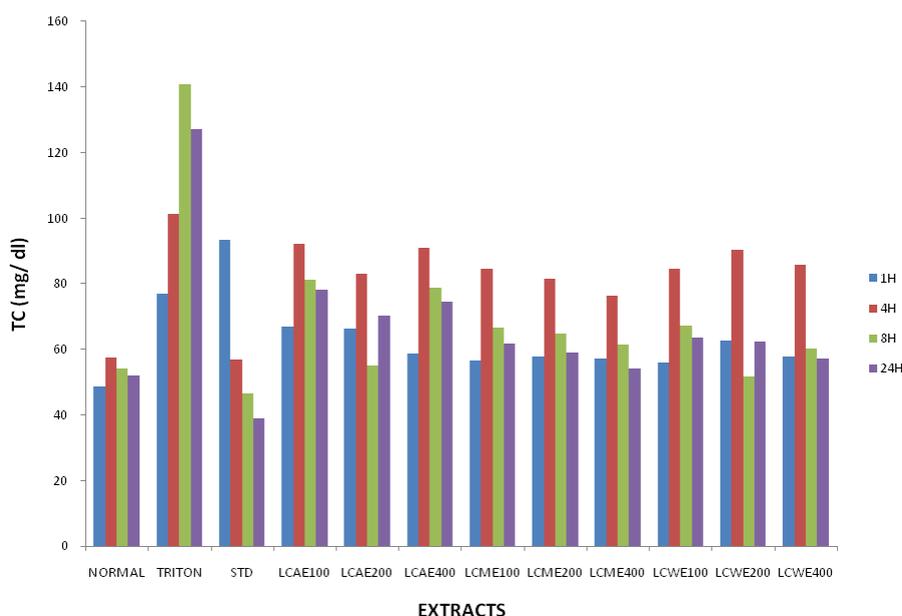
Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + acetone extract; LCME: Triton + methanol extract; LCWE: Triton + aqueous extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein; LDL: Low Density Lipoprotein; a: Changes triton versus normal; b: Changes triton versus other groups; ns: non-significant; *: p < 0.05; **: p < 0.01

Table 1: Lipid levels after 24 hours of treatment with extracts and atorvastatin.

Phytochemicals	LCAE	LCME	LCWE
TPC (mg gallic acid equivalent/g of sample)	608	188	79
TFC (mg quercetin equivalent/g of sample)	128	31	12
	In seeds		
TFC (% w/w)	6.8		
Total saponin content (% w/w)	6.7		

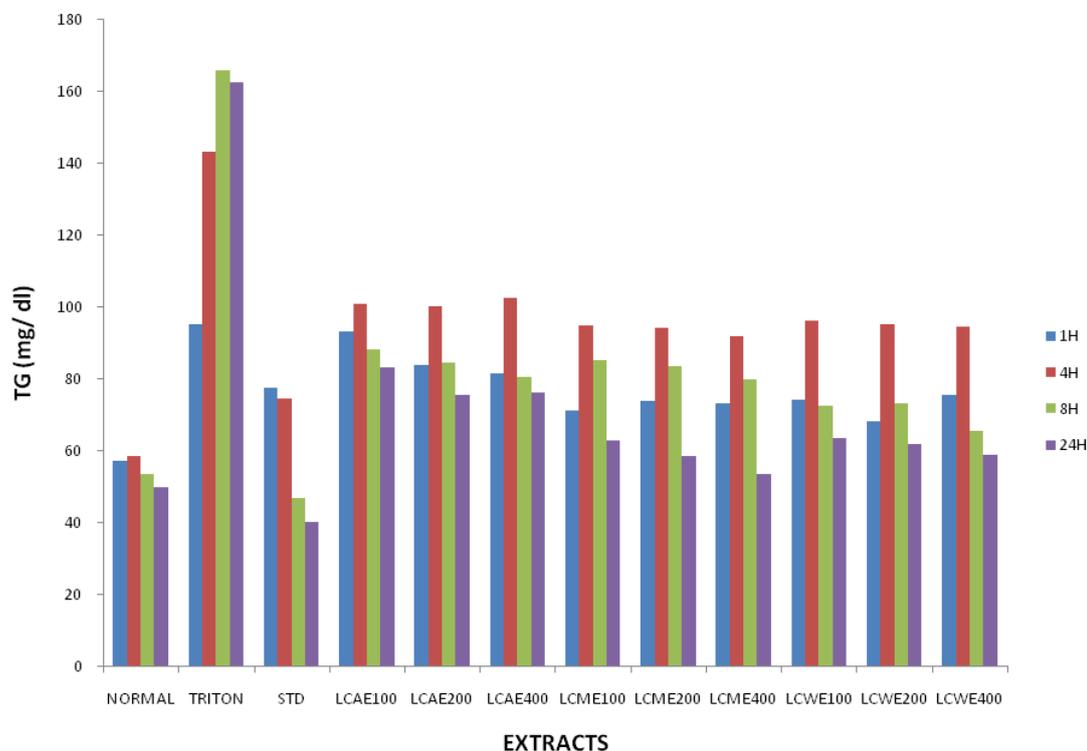
TPC: Total Phenolic Content; TFC: Total Flavonoid Content; LCAE: Acetone Extract; LCME: Methanol Extract; LCWE: Aqueous Extract

Table 2: Total phenolic, flavonoid and saponin content.



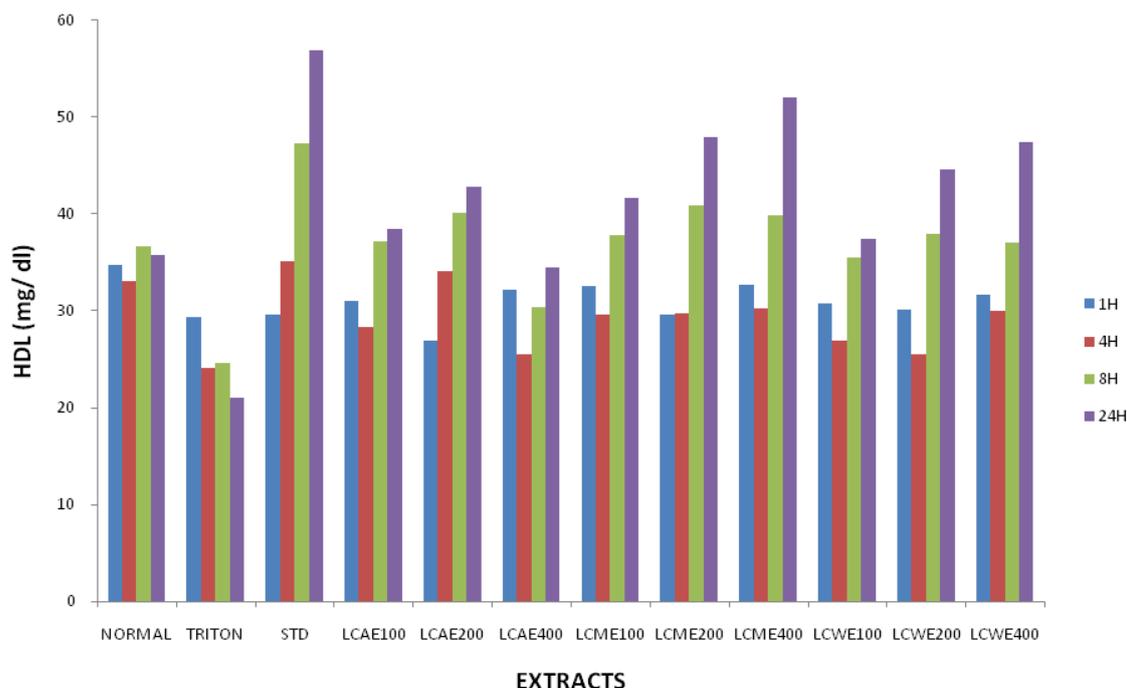
Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + acetone extract; LCME: Triton + methanol extract; LCWE: Triton + aqueous extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; TC: Total Cholesterol

Figure 1: Total cholesterol level in all the groups after 1, 4, 8 and 24 hours of treatment.



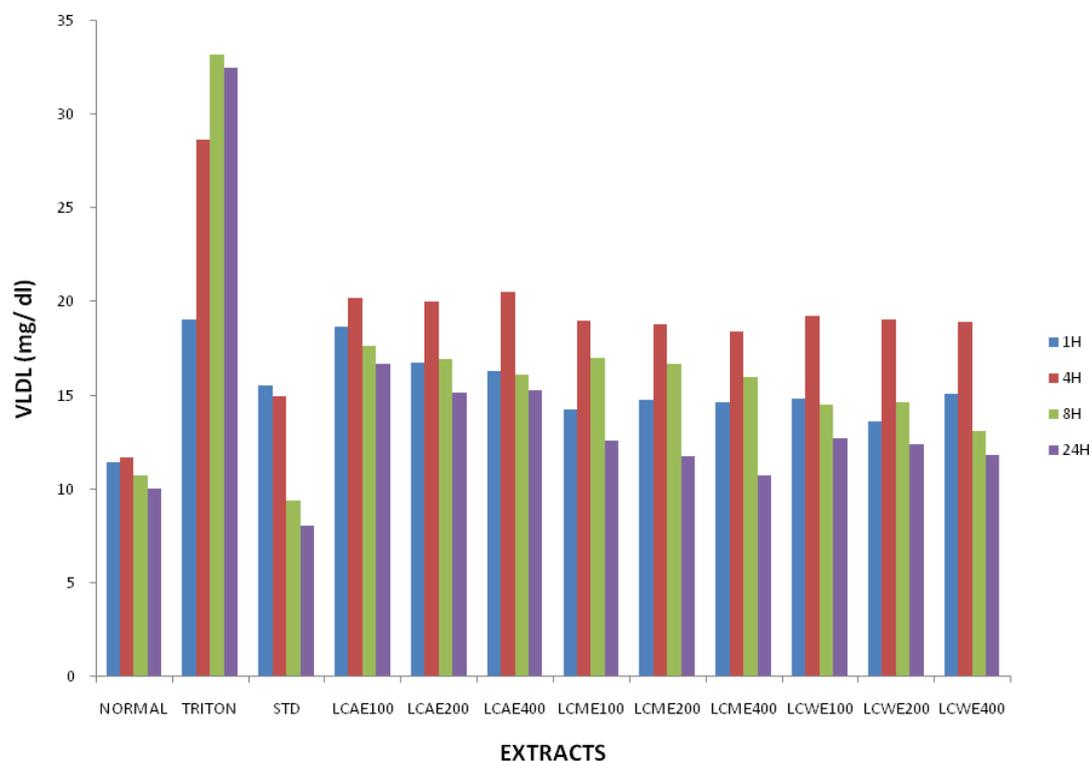
Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + acetone extract; LCME: Triton + methanol extract; LCWE: Triton + aqueous extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; TG: Triglyceride

Figure 2: Triglyceride level in all the groups after 1, 4, 8 and 24 hours of treatment.



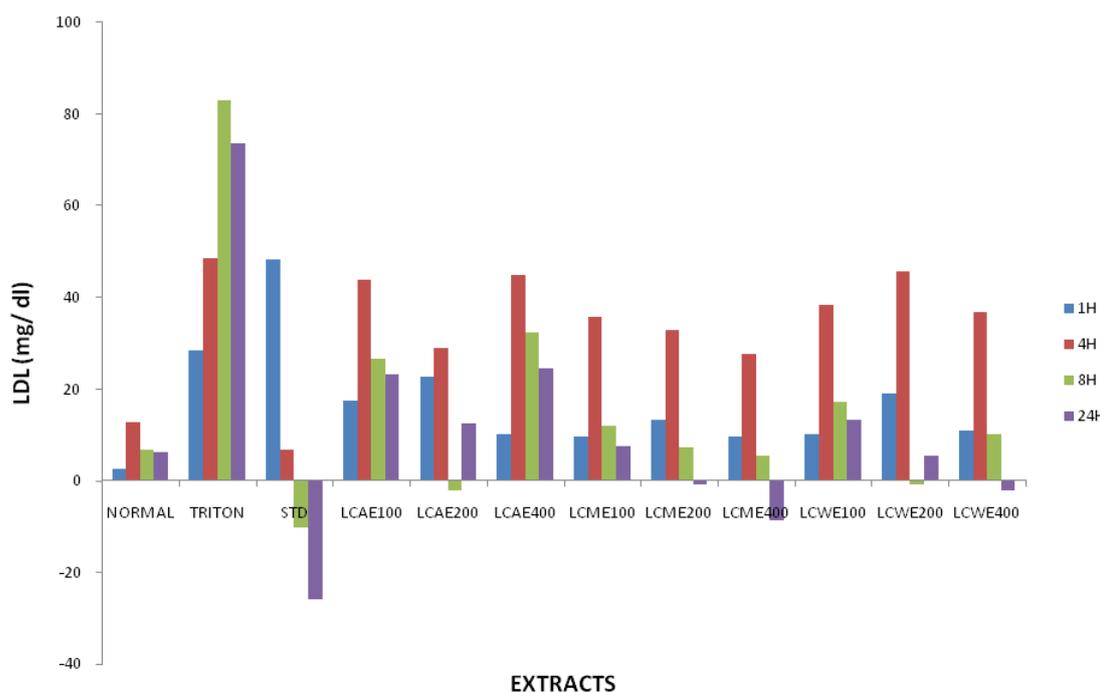
Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + acetone extract; LCME: Triton + methanol extract; LCWE: Triton + aqueous extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; HDL: High Density Lipoprotein

Figure 3: High density lipoprotein level in all the groups after 1, 4, 8 and 24 hours of treatment.



Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + acetone extract; LCME: Triton + methanol extract; LCWE: Triton + aqueous extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; VLDL: Very Low Density Lipoprotein

Figure 4: Very low density lipoprotein level in all the groups after 1, 4, 8 and 24 hours of treatment.



Normal: Normal group; Triton: Hyperlipidemic group; Std: Triton + atorvastatin; LCAE: Triton + Acetone Extract; LCME: Triton + Methanol Extract; LCWE: Triton + Aqueous Extract; 100: 100 mg/Kg; 200: 200 mg/Kg; 400: 400 mg/Kg; LDL: Low Density Lipoprotein

Figure 5: Low density lipoprotein level in all the groups after 1, 4, 8 and 24 hours of treatment.

Discussion

Triton WR-1339 has been widely used as a model (*in vivo*) for induction of acute hyperlipidemia in animals. It increases the level of lipids by blocking the clearance of triglyceride-rich lipoproteins [21]. The convenience associated with this model, in terms of length of treatment period and handling, has increased its popularity for screening natural or chemical hypolipidemic as well as antihyperlipidemic drugs [4,13,22-25]. Schurr et al. demonstrated the use of Triton WR-1339 for induction of hyperlipidemia, where administration of Triton increases the plasma triglycerides and total cholesterol levels to maximum in 20 hours, followed by a decline to normal values [25]. In the present study, the Triton WR-1339 model gave similar pattern of changes in lipid profile, at both time periods, i.e., 8 and 24 hours (after treatment). There was an increase in VLDL and LDL levels observed after 4 hours as compared to the levels after 1 hour of treatment. This may be due to the hyperlipidemic activity of Triton to delay the removal of lipoproteins. This demonstrates the feasibility of using Triton WR-1339 to assess the antihyperlipidemic activity of various *Lens culinaris* Medikus seed extracts.

The LCME at a dose of 400 mg/Kg was found to possess more significant antihyperlipidemic activity than other extracts as it significantly lowered all the lipid levels after 8 and 24 hours of treatment. It also increased the HDL cholesterol significantly after 24 hours of treatment as compared to hyperlipidemic group. LCWE at a dose of 400 mg/Kg also showed a potent lipid lowering effect but it was found to be less active than LCME 400. The underlying mechanism was not elucidated in the present study; however, the extracts might have the ability to inhibit the increased synthesis of cholesterol and triglycerides in the body [25]. The reduction of plasma TC by the *Lens culinaris* Medikus seeds extract was also associated with a decrease in its LDL fraction. This might be due to the rapid catabolism of LDL through its hepatic receptors for final elimination in the form of bile acids [13].

The results clearly show that *Lens culinaris* Medikus seeds have the potential to lower the plasma lipid levels significantly ($p < 0.05$). All the extracts, i.e., LCAE, LCME and LCWE possess the antihyperlipidemic activity, though the potential to lower the lipids varied dose dependently as well as according to the antihyperlipidemic activity of the extracts.

The results found clearly demonstrate that the bioactive compounds present in extracts possess cholesterol-suppressive capacities and ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemia models. The compounds isolated from lentil seeds such as soyasaponins and trigonelline have reported lipid lowering activity [26,27]. As determined by qualitative phytochemical analysis, the LCME was found to be rich in saponins, flavonoids, carbohydrates, triterpenoids and proteins. In earlier studies, plant polyphenols and saponins have exhibited a variety of pharmacological activities, including the anti-atherogenesis effect [28]. The results strongly suggest that the antihyperlipidemic activity of this medicinal plant could be attributed to the presence of the valuable polyphenolic compounds and saponins. The saponins and flavonoids have reported antihyperlipidemic property as they lower cholesterol levels [26].

Flavonoids from the plant seed extract may augment the activity of Lecithin acyl transferase or assist in incorporating free TC into HDL (this might increase HDL) and transferring it back to VLDL and LDL, which are taken back later in liver cells [29,30]. Isolated saponins and foods containing saponins have been shown to lower plasma TC concentrations in a number of animal species, thus such items could be important in formulating hypocholesterolemic diets for human

consumption [26]. Previous studies have also reported the presence of saponins in lentils [31]. Saponins act as antihyperlipidemics either by binding with cholesterol in intestinal lumen, or by increasing metabolism of cholesterol to sterols through the faecal matter. Increase in bile acid excretions by enhanced synthesis of cholesterol in the liver consequently lowers the plasma cholesterol. They may also act by increasing the lipoprotein lipase activity which helps in faster removal of free fatty acids from cholesterol which in turn decreases the TC level [26,32,33]. The soluble dietary fibres present in the seeds of *Lens culinaris* Medikus seeds may also contribute to lowering of lipid levels. However, these hypothesis need to be validated by an experimental study.

Conclusion

The results validate that the lentil seeds possess antihyperlipidemic activity. This is the first study which investigates the antihyperlipidemic activity of various extracts of *Lens culinaris* Medikus seeds and the results found are encouraging enough for further assessment to elucidate both, the underlying mechanisms for identification of the bioactive compounds, as well as the antihyperlipidemic activity of lentils on chronic hyperlipidemia models.

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