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Antihyperlipidemic Effect of Flax Lignan Concentrate in Triton Induced Hyperlipidemic Rats

[1]A.A. Zanwar, [2]M.V. Hegde and [1]S.L. Bodhankar
[1]Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Paud Road, Erandawane, Pune-411 038, Maharashtra, India
[2]Interactive Research School for Health Affairs, Medical College Campus, Bharati Vidyapeeth Deemed University, Dhankawadi, Pune-411 043, India

Abstract: *Linum usitatissimum* commonly known as flax (also known as linseed) is a member of the genus *Linum* in the family Linaceae. The objective of study was to investigate the antihyperlipidemic effect of Flax Lignan Concentrate (FLC) in triton WR-1339 induced hyperlipidaemic rats. Hyperlipidemia was induced by intraperitoneal injection of triton WR-1339 (200 mg kg^{-1}). Triton WR-1339 (200 mg kg^{-1}, i.p.) injection significantly ($p<0.001$) increased total cholesterol, triglyceride, very high density lipoprotein at 24 h and thereafter at 48 h reduction was observed. FLC showed dose dependant decrease in the total cholesterol, triglyceride and very high density lipoprotein. FLC increased HDL-cholesterol whereas HDL decreased in the rats treated with triton alone. Atorvastatin was more effective than FLC in all the respect. Administration of triton alone increased both coronary risk index and low density lipoprotein and FLC and atorvastatin treatment decreased both the indices. It is concluded that FLC reduces total cholesterol, triglyceride, very high density lipoprotein, low density lipoprotein, coronary risk index and increased HDL in triton induced hyperlipidaemia in rats.

Key words: Atherosclerosis, flax lignan concentrate, hyperlipidemia, flaxseed, *Linum usitatissimum*, triton WR-1339

INTRODUCTION

Elevated levels of serum lipids (cholesterol and triglycerides) are extremely common and are one of the most important of the heart disease risk factors. It is now well established that hyperlipidaemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications (Stokes *et al*., 1987; Jalali-khanabadi *et al*., 2006). Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths (Rosmund *et al*., 1998). Hyperlipidemia is the most common risk factors in myocardial infarction (Ilali and Taraghi, 2010) and plays key role also in acute myocardial infarction also (Motawi *et al*., 2001).

Linum usitatissimum (Linn.), commonly known as flaxseed or linseed. In ancient times, around 500 B.C.E., Hippocrates wrote about the value of flax in relieving abdominal distress. Flaxseed has also been used as a laxative and relieves itching. Flaxseed is also used as folk remedy for cough, gallstone, lung disorder and digestive disorder including constipation. Cold-pressed linseed oil was recommended for diseases of the chest and lungs such as asthma, cough, tuberculosis, pneumonia and pleurisy (Vaisey-Genser and Morris, 2003). Mainly flaxseed is currently receiving attention of scientific community as a functional food for prevention of hypercholesterolemic atherosclerosis. Flax seed is a rich source of ω-3 fatty acid and plant lignan (Hunter, 1990). The flax seed mainly contains 35% oil of which 55% is α-linolenic acid (ω-3 fatty acid) (Carter, 1993). Moderate amount of flaxseed (10 to 20%) proved to have favorable baked products in terms of taste and health benefits also (Bashir *et al*., 2006). The ethanolic extract of flaxseed, high mucilage low protein product from detoxified flaxseed meal and phenolic components from n-butanol fraction of defatted flaxseed meal exhibited antioxidant activity

Corresponding Author: S.L. Bodhankar, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411 038, India
Tel: +91-20-25437237/29 Fax: +91-20-25439386

(Zanwar *et al*., 2010; Singer *et al*., 2011; Kasote *et al*., 2011). *In vivo* antidiabetic (Prasad *et al*., 2000), antiatherosclerotic (Prasad, 1997) activities of flaxseed have been reported. Moreover, flaxseed in combination with garlic found to be protective in hyperlipidemia (Abdel-Rahman *et al*., 2009) and protective role in osteoporosis (Boulbaroud *et al*., 2008a, b), diabetic complications (Abuelgassim, 2010) is also reported.

In our previous study, we have reported the antioxidant and cardioprotective activity of flaxseed (Zanwar *et al*., 2010, 2011). Anti-atherosclerotic activity of flaxseed supplementation to hypercholesterolemic rabbit has been reported by Prasad (1997, 1999, 2007). The objective of the present investigation was to study the effect of Flax Lignan Concentrate (FLC) for possible antihyperlipidemic activity in triton WR-1339 induced hyperlipidaemic rats.

MATERIALS AND METHODS

Collection and authentication of plant: Authenticated seeds of *Linum usitatissimum* were obtained from Dr. P.B. Ghorpade, Principal, Scientist and Linseed breeder, Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture, Nagpur, India, Maharashtra State, India and voucher specimen was deposited at the institute.

Drugs and chemicals: Triton WR-1339 (Tyloxapolol, Himedia). Absolute alcohol (Changshu Yangyuan Chemicals, China). The n-hexane, hydrochloric acid, sodium hydroxide, methanol, hydrochloric acid and sodium chloride of analytical grade (Qualigene fine-chem. Ltd., Mumbai, India) were used in the investigations.

Preparation of flax lignan concentrate: The authenticated seeds of *Linum usitatissimum* (variety NL-97) were processed for extraction of oil at our Omega-3-oil unit, Sangamner, Maharashtra, India. This processing plant is set up under National Agriculture Innovation Project funded by Indian council of Agricultural Research, New Delhi, India. The double cold pressed flaxseed cake/meal obtained from this oil unit was defatted by n-hexane in a Soxhlet apparatus to remove residual oil. The defatted cake was then hydrolyzed with 1 M aqueous sodium hydroxide for 1 h at room temperature with intermittent shaking, followed by extraction with 50% ethanol. After filtration the filtrate was acidified to pH 3 using 1 M hydrochloric acid. The filtrate was dried on tray dryer at 50°C. The yield of dry FLC powder was 14.81% w/w. Weighed quantity of FLC powder was dissolved in distilled water to prepare the different doses of FLC for pharmacological studies. The FLC samples were analyzed by high performance thin layer chromatography and secoisolaricinol diglucoside (SDG) lignan content in FLC was 40 mg g^{-1} (Zanwar *et al*., 2011).

Preliminary phytochemical screening of FLC: The qualitative analysis for the presence of alkaloid (Mayer's, Hager's, Dragendorff's, Wagner's test), flavonoids (Shinoda test), steroids (Salkowski, Liebermann-Burchard, Liebermann's test), phenolic compounds, glycosides and volatile oils in FLC was carried out by methods described by Khandelwal (2002).

Research protocol approval: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of our college constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

Experimental animals: Male Wistar rats weighing between 180 and 220 g were purchased from National institute of toxicology, Pune, India. The animals were housed at an ambient temperature 25±2°C and relative humidity 40-50% and light and dark cycle (12 h light/dark). The animals had access to pellet diet (Chakan oil mills, Pune) and water *ad libitum*.

Triton model of hyperlipidaemia: Triton WR-1339 (tyloxapol, isooctylpolyoxyethylene phenol) was dissolved in normal saline and administered intraperitoneal (i.p.) to the rats (200 mg kg^{-1}, body weight) in order to develop an acute hyperlipidemia in them (Harnafi *et al*., 2007).

Experimental design and protocol: Overnight fasted rats were divided into six groups containing six rats in each group. Group I control (CG), received intraperitoneal administration of normal saline and distilled water orally. Animals of group II to VI were treated with intraperitoneal injection of triton WR-1339 (200 mg kg^{-1}) to produce hyperlipidemia and randomly divided into remaining five groups (Group II to VI). Group III, IV and V received increasing doses of FLC i.e., 125, 250 and 500 mg kg^{-1}, p.o., respectively and Groups VI rats received atorvastatin (ATR) (10 mg kg^{-1}) p.o. During study period animals had access only to water. At 0 h (before test drug administration) and after 6, 24 and 48 h of treatments,

animals were lightly anaesthetized with anesthetic ether and blood was withdrawn retro orbital plexus. The blood samples were centrifuged (2500 rpm/10 min at 4°C) and serum was used for lipid analysis.

Biochemical analysis of serum: Analytical procedures serum triglycerides, cholesterol and HDL-C were quantified by an enzymatic method using Accurex biomedical kit (Accurex biomedical Pvt. Ltd., India). Briefly, serum triglycerides, after enzymatic hydrolysis with lipases, which is converted by glycerol kinase into glycerol-3-phosphate which is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red colored compound and spectrophotometrically measured at 540 nm.

Total cholesterol levels were determined by the cholesterol oxidase enzymatic method. Cholesterol was hydrolyzed and in the presence of phenol, the quinone imine as indicator was formed from hydrogen peroxide and 4-aminoantipyrine via peroxidase catalysis and spectrophotometrically measured at 510 nm.

HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with Phosphotungstic Acid (PTA) and magnesium chloride.

Serum LDL-C and VLDL-C was estimated using Frieldwann's equation (Friedewald *et al*., 1972):

$$LDL\text{-}C = [TC-(HDL+TG/5)]; VLDL\text{-}C = TG/5$$

Coronary Risk Index (CRI) was calculated by the following formula (Adeneyea and Olagunjub, 2009):

$$CRI = \text{Total cholesterol/HDL-cholesterol}$$

Statistical analysis: Data were expressed as the Mean±SEM. Statistical analysis was carried out by one-way ANOVA followed by *post hoc* Bonferroni test using graphPad prism 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The p value was considered significant when $p<0.05$.

RESULTS

Preliminary phytochemical analysis of FLC showed presence of tannins, phenol and flavonoids. Triton WR-1339 (200 mg kg^{-1}, i.p.) injection significantly ($p<0.001$) increased total cholesterol, triglyceride and very high density lipoprotein at 6 h (Fig. 2) and 24 h (Fig. 3), compared to 0 h (Fig. 1) and thereafter at 48 h reduction was observed (Fig. 4). The coronary risk index showed a peak effect at 6 h and gradual decline at 24 h and 48 h. The LDL level was significantly ($p<0.001$) increased at 24 h. At 6 h, FLC at all doses and ATR significantly decreased TG dose dependently ($p<0.001$) and HDL-C was increased significantly ($p<0.001$), however there was slight significant decrease in case of TC and VLDL-C and non-significant changes were observed in case of LDL and CRI. The result obtained at 24 h was considered for comparison with the test group. FLC showed dose dependant decrease in the TG. Higher doses of FLC 500 mg kg^{-1} showed statistically significant ($p<0.01$) reduction in TC, whereas reduction produced by lower doses i.e., 125 and 250 mg kg^{-1} were non-significant. Atorvastatin (10 mg kg^{-1}) was more effective in reducing the TC ($p<0.01$) than FLC. FLC 250 and 500 mg kg^{-1} reduced TG significantly ($p<0.001$). Atorvastatin (ATR) was more effective than FLC in this respect also ($p<0.001$). FLC 500 mg kg^{-1} significantly increased ($p<0.001$) HDL-cholesterol which was significantly decreased ($p<0.05$) in triton alone. ATR was also more effective than FLC in this respect too. The VLDL-cholesterol was significantly increased ($p<0.001$) by triton and FLC treatment reduced it dose dependently at 250 and 500 mg kg^{-1} doses. ATR was more effective in case of VLDL also. The results obtained thus indicate effective doses are in the range of 250 and 500 mg kg^{-1} in exhibiting the hyperlipidemic activity. Administration of triton alone increased CRI and LDL ($p<0.001$), FLC and ATR treatments significantly decreased CRI the index ($p<0.001$) and LDL was non-significantly decreased.

DISCUSSION

In the present investigation, the method was successfully used to produce hyperlipidemia as the results indicated increased total cholesterol and triglyceride in the serum samples at 24 h. Previously this model has been successfully used in evaluation of antihyperlipedemic action of number of medicinal plants such as *Erica multiflora* (Harnafi *et al*., 2007), Indian black tea (Chander *et al*., 2005), *Terminalia arjuna* (Chander *et al*., 2004), *Lagenaria siceraria* (Ghule *et al*., 2006) has been assessed for the hypolipidemic activity in a Triton WR-1339-induced hyperlipedemic model. Prasad (1997) in his pioneering experiments investigated the

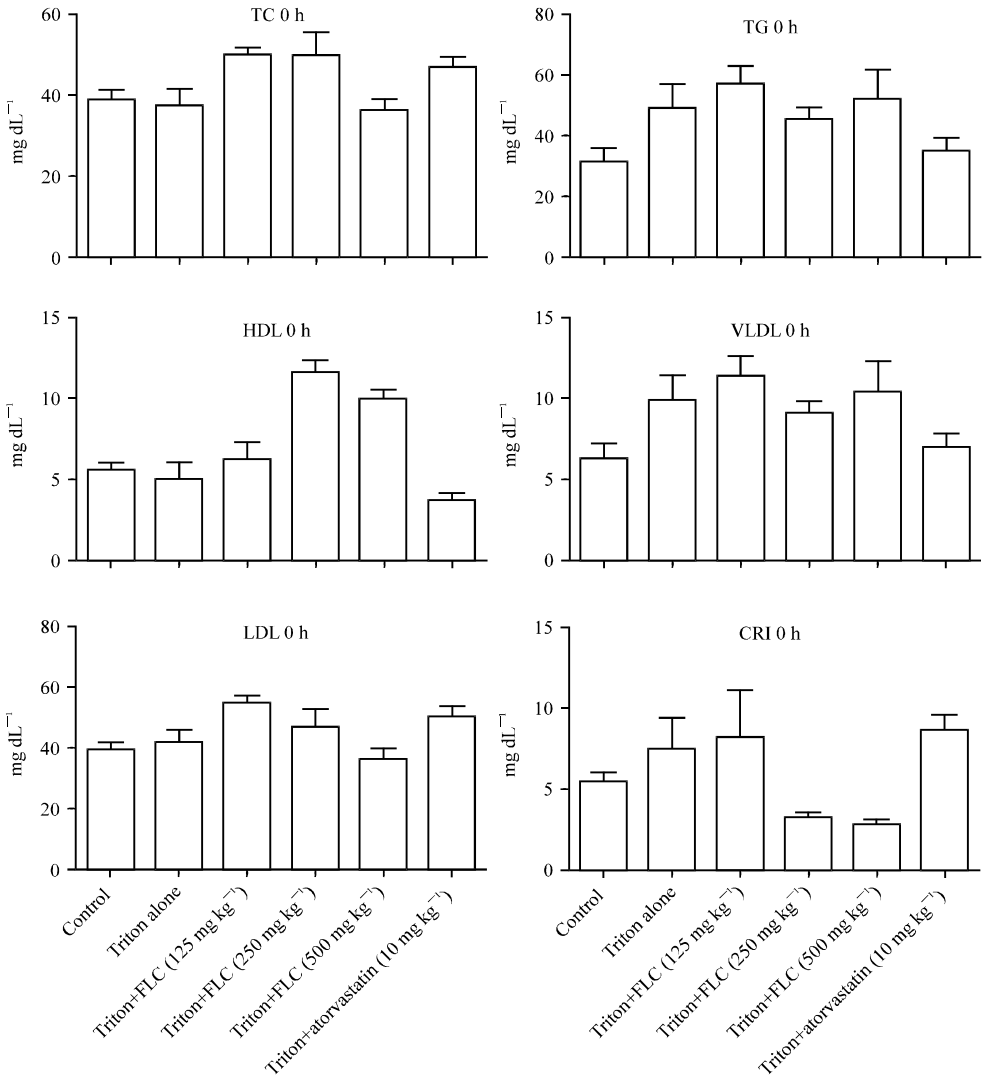

Fig. 1: Effect of FLC on serum lipid levels in Triton WR-1339-induced hyperlipedemic in rats at 0 h. Values are expressed as Mean±SEM from six animals in each group. Data was analyzed by One-way ANOVA followed by post hoc Bonferroni test. $p<0.05$ considered as significant compared to Triton alone group. TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol

effect of dietary flaxseed supplementation and demonstrated the effectiveness of flaxseed in reducing hypercholesterolemia in rabbits. Later, Prasad *et al.* (1998) he used isolated SDG lignan and supplemented it in diet and reported reduction of hypercholesteromic atherosclerosis in rabbits (Prasad *et al*., 1998). Present study differed from that of Prasad whereby we administered the SDG orally in rats. The model of inducing hypercholesterolemia was also different than that of Prasad (1997).

In the present investigation, we observed that FLC reduced triglyceride more effectively than total cholesterol. It has been reported by Hokanson and Austin (1996) that elevated plasma triglyceride levels were associated with increased incidence of coronary artery disease. The higher TG levels have been attributed mainly to an increased population of small, dense LDL deposits which are atherogenic (Austin *et al*., 1994) and enhanced cholesteryl ester mass transfer from apolipoprotein

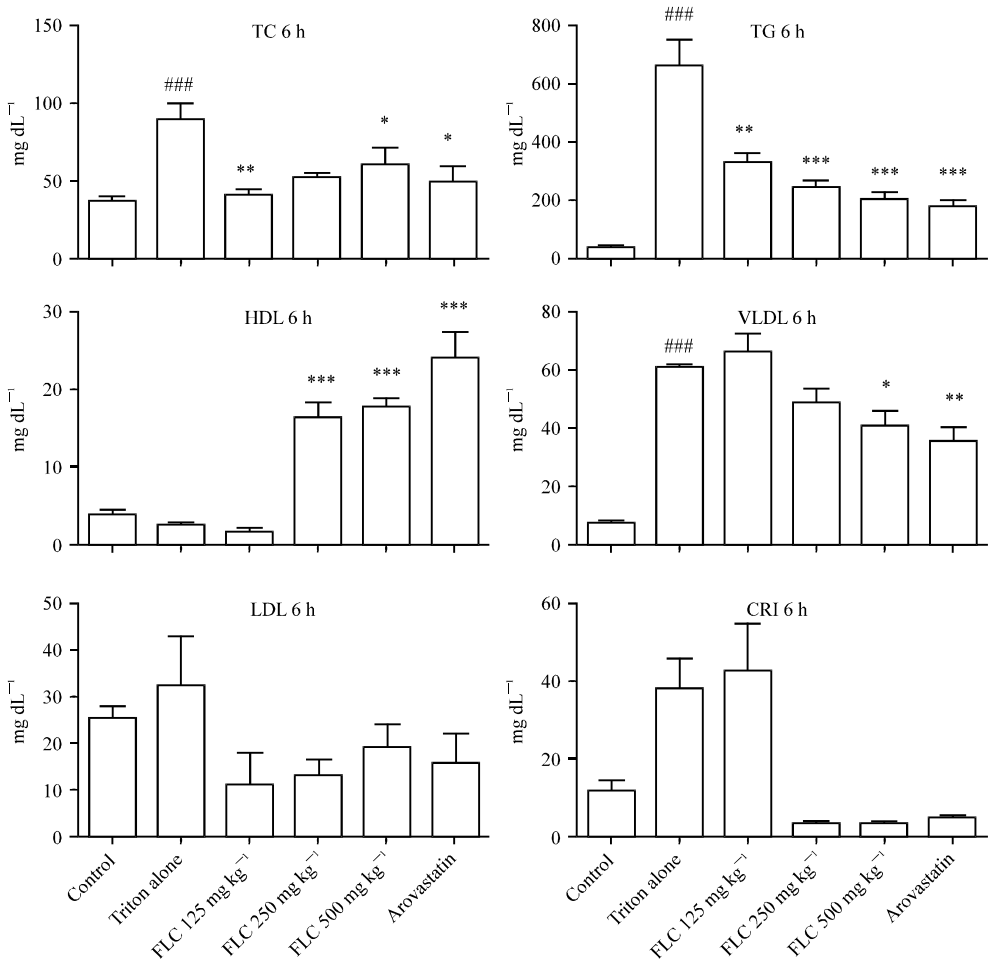

Fig. 2: Effect of FLC on serum lipid levels in Triton WR-1339-induced hyperlipedemic in rats at 6 h. Values are expressed as Mean±SEM from six animals in each group. Data was analyzed by One-way ANOVA followed by post hoc Bonferroni test. $p<0.05$ considered as significant compared to Triton alone group. TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, ns: Not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$ considered as significant compared to Triton alone group, ###$p<0.001$ compared to control group

containing lipoprotein VLDL and LDL. TGs have been proposed to be major determinants of cholesterol esterification, its transfer and remodeling in human plasma (Murakami *et al*., 1995).

Reduction of elevated serum triglyceride by FLC thus appears to be due to increased breakdown of triglyceride. In the present investigation FLC treatment reduced serum TC and also increased serum HDL-cholesterol. Increased in HDL-cholesterol is regarded as a health promotery effect of anti-hyperlipidemic drugs. Malloy and Kan (1994) have reported that independent relationship between blood HDL-cholesterol levels and cardiovascular disease risk. HDL is called good cholesterol as it facilitates the mobilization of TG and TC from plasma to liver where it is catabolised and eliminated in the form of bile acids. Involvement of Lecithin Cholesteryl Acyl Transferase (LCAT) in the regulation of blood lipids has been suggested. LCAT play a key role in lipoprotein metabolism and most of lipoprotein changes are outcome of primary abnormality owing to liver diseases (Khanna *et al*., 2002).

Low density lipoprotein and coronary risk index are two derived parameters to access the risk of atherosclerosis in the coronary arteries. Increases in the both indices in triton treated rats indicated increased risk of coronary artery disease. FLC treatment reduced both

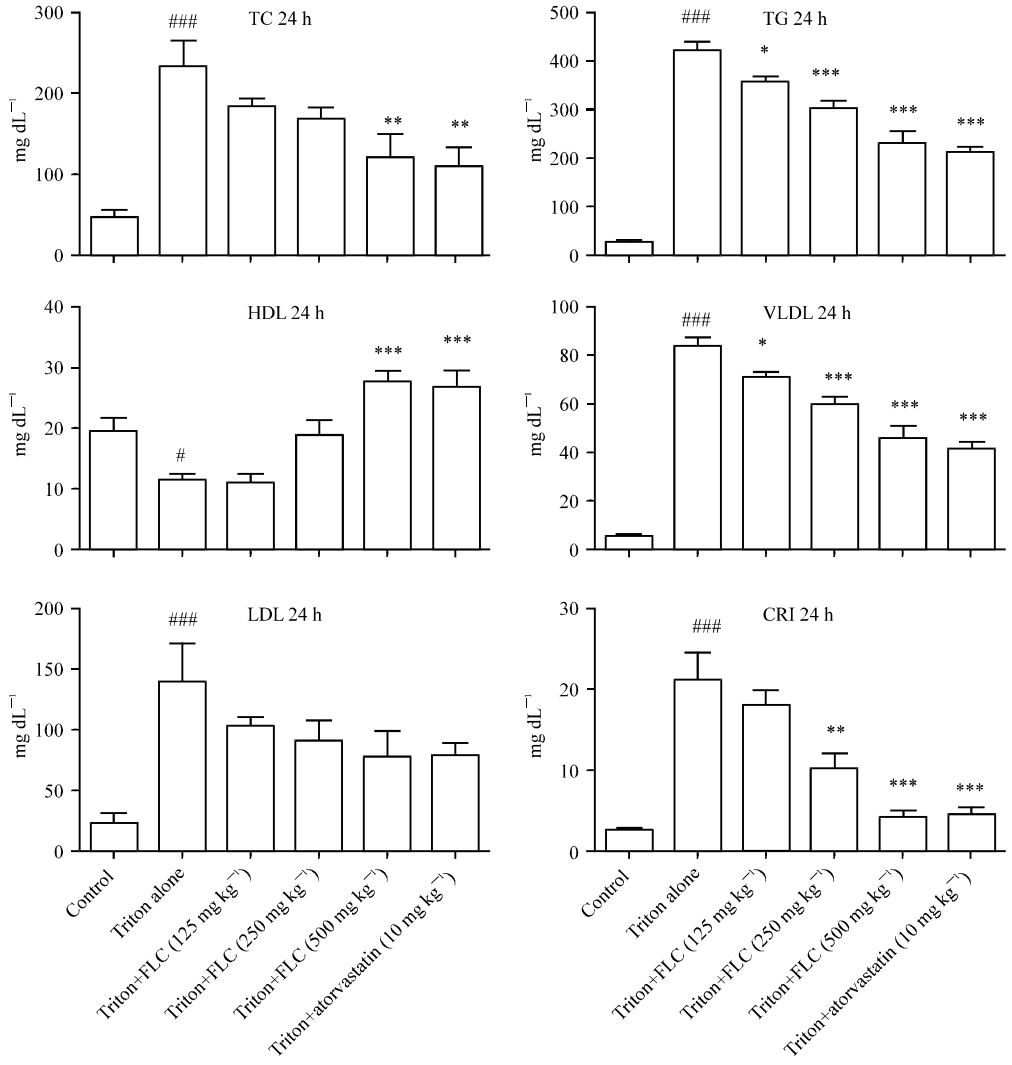

Fig. 3: Effect of FLC on serum lipid levels in Triton WR-1339-induced hyperlipedemic in rats at 24 h. Values are expressed as Mean±SEM from six animals in each group. Data was analyzed by One-way ANOVA followed by post hoc Bonferroni test. $p<0.05$ considered as significant compared to Triton alone group. TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, ns: Not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$ considered as significant compared to Triton alone group, $^{###}p<0.001$ compared to control group

indices therefore reduced risk of coronary artery disease. In the present investigation atorvastatin was used as positive control. The statins are drug of first choice for treating primary hyperlipidemia with high levels of LDL and TC and moderate TG levels (type 2A, 2B V) as well as for secondary (diabetes, nephritic syndrome) hypercholesterolemia. Effect of statin in reducing LDL-cholesterol associated mortality and morbidity is now well established. Atorvastatin is more potent new statin which have highest LDL-cholesterol lowering efficacy. Our result indicted that FLC was comparable in reducing serum TC, TG, VLDL compared to atorvastatin. FLC was less effective in reducing LDL and CRI.

Preliminary phytochemical analysis of FLC showed the presence of flavonoids and phenolics. Flavanoids and phenolics are potent antioxidants and are known to modulate the activities of various enzymes systems due to their interaction with various biomolecules (Harnafi *et al*., 2007; Koshy *et al*., 2001; Javanmardi *et al*., 2003). In fact, flavonoids and plant polyphenols, exhibit different pharmacological activities, including hypolipidemic and anti-atherogenic effects

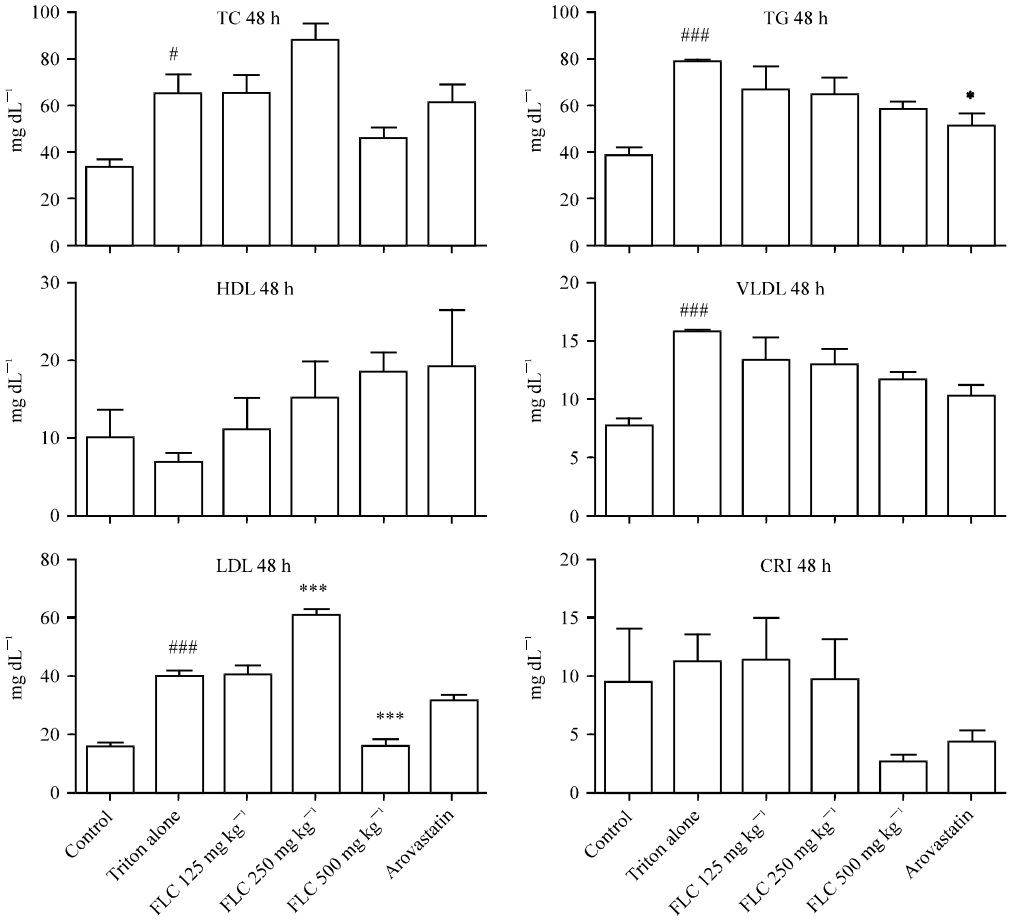

Fig. 4: Effect of FLC on serum lipid levels in Triton WR-1339-induced hyperlipedemic in rats at 48 h. Values are expressed as Mean±SEM from six animals in each group. Data was analyzed by One-way ANOVA followed by post hoc Bonferroni test. $p<0.05$ considered as significant compared to Triton alone group. TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, ns: Not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$ considered as significant compared to Triton alone group, $^{###}p<0.001$ compared to control group

(Sudheesh *et al*., 1997; Anila and Vijayalakshmi, 2002). Thus the hypolipidemic action of oral administration of FLC may be attributed to flavonoids in FLC along with synergistic action of SDG.

CONCLUSION

In conclusion, FLC 250 and 500 mg kg^{-1} reduced TC, TG, VLDL, CRI and raised HDL in triton induced hyperlipidemia in rats. The antihyperlipidemic effect appears to be due to the antioxidant activity of flavonoids and SDG and cardioprotective activity of SDG lignan.

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