CHAPTER 5

Identification and isolation of active constituents present in the Cassia auriculata and their potential to treat Triton WR – 1339 induced hyperlipidemia in male Wistar rats

1. ABSTRACT

Flavonoids have been reported to exhibit several pharmacological properties. In our present study, we observed that rutin, a known glycosylated flavonoid and an active component isolated from Cassia auriculata flowers, lowers plasma triacylglycerol levels. The present work investigated the anti-hyperlipidemic effect of rutin in hyperlipidemia induced male albino Wistar rats (by a single intra-peritoneal (IP) injection of Triton-WR 1339 (300mg/kg/b.wt). Hyperlipidemic rats showed sustained elevated levels of serum cholesterol and triacylglycerol and administration of rutin (10, 25, 50,100 mg/kg b.wt.) exhibited a significant reduction in serum lipid parameters such as total cholesterol, triacylglycerol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), but increased high density lipoprotein (HDL) in comparison with control rats. The anti-hyperlipidemic effect was assessed by evaluating the lipid profile (Total cholesterol, TG, VLDL, LDL, HDL), expression of regulatory genes of lipid metabolism in liver namely, sterol receptor element binding protein type 1c(SREBP1c), acetyl-CoA carboxylase type 1 (ACC1), sterol receptor element binding protein type 2 (SREBP2), hydroxy methyl glutaryl (HMG) Co-A reductase (HMGCR), HMG-Co-A synthase (HMGCS), Cholesterol 7 α-hydroxylase (CYP7A1), ATP-binding cassette transporter 1 (ABCA1) and by histopathological studies of liver. The drug has the potential to act as an anti-hyperlipidemic drug. Pronounced changes were observed at 10 mg /kg /b.wt. rutin and it was comparable to the standard drug atorvastatin. Therefore, rutin seems to be a selective drug for hyperlipidemia, which could be promising in the development of new drug.

Key words: Rutin, Anti-hyperlipidemia, Triacylglycerol, Atorvastatin, Triton WR-1339.
2. **INTRODUCTION**

Hyperlpidemia is considered as one of the most familiar metabolic disorders and it is closely associated with obesity, diabetes mellitus, and several other metabolic syndromes (Farrell et al., 2008; Trauner et al., 2010). The Liver plays an essential role in lipid metabolism by regulating lipogenesis (Madan et al., 2006). With the current sedentary life style, stress and food habits there is risk for developing diabetes, hypertension, dyslipidemia, insulin resistance, dyspnoea and apnea especially in obese individuals (Bray GA 2000). The development of prolonged oxidative stress, compounded by defective desaturation and dietary imbalance promotes hepatic steatosis (Araya et al., 2010; Videla et al., 2006). The developments in the lifestyles and dietary habitats caused drastic changes as with many developed societies and these changes increased the physical disorders such as obesity and NAFLD (Non alcoholic fatty liver disease). HDL is beneficial at higher levels and reduced the risk with cardiovascular events, by promoting the reverse cholesterol transport, an anti-atherogenic process resulting in the transport of cholesterol from peripheral tissues to the liver for subsequent processing and degradation. High cholesterol diet leads to dyslipidemic syndrome and hyperlipidemia is characterized by an increase in TAG and decrease in HDL-cholesterol (Bloomgarden ZT 2004). Although numerous synthetic lipid-lowering drugs, such as fibrates, statins, and bile acid sequestrants, have been developed to combat hyperlipidemia; drug management without accompanying side effects is still a challenge (Eghdamian and Ghose 1998). Statin is the main drug for the treatment of cardiovascular disease; however, statins are associated with a number of adverse events. These include muscle-related complaints, such as rhabdomyolysis, myalgia, cramps, and muscle weakness (i.e., myopathy) (Parker and Thompson, 2012; Sewright et al., 2007). Statins also provoke liver dysfunction and renal failure (Fernandes et al., 2012; Beltowski et al., 2009). The number of patients with statin-related disturbances has recently increased, due to the widespread use of this drug as a
blood cholesterol-lowering agent. Therefore, the development of promising cholesterol-lowering treatments that are alternatives to statin is of utmost importance. Rutin is a flavonol glycoside (glycosylated flavonoid) consisting of the flavonol quercetin and the disaccharide rutinose. Rutin is widely distributed in various plants, and is known for its antioxidant and anti-inflammatory activity (Middleton-Junior et al. 2000). Recent studies on fatty liver in food science have focused on the searching for functional food ingredients or herbal extracts that can suppress the accumulation of hepatic lipid. Rutin is a common dietary flavonoid that is available in fruits, including tomatoes, vegetables, and plant-derived beverages such as tea and wine. Rutin has several pharmacological properties including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombic, vasoprotective, and cardioprotective activities (La Casa et al., 2000; Sheu et al., 2004; Madan et al., 2006). The non-ionic detergent, Triton WR 1339 is used to induce acute hyperlipidemia and it increases the hepatic synthesis of cholesterol and triacylglycerol (Vogel HG and Vogel WH 1997; Kumar et al., 2011). Triton blocks the uptake of triacylglycerol-rich lipoproteins from plasma by peripheral tissues in order to produce acute hyperlipidemia in animal model. This model is used for screening anti-hyperlipidemic drugs (Schurr et al., 1972) and many medicinal plants have been assessed for their anti-hyperlipidemic activity (Khanna et al., 2002). Hyperlipidemia was induced by Triton, and we evaluated the effect of rutin for its anti-hyperlipidemic activity by measuring the expression pattern of the genes involved in cholesterol metabolisms such as HMGCR, HMGCS, SREBP-1c, SREBP-2, ACC1, CyP7A1, and ABCA1.
3. MATERIALS AND METHODS

3.1. HPLC Analysis

3.1.1. Reagents and chemicals

Methanol, acetonitrile and acetic acid were of HPLC grade (Tedia Company, USA). Ethanol was of analytical grade (Beijing Chemical Reagents Company, China). Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, and USA). RU, QU standards were purchased from Sigma Company (USA).

3.1.2. Chemicals

Atorvastatin, Triton WR-1339 (Tyloxapol) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Atorvastatin and Triton WR-1339 were dissolved in saline solution (pH 7.4). The doses of the rutin used in this study were 10 mg, 25 mg, 50 mg, 100 mg/kg b.wt. Atorvastatin (10 mg/kg b.wt.) was used as the reference standard drug for evaluating the antihyperlipidemic activity (M.S. Sikarwaret al., 2012). Atorvastatin reduces plasma cholesterol by inhibiting HMG-CoA reductase activity and reduces the risk of coronary events.

3.2 Induction of hyperlipidemia

3.2.1 Experimental design

Male albino rats of Wistar strain, 150–200 g of body weight were selected under hygienic conditions and kept under standard environmental conditions (temperature: 24 ± 1 °C, light/dark cycle: 12/12 h) in Central Animal Facility of Bharathidasan University. All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (CPCSEA permission No. BDU/IAEC/ 2016 / OE /02/ Dt.17.3.16) of Bharathidasan
University, Tiruchirappalli, India. The rats were fed at *ad libitum* and were randomly divided into 8 Groups, comprising of six rats each.

Hyperlipidemia was induced in the experimental rats by a single intraperitoneal (IP) injection of Triton-WR 1339 (300 mg/kg b.wt.) and after 48 h rats depicted elevated levels of serum cholesterol and triacylglycerol (Okazaki et al., 1990). The hyperlipidemic animals were given atorvastatin (10mg/kg/b.wt.) for 14 days or rutin. Rutin was given initially at a dose of (2.5, 5 & 10 mg/kg/b.wt.) and the lipid lowering effect (data not shown) in the serum was observed and rutin at 10 mg/kg/b.wt. Alone showed lipid lowering effect. Hence we decided to check the effect of rutin at a higher concentration (10, 25, 50, & 100mg/kg/b.wt.). Blood samples were collected at three different time intervals such as baseline (day 0, before rutin administration), day 8, and day 15 from both control and experimental rats. On the 15th day, after an overnight fast the rats were sacrificed by decapitation and the liver was removed from each rat for further analysis. The following treatment schedule was followed.

Group-I: Male Wistar rats were fed with control diet *ad libitum* (control animals) for 14 days.

Group-II: Control rats were administered with atorvastatin (10 mg/kg b. wt.) for 14 days.

Group-III: Control rats were administered with rutin (10 mg/kg b. wt.) for 14 days.

Group-IV: Hyperlipidemia in rats was induced by Triton-WR 1339 (300 mg/kg b. wt.).

Group-V: Hyperlipidemic rats were administered with atorvastatin (10 mg/kg b.wt.) for 14 days.

Group-VI: Hyperlipidemic rats were administered with rutin (10 mg/kg b.wt.) for 14 days.
Group-VII: Hyperlipidemic rats were administered with rutin (25 mg/kg b.wt.) for 14 days.

Group-VIII: Hyperlipidemic rats were administered with rutin (50 mg/kg b.wt.) for 14 days.

Group-IX: Hyperlipidemic rats were administered with rutin (100 mg/kg b.wt.) for 14 days.

3.2.2 Preparation of liver samples

The liver samples (100 mg/ml) were homogenized in 50 mM phosphate buffer (pH 7.4), and the homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant obtained was used for biochemical analysis.

3.3 Biochemical analysis

Total cholesterol (TC), triacylglycerol, and HDL in serum and tissues were determined using enzymatic kits (BioSystems Spain) following the manufacturer’s instructions. The atherogenic index (AI) was calculated as $AI = (TC - HDL)/HDL$ and the LDL was calculated by Friedewald’s formula (1972).

3.4 Analysis of mRNA expression of hepatic genes

3.4.1 RNA isolation and RT-PCR

The liver tissue was minced in polytron tube and passed through pipette several times. Trizol (sigma, USA) was added to the tissue (25 -100mg) and mixed well. Then chloroform (0.2 ml) was added, and the samples were shaken vigorously for 15 sec and kept at room temperature (RT) for 15 min. The mixture was centrifuged at 12000 x g for 15 min at 4 °C. The aqueous phase was transferred to a fresh tube and isopropanol (0.5ml) was added, mixed and incubated at RT for 10min. The RNA was pelleted by centrifugation (12000 x g) and the pellet was washed with ethanol (75%), air dried and
suspended in DEPC- treated water, and total RNA was estimated by spectrophotometry (A$_{260nm}$).

### 3.4.2 Western Blotting analysis

Liver tissue (60 mg protein) was suspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail) and subjected to SDS-PAGE. For immunoblot analyses, proteins were transferred to a nitrocellulose membrane at 120 V for 2 h and the membrane was incubated with the following primary antibodies (1:1000 dilutions) for 2h. Primary antibodies were anti-HMGCR, anti-SREBP-2, anti-ACC1, anti-CyP7A1, anti-ABCA1 (ABCAM, USA) and anti-β-actin (Sigma-Aldrich, St.Louis, MO, USA). This was followed by an ALP-conjugated goat anti-rabbit IgG (1:2500) for 1 h. The membrane was treated with a BCIP/NBT substrate system. The “Image J” software was used to quantify the band intensity.

### 3.5 Hepatic histological analysis

The liver was removed and fixed in a buffer solution containing 10% formalin, after which the fixed tissues were paraffin embedded, and 3-5-mm sections were prepared and stained with hematoxylin and eosin. The stained area was viewed using a microscope at a magnification of 40x.

### 3.6 Statistical analysis

Data were analyzed using the programs of GraphPad Prism 6.0 software package for Windows. All the values reported in the article were the mean of six animals and each experiment performed in replicates. Statistical analysis was carried out by post hoc testing and, for intergroup comparisons, using Tukey’s multiple comparisons test. Values are statistically significant at **p<0.01 and *p<0.05.
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4. RESULTS

4.1 Analysis of Active principle by HPLC

For the isolation and identification of the active compound, various proportions of methanol–water or acetonitrile–water system was chosen as a mobile phase, but separation was not satisfactory. Hence in succession, a mixture of methanol, acetonitrile, and water in different ratios was tested. Eventually, it was found that methanol–acetonitrile–water (40:15:45, v/v/v) system gave a much better separation under this system, with slight trailing peaks for rutin and quercetin were observed in the chromatograph (Fig. 1).

![Chromatogram of rutin (RU) and quercetin (QU), methanol–acetonitrile–water (40:15:45, v/v/v) methanol, acetonitrile or water and standard mixture (adding no acetic acid to mobile phase), Peak 1 for RU, usually used as a component of the mobile Peak 2 for QU.](image)

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**Totals**

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**Fig. 1** Chromatogram of rutin (RU) and quercetin (QU), methanol–acetonitrile–water (40:15:45, v/v/v) methanol, acetonitrile or water and standard mixture (adding no acetic acid to mobile phase), Peak 1 for RU, usually used as a component of the mobile Peak 2 for QU.
Ethanolic extract of *Cassia auriculata* was injected directly and separated under the optimum conditions as mentioned earlier. The typical chromatogram of the Et- CAF extract is shown in (Fig.2)

**Fig. 2** Chromatograms of a sample of ethanolic extract in *Cassia auriculata* flower (Arrow indicated Peak for rutin present in the sample)

### 4.2 Effect of rutin on serum biochemical parameters

The lipid profile in serum with animals from Group I (control), II (control + atorvastatin) and III (control + rutin 10 mg/kg/b. wt) were almost similar (Fig.1). The Triton WR-1339 increased the lipid levels significantly (Group IV) when compared with Group I, II and III rats. The lipid profile of the hyperlipidemic rats was treated with different doses of rutin and the lipid parameters analyzed. Group VI animals (rutin 10 mg/kg/b. wt) showed a significant reduction in triacylglycerol (40%), cholesterol (37%), LDL (60%) and VLDL (42.7%) levels, but a marked increase in HDL (87%) after 14 days when compared to Group I (Fig.1A). The groups II and III behaved similar to Group I in all the experiments and so for convenient reasons the Groups II and III were not included in the further experiments. Among the rutin treatment groups (VI to
IX with 10, 25, 50 & 100 mg/kg b.wt. respectively) the group VI (10 mg/kg b. wt.) showed the maximum anti-hyperlipidemic activity, and so this concentration was alone used for the further analyses.

A) Triacylglycerol

B) Cholesterol
C) HDL

![Graph showing HDL levels over time for different groups.]

D) LDL

![Graph showing LDL levels over time for different groups.]

Control, Statin, Rutin, HC, HC+Statin, HC+Rutin 10mg, HC+Rutin 25mg, HC+Rutin 50mg, HC+Rutin 100mg
Fig.1 Serum lipid levels in hyperlipidemic rats: Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with drug statin and rutin (10, 25, 50, 100 mg/kg b. wt.). The triacylglycerol and cholesterol (total and in various lipo-proteins) were measured in serum. The experiments were performed in triplicates, and the values (mean ± SD) are representative of at least three independent experiments. The control group was compared with the hyperlipidemic group (###p < 0.001) and the hyperlipidemic group was compared with the statin group (**p < 0.01) or rutin treated group (###p < 0.01) (**p < 0.01, *p < 0.05 and NS-not significant).

4.3 Effect of rutin on the hepatic mRNA expression of lipogenic genes

The SREBP-1c and ACC-1 regulate the de novo lipogenesis (Gondr et al., 2001; Kim and Spiegelman, 1996). Induction of hyperlipidemia (Group IV) significantly up regulated the mRNA expression of SREBP-1c and ACC-1 in the liver; whereas atorvastatin and rutin markedly reduced them (Fig.2A&B).
Fig. 2 Effect of rutin on hepatic mRNA expression of lipogenic genes. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed using statin and rutin (10 mg/kg b.wt.). The mRNA expression levels of (a) SREBP-1c, (b) ACC1 were quantified using RT-PCR and were normalized with actin. The experiments were performed in triplicates and the values (mean ± SD) are representative of at least three independent experiments. The control group was compared with the hyperlipidemic group (**p < 0.001), and the hyperlipidemic group was compared with the statin group (***p < 0.001) rutin treated group (##p < 0.01) (###p < 0.001, **p < 0.01, *p < 0.05 and NS-not significant.

4.4 Effect of rutin on the hepatic expression of cholesterol biosynthetic genes

HMGR is the regulating enzyme in the mevalonate (required for cholesterol and other isoprenoids) synthesis. Besides HMGCR, the SREBP-2 is also known to up-regulate the genes involved in cholesterol biosynthesis and uptake. We measured the expression of HMGCR, HMGCS, and SREBP-2 in Triton WR-1339 induced hyperlipidemic rats with or without rutin treatment. The HMGCR mRNA expression was up-regulated in the hyperlipidemic rats, and the treatment with atorvastatin or rutin reverted (10 mg/kg b.wt.) the aberrant mRNA expression levels back to control levels. Similar results were also found for SREBP-2 expression. These results suggested that atorvastatin and rutin suppressed the HMGCR, HMGCS, and SREBP-2 expression compared to the hyperlipidemic group. (Fig.3 A, B &C).
A) SREBP-2

B) HMGS

C) HMGR

Fig. 3 Effect of rutin on the expression of cholesterol regulatory genes in the liver. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with drug statin and rutin (10mg/kg b.wt). The mRNA expression levels of (a) SREBP-2, (b) HMGCS & c) HMGCR were determined using RT-PCR and were normalized with actin. The experiments were performed in triplicates and the values (mean ± SD) are representative of at least three independent experiments. The control group was compared with the hyperlipidemic group (**p < 0.001), and the hyperlipidemic group was compared with the statin group (**p < 0.001) or rutin treated group (**p < 0.001) (**p < 0.001, **p < 0.01, *p < 0.05 and NS-not significant).
4.5  Effect of rutin on the expression of bile acid synthesizing genes in the liver

The enzyme, CYP7A1 would increase bile acid production and decrease cholesterol accumulation in the liver, leading to down regulation of LDL receptors. The gene expression and protein expression of CYP7A1 was up regulated in the liver of hyperlipidemia (Triton WR- 1339 treated) induced rats and rutin (10 mg/kg/b.wt.) administration reverted them back to control levels (Fig.4).

![Image](441x528)

**Fig.4 Effect of rutin on the expression of bile acid synthesizing genes in the liver.** Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with statin and rutin (10mg/kg b.wt). The mRNA expression level of Cyp7A1 was determined using RT-PCR and were normalized with actin. The experiments were performed in triplicates and the values (mean ± SD). The control group were compared with the hyperlipidemic group (**p < 0.001), and the hyperlipidemic group was compared with the statin group (**p < 0.001) or rutin treated group (***p < 0.001) (**p < 0.001, *p < 0.01, *p < 0.05 and NS- not significant).
4.6 Effect of rutin on the gene expression of ABCA1 (involved in cholesterol efflux) in the liver

The mRNA expression levels of ABCA1 were measured in the Triton WR–1339 induced hyperlipidemic rats (Fig.5). There was a down-regulation in the expression levels of ABCA1 in the liver of the hyperlipidemia induced rats, and administration of rutin (10 mg/kg/b. wt) increased the expression compared to the hyperlipidemic group (Fig.5).

Fig.5 Effect of rutin on the expression levels of cholesterol efflux genes in liver. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with drug statin and rutin (10mg/kg b.wt). The mRNA expression levels of ABCA1 was determined using RT-PCR and were normalized with actin. The values (mean ± SD) are representative of at three independent experiments. The control group were compared with the hyperlipidemic group (*p < 0.05), and the hyperlipidemic group were compared with the statin group (**p < 0.01) or rutin treated group (###p < 0.01) (###p < 0.001, **p < 0.01, *p < 0.05 and NS-not significant)
4.7 Effect of rutin on the protein expression for the cholesterol biosynthetic genes in rat liver

HMGR is the regulatory enzyme involved in the mevalonate (required for cholesterol and other isoprenoids) synthesis. Besides HMGR, the SREBP-2 is also known to up-regulate the genes involved in cholesterol biosynthesis and uptake. We measured the expression of HMGR, and SREBP-2 in Triton WR-1339 induced hyperlipidemic rats with or without rutin treatment. The protein expression of HMGR was up-regulated in the hyperlipidemic rats; atorvastatin and rutin reverted (10 mg/kg b.wt.) the protein expression levels back to control levels (Fig.6). Similar results were also found for SREBP-2 expression. These results suggested that atorvastatin and rutin suppressed the ACC1, CyP7A1, HMGR, and SREBP-2 expression compared to the hyperlipidemic group. (Fig. 6).
Fig. 6 Effect of rutin on the cholesterol biosynthetic genes in the liver. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with statin and rutin (10 mg/kg b.wt.). The protein levels of ACC1, CyP7A1, SREBP-2, HMGCR was determined using western blot analysis after normalizing with actin. The experiments were performed in triplicates and the values (mean ± SD) are representative of at least three independent experiments. The control group were compared with the hyperlipidemic group (###p < 0.001), and the hyperlipidemic group were compared to the statin group (**p < 0.001) or rutin treated group (##p < 0.01),(**p < 0.001, *p < 0.05 and NS-not significant).
4.8 Effect of rutin on the protein expression of ABCA1 (involved in cholesterol efflux) in the liver

The protein level of ABCA1 was measured in the Triton WR–1339 induced hyperlipidemic rats (Fig. 7). There was a down-regulation in the mRNA expression levels of ABCA1 in the liver of the hyperlipidemic rats, and with rutin treatment (10 mg/kg/b. wt) both the mRNA expression and the protein expression were increased when compared with the hyperlipidemic group (Fig. 7).

Fig. 7 Effect of rutin on the cholesterol efflux protein in the liver. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with drug statin and rutin (10 mg/kg b.wt). The protein expression level of ABCA1 was determined using western blot analysis after normalizing with actin. The experiments were performed in triplicates, and the values (mean ± SD) are representative of at least three independent experiments. The control group were compared to the hyperlipidemic group (**p < 0.001), and the hyperlipidemic group were compared to the statin group (***p < 0.001) or rutin treated group (###p < 0.001) (**p < 0.001, *p < 0.01, *p < 0.05 and NS - not significant).
4.9 Effect of rutin on liver histopathology of Triton induced hyperlipidemic condition

In Triton induced hyperlipidemic rats, fat deposit in the liver tissue was significantly larger than those in the control group Fig. 3(a) (b) (c) and (d). However, the data clearly showed that lipid droplet in liver was significantly decreased after treatment with rutin (10 mg/kg/b.wt.).

Fig. 8 Effect of rutin on the Triton induced hyperlipidemic rats and histopathological changes in the liver. Hyperlipidemia was induced in rats by Triton WR-1339. The anti-hyperlipidemic effect was analyzed with statin and rutin (10 mg/kg b.wt.). The liver sections of (a) Control (b) hyperlipidemic rats (c) Hyperlipidemic rats treated with statin (10mg/kg/b. wt.); (d) Hyperlipidemic rats treated with rutin (10mg/kg/b.wt.).HE (Haematoxylin Eosin) were stained with rutin and the different groups compared. The red arrows indicated the fat droplets in the liver sections.
5. DISCUSSION

Obesity, hypertriglyceridemia, and hypercholesterolemia are the common causes for many diseases such as cardiovascular and liver diseases. High cholesterol diet leads to dyslipidemic syndrome, and hyperlipidemia is characterized by increase in TAG and decrease in HDL-cholesterol. (Bloomgarden ZT 2004). Hyperlipidemia is a major cause of cardiovascular disease (Fodor et al., 2000; Gotto and LaRosa 2005). Treatment of hyperlipidemia ideally reduced the levels of low-density lipoprotein cholesterol (LDL-C, or “bad” cholesterol) in the blood and attenuates the risk of the disease (Ballantyne, 2007). Statin trials are the mainstays for the treatment of cardiovascular disease; however, statins are associated with a number of adverse events. These include muscle-related complaints, such as rhabdomyolysis, myalgia, cramps, and muscle weakness (i.e., myopathy) (Parker and Thompson, 2012; Sewright et al., 2007). The number of patients with statin-related disturbances has recently increased, due to the widespread use of these drugs as blood cholesterol-lowering agents Therefore; the development of promising cholesterol-lowering treatment alternatives to statins is of utmost importance. The Triton WR-1339, a non-ionic detergent (oxyethylatedtertiaryoctylphenolformaldehyde polymer), provokes acute hyperlipidemia in animal models, and thus has been widely used as a hyperlipidemia inducing agent to study lipid metabolism and the metabolic inter-relationship between plasma lipoproteins. (Zarzecki et al., 2014; Ghatak and Panchal 2012). Recent studies on fatty liver in food science have focused on searching functional food ingredients or herbal extracts that can suppress the accumulation of hepatic lipid. Rutin is a common dietary flavonoid that is present in fruits, including tomatoes, vegetables, and plant-derived beverages such as tea and wine. It has been reported that rutin has several pharmacological properties including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombic, vasoprotective, and cardioprotective activities (La Casa et al., 2000; Sheu et al., 2004; Mellou et al., 2006; Madan et al., 2006). In the current study,
rutin-supplement attenuated Triton WR-1339-induced hyperlipidemia by lowering the concentrations of total cholesterol, LDL and triacylglycerol. When compared to Triton WR-1339-induced hyperlipidemic group the rutin treatment reduced the hepatic lipid levels, serum triacylglycerol (40%), cholesterol (37%), LDL (60%) and VLDL (42%), but a significant increase of HDL (87%) was observed (Fig. 1). This pattern was similar to that observed with statin treatment and the levels were near control levels. Alteration of lipid and lipoprotein levels (Fig. 1A to E) were observed in the hyperlipidemic group and were reverted to near control values with rutin treatment as observed in statin. The rutin lowers the lipid components in the serum of hypercholesterolemic rats, probably by reducing the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase (Ziaee et al., 2009). Studies have shown that sterol regulatory element-binding proteins (SREBPs) regulate lipid metabolism. SREBP-1c plays an essential role in the regulation of lipogenesis involved in fatty acid synthesis, SREBP-1c is a well-known transcription factor regulating the gene expression of the lipogenic enzymes in the liver (7). The expression of SREBP-1c was increased in the liver of the hyperlipidemic rats (J.D. Horton, et al., 1998) and in our hyperlipidemic rats also we observed a similar pattern and was reverted to near control levels with the statin or rutin treatment. (Fig. 2 A&B).

The SREBP-2 regulates the transcription of genes involved in cholesterol biosynthesis, i.e. low-density lipoprotein receptor (LDLR), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGCR), and SREBP-2 (8 & 9). In the hyperlipidemic animals, the SREBP2 levels (both protein and mRNA expression) were reduced and were reverted to near normal levels with rutin treatment (Fig. 3A, B&C). The enzyme ACC1 was transcriptionally regulated by sterol regulatory element binding protein-1c (SREBP-1c). Both the ACC1 & SREBP-1c mRNA expression were up-regulated during hyperlipidemic condition and was reverted to near normal levels with rutin treatment. The bile acids play a critical role in the maintenance of mammalian cholesterol and administration of rutin decreased the expression of CyP7A1 when compared to Triton
WR -1339 induced hypercholesterolemic rats (Fig.4&6). ABCA1 is a trans-membranous protein and defect of the ABCA1 decreased serum HDL level, resulting in lipid accumulation. In cardiovascular diseases, serum HDL level decreased and led to precipitation of intracellular cholesterol in peripheral cells due to a defect of ABCA1 (S.M. Clee, et al., 2000). The Triton WR-1339 induced rats depicted a down-regulation of ABCA1 in the liver (Fig.5&7) and the level was reverted to normal with the administration of statin or rutin (10 mg/kg/b.wt.). The efflux of cholesterol was positively correlated with the levels of ABCA1 (Fig.5 & 7).

To conclude, the hyperlipidemia induced rats with the statin or rutin treatment depicted a reduction in the lipid levels. The treatment with rutin (10 mg/kg/b.wt.), reduced the serum lipids, LDL and VLDL but increased HDL levels as observed with hyperlipidemic animals with statin treatment. The expression of SREBP1, ACC-1, SREBP-2, HMGR, HMGS, CyP7A1, and ABCA1 all reverted as in statin treated animals and similar to those in the control animals. Thus rutin aids as a potential anti-hyperlipidemic drug and can be exploited commercially in future.
Summary

10 mg RUTIN

HDL-C level was increased

Expression of cholesterol synthesizing genes (mRNA & protein) reverted to control levels

Normal liver histopathology
Summary and Conclusion

Hyperlipidemia, is the most common form of dyslipidemia, and increases the risk of developing cardiovascular diseases. So the demand of finding natural therapeutics is in rise. Traditional Indian medicine has employed *Cassia auriculata* (*a herb, belonging to the family of Caesalpiniaceae*) to treat a range of disorders including hyperlipidemia. Here, we took the flower of *Cassia auriculata* for assessing its role in treating hyperlipidemia. In this study, we extracted solvent fractions (ethanol, ethyl acetate, acetone, and chloroform) of *Cassia auriculata* flower and determined which solvent exerts the maximum efficacy in treating hyperlipidemia. We found the ethanolic extract of *Cassia auriculata* flower (Et-CAF) to be the most effective solvent extract in alleviating hyperlipidemia induced by oleic acid in the model system *Saccharomyces cerevisiae*.

Et-CAF lowers the neutral lipid synthesizing genes (*LRO1, DGA1, ARE1* and *ARE2*) thereby reducing the triacylglycerol (TAG) and sterol ester (SE) levels. The
results were further supported by quantifying the BODIPY stained lipid droplets (LDs) of Et-CAF treated hyperlipidemic yeast cells which were considerably attenuated in size as well as numbers when compared to hyperlipidemic cells.

Rat model has intricate homoeostasis analogous to human; and the fate of cholesterol synthesis, logistics and degradation is tightly regulated. In order to substantiate our findings we used TritonWR-1339 to induce hyperlipidemia in male Wistar rats and the role of Et-CAF as an anti-hyperlipidemic drug was evaluated. The hyperlipidemic rats showed a significant surge in the expression of cholesterol synthesizing genes (SREBP2, HMGR, and HMGS), fatty acid synthesizing genes (ACC1, SREBP1c) and bile acid synthesising gene (CYP7A1) and down regulation of the cholesterol transport gene (ABCA1). Levels of low density lipoprotein (LDL), very low intensity lipoprotein (VLDL), triacylglycerols (TAG) were also increased. However the high density lipoproteins (HDL) were decreased. Histopathological study of rat liver showed an increase in fat lipid droplets (LDs) size, and number. The LDs were stained with HE stain (Haematoxylin Eosin). Et-CAF treatment restored the elevated expression of above mentioned genes (SREBP2, HMGR, HMGS, ACC1, SREBP1c, and CYP7A1), lipoproteins (LDL, VLDL) and TAG levels to near normal levels. The HDL levels and expression of ABCA1 was increased.

HPLC analysis revealed the active compound that possesses the anti-hyperlipidemic activity in Et-CAF to be rutin. So, we continued checking whether rutin exerts same magnitude of anti-hyperlipidemic potential as Et-CAF. The results exactly replicated the effect of Et-CAF in treating hyperlipidemia. Thus, rutin is the main therapeutic factor present in Et-CAF extract.
Finally, we conclude that Et-CAF effectively treats both hyperlipidemic *Saccharomyces cerevisiae* as well as the rat model.