Chapter 4

*Cassia auriculata* flower extract attenuates hyperlipidemia in male Wistar rats by regulating the hepatic cholesterol metabolism

1. **ABSTRACT**

Hyperlipidemia in the male albino Wistar rats were induced by Triton WR – 1339. The treatment of the hyperlipidemic animals with the ethanol extract of *Cassia auriculata* flower (Et-CAF) exhibited a dose dependent reduction in serum triacylglycerol, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) similar to the hyperlipidemic animals treated with standard drug atorvastatin. Hyperlipidemia altered the protein and mRNA expression levels of the key genes (SREBP-1c, ACC1, SREBP-2, HMGCR, HMGCS, CYP7A1, and ABCA1) in lipid metabolism and the treatment with Et-CAF (300 mg /kg b. wt) reverted these levels similar to that observed with atorvastatin treated hyperlipidemic animals. These results revealed that Et-CAF extract served as an efficient anti-hyperlipidemic drug.

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

Hyperlipidemia is characterized by elevated serum total cholesterol, increased LDL cholesterol and decreased HDL cholesterol (Saravanakumar et al., 2008). Hyperlipidemia with the accumulation of cholesterol, triacylglycerol, and defect in the transporting lipoproteins is considered to be a causative factor for arteriosclerosis (Ogel H.G.V. 1997). The lipoproteins serve as lipid carriers in the blood and are divided into chylomicrons, chylomicron remnants, VLDL (very low density lipoprotein), IDL (intermediate density lipoprotein), low density lipoprotein (LDL) and HDL (high density lipoprotein). HDL removes excess cholesterol from peripheral cells and delivers it to the liver, and so is a desirable lipoprotein. Apart from hyperlipidemia, hypercholesterolemia also plays an important etiological role in a variety of diseases, including hypertension, type 2 diabetes, coronary heart disease (CHD), and stroke (Pis-Sunyer FX 1993). The statins are widely used for treating cardiovascular diseases and has side effects; hence cholesterol-lowering drugs that are alternative to statin are sought. The natural products have been screened for lipid lowering and antioxidant activities and the World ethnobotanical information provides some herbal medicines from plants and vegetable sources that can control hyperlipidemia and related complications (Dahanukar et al., 2000). India is gifted with huge resources of medicinal and aromatic plants. There are numerous studies examining the role of C. auriculata (Cesalpinaceae, common name Tanner’s Cassia) as a therapeutic agent. It is a common plant in India and has been widely used in Ayurvedic medicine. The aqueous extract of C. auriculata (CA) controls diabetes in alloxan induced diabetic rats at a dose of 0.5 g/kg body weight (Pari et al., 2002; Uma et al., 2006; Lukmanul et al., 2007; Kumaran et al., 2007), by controlling blood glucose (Surana et al., 2008), dyslipidemia, cardiovascular risk (Javekar et al., 2006), rheumatism, and conjunctivitis (Joshi SG 2000). The alcoholic extract of CA leaf was effective against alcoholic liver injury (Kumar Rajagopal et al., 2003), and cancer (Prasanna et al., 2009). CA seeds are
used in treating ophthalmia, dysentery, and the root is used in the treatment of skin disease, leprosy, tumors, antipyretic, antiulcer, asthma and renal injury (Qadry S 2005; Annie et al., 2005; Nageswara Rao et al., 2000). The ethanolic extract of *C. auriculata* flower (Et-CAF) is reported to possess potent anti-hyperlipidemic and antioxidant activity along with anti-hyperglycemic effect and these beneficial activities may contribute to its cardio protective and anti-atherosclerotic role (Vijayaraj et al., 2013), but the molecular mechanism is yet to be studied. In this study, hyperlipidemia in male albino Wistar rats were induced by Triton WR-1339 (Schurr et al., 1972), and we investigated the protective effects of Et-CAF by examining the serum lipid profile, gene and protein expression of key cholesterol metabolic genes such as HMGR, HMGS, SREBP-1c, SREBP-2, ACC1, CyP7A1, and ABCA1 which are all involved in cholesterol metabolism.

3. MATERIALS AND METHODS

Materials

3.1 Chemicals

Atorvastatin and Triton WR-1339 (Tyloxapol) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Atorvastatin and Triton WR-1339 were dissolved in saline solution (pH 7.4). The doses of the Et-CAF extract used in this study are 100 mg, 200 mg, and 300 mg/kg b.wt. And the standard drug atorvastatin (10 mg/kg b.wt.) at the suggested dosage (Sikarwar et al., 2012), reduces plasma cholesterol effectively by inhibiting the HMG-CoA reductase. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

3.2 Preparation of ethanolic extract of *C. auriculata* flower

Fresh *C. auriculata* flowers (CAF) were collected from Bharathidasan University campus, Tiruchirappalli, Tamilnadu, India. The plant was identified and authenticated by Dr. S. John Britto, The Director, The Rapinat Herbarium and Centre
for Molecular Systematics St Joseph’s College Tiruchirappalli, Tamilnadu India with a voucher specimen (No.RVK 001). The flowers were dried under shade thoroughly and powdered. About 150 g of the dry CAF powder was extracted using different solvents (chloroform, acetone, ethyl acetate and ethanol). The anti-hyperlipidemic effect was maximum in the ethanol extract (Et-CAF) and so used in further studies (Vijayakumar et al., 2017). The combined ethanol extracts were concentrated in a rotary evaporator at reduced pressure to obtain about 33.50 g (22.33%, w/w) and stored at 4 °C until further use. During the study, the residual extract was suspended in saline and orally administered to the animals (Pari L and Latha M 2002).

3.3 Induction of Hyperlipidemia

Experimental design

Male albino rats of Wistar strain, 150–200 g body weight were 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 ad libitum and randomly divided into 8 Groups, comprising of six rats each.

Hyperlipidemia was induced in the experimental rats by a single intra peritoneal (IP) injection of Triton-WR 1339 (300 mg/kg b.wt.), and hyperlipidemia was observed after 48 h [18], and the rats depicted elevated levels of cholesterol and triglyceride in serum (Okazaki et al., 1990).

The hyperlipidemic animals were given Et-CAF (100, 200 & 300mg/kg/b.wt.) or atorvastatin (10mg/kg/b.wt.) orally for two weeks. Blood samples were collected at three different time intervals such as baseline (day 0, before CAF administration), day
8, and day 15 from both control and experimental rats. On the 15th day, after overnight fasting, 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).

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

Group-III: Control rats were administered with Et-CAF (300 mg/kg b. wt.) for 14 days.

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

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

Group-VI: Hyperlipidemic rats administered with Et-CAF (100 mg/kg b.wt.) for 14 days.

Group-VII: Hyperlipidemic rats administered with Et-CAF (200 mg/kg b.wt.) for 14 days.

Group-VIII: Hyperlipidemic rats administered with Et-CAF (300 mg/kg b.wt.) for 14 days.

### 3.4 Biochemical analysis

Total cholesterol (TC), triglyceride, and HDL in serum 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).
3.5 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 molecular analysis.

3.6 Analysis of mRNA expression of hepatic genes

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. 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 ºC. 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 down 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}). List of primer sequence is mentioned in Table 1.

3.7 Western Blotting analysis

Liver tissue (60 mg protein) was suspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mMNaCl, 5 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail) and subjected to SDS-PAGE. For immune blot analyses, proteins were transferred to a nitrocellulose membrane at 120 V for 2 h, and the membrane was incubated with the primary antibody (1:1000 dilution) for 2 h. Primary antibodies used 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 the secondary 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.
3.8 Histo-pathological studies

Conventional techniques of paraffin-wax sectioning and haematoxylin-eosin (HE) staining were used for histological studies. Slices of fresh hepatic tissue were cut and fixed in buffered neutral formalin fixative for 24 h. Following fixation, the tissue slices were washed and processed through an ascending series of alcohol (30%, 50%, 70%, 90%, and 100%), cleared in methyl salicylate, and infiltrated with wax at 57°C. The hepatic tissue, thus cleared, was embedded in paraffin. Sections of 6–8 μm thickness were cut, stained by aqueous haematoxylin and alcoholic-eosin, and then examined by bright-field microscopy (200x) (CarlZeiss Axioskop 2 plus; Jena, Germany).

3.9 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<0.0001, ***p<0.001, **p<0.01 and *p<0.05.
Table 1. Primer sequences and expected product sizes of the genes amplified

<table>
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<th>S.No</th>
<th>Gene</th>
<th>Primer</th>
<th>Size(Bp)</th>
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<td>1</td>
<td>HMGR</td>
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</tr>
<tr>
<td>2</td>
<td>HMGS</td>
<td>5’AGGCACAAAGACCTACACAGAG‘3 5’ GGCTGCTCTACCTCCGTCA‘3</td>
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<tr>
<td>3</td>
<td>ACC-1</td>
<td>5’ CTGGGGTGGTGGATTCCCATT’3 5’ GGTGGGCTTTAAACCCCCTCAT’3</td>
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</tr>
<tr>
<td>4</td>
<td>CyP7A1</td>
<td>5’ CCAAGTAAGTGCTCCACCCCTC‘3 5’ GTTCGCCTGTCCACCAACG‘3</td>
<td>119</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
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<tr>
<td>8</td>
<td>β-Actin</td>
<td>5’ ACGTGCCTTGGACTTTGAA 5’AGATGGAGGCAAAAGCGGTGA</td>
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4. RESULTS

4.1 Effect of Et-CAF extract on serum biochemical parameters

The serum lipid profile with the animals from Group I (control), II (control + atorvastatin) and III (control + Et-CAF 300 mg/kg/b. wt) were almost the same (Fig.1). The Triton WR-1339 increased the lipid levels significantly (Group IV) when compared with the control. The hyperlipidemic rats were treated with different doses of Et-CAF. Group VIII animals (Et-CAF 300 mg/kg/b. wt) showed a significant reduction in triacylglycerol (44.7%), cholesterol (34.11%), LDL (60%) and VLDL (44.7%) levels, but a marked increase in HDL (85%) after 15 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.

(A) Triacylglycerol  

(B) Cholesterol
Fig. 1 Serum lipid levels in hyperlipidemic rats. Hyperlipidemia was induced in rats by Triton WR-1339 and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. The triacylglycerol and cholesterol (total and in various lipoproteins) were measured in serum. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group was compared with the hyperlipidemic group (with statistical difference of ###p < 0.001) and the hyperlipidemic group was compared with the statin /ET-CAF treated group (with statistical difference of ***p < 0.001, **p < 0.01, *p < 0.05 and NS - not significant).
4.2 Effect of Et-CAF on the hepatic mRNA expression of lipogenic genes

The SREBP-1c and ACC-1 regulate the denovo lipogenesis (Kim et al., 1996). Induction of hyperlipidemia (Group IV) significantly up regulated the mRNA expression of SREBP-1c and ACC-1; whereas atorvastatin and Et-CAF markedly reduced those (Fig.2A&B). The mRNA and protein expression of ACC-1 (Fig. 6) were more significant with Et-CAF (300 mg/kg/b. wt).

![A) B) Fig.2 Effect of Et-CAF on hepatic mRNA expression of lipogenic genes. Hyperlipidemia was induced in rats by Triton WR-1339, and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. Using RT-PCR the mRNA expression levels of (a) SREBP-1c, and (b) ACC1 were determined after normalizing with actin. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared with the hyperlipidemic group (with statistical difference of ###p < 0.001), and the hyperlipidemic group were compared with the statin/ET-CAF treated group (with statistical difference of ***p < 0.001, **p < 0.01, *p < 0.05 and NS - not significant).]
4.3 **Effect of Et-CAF on the mRNA and 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, HMGS, and SREBP-2 in Triton WR-1339 induced hyperlipidemic rats with or without Et-CAF treatment. Their mRNA expression of HMGR was up-regulated in the hyperlipidemic rats; Atorvastatin and Et-CAF reverted (300 mg/kg b.wt.) the mRNA expression levels back to control levels (Fig. 3A-C). Similar results were also found for SREBP-2 expression. These results suggested that atorvastatin and Et-CAF suppressed the HMGR, HMGS, and SREBP-2 expression compared to the hyperlipidemic group. (Fig.3A-C).

A) SREBP-2  
B) HMGS
C) HMGR

**Fig. 3 Effect of Et–CAF on the expression of cholesterol regulatory genes in the liver.** Hyperlipidemia was induced in rats by Triton WR- 1339 and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. Using RT –PCR the mRNA expression levels of (a) SREBP-2, (b) HMGS & c) HMGR were determined after normalizing with actin. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared with the hyperlipidemic group (with statistical difference of ***p < 0.001), and the hyperlipidemic group were compared with the statin/ET-CAF treated group (with statistical difference of **p < 0.01, *p < 0.05 and NS - not significant).

**4.4 Effect of Et-CAF on the gene and protein expression of bile acid synthesizing gene in the rat liver**

The enzyme encoded by CYP7A1 increases bile acid production, and the liver of hyperlipidemia (Triton WR- 1339 treated) induced rats depicted an up regulation of the gene and protein expression of CYP7A. The Et-CAF (300 mg/kg/b.wt.) administration reverted it back to control levels (Fig.4).
Hyperlipidemia was induced in rats by Triton WR-1339, and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. Using RT-PCR the mRNA expression levels of Cyp7A1 was determined after normalizing with actin. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared with the hyperlipidemic group (with statistical difference of \(### p < 0.001\)), and the hyperlipidemic group were compared with the statin/ET-CAF treated group (with statistical difference of \(**p < 0.01, *p < 0.05\) and NS - not significant).

4.5 Effect of Et-CAF on the gene and 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.5). There was a down-regulation in the expression levels of ABCA1 in the liver of the hyperlipidemic rats, and expression was increased with Et-CAF (300 mg/kg/b. wt) administration as compared with the hyperlipidemic group (Fig.5)
Fig. 5 Effect of Et–CAF on the expression levels of cholesterol efflux gene in the liver. Hyperlipidemia was induced in rats by Triton WR-1339, and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. Using RT -PCR the mRNA expression levels of ABCA1 was determined after normalizing with actin. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared with the hyperlipidemic group (with statistical difference of *p < 0.05), and the hyperlipidemic group were compared with the statin /ET-CAF treated group (with statistical difference of **p < 0.01, ***p < 0.001, *p < 0.05 and NS - not significant).

4.6 Effect of Et-CAF 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 Et-CAF treatment. The protein expression of HMGR was up-regulated in the hyperlipidemic rats; atorvastatin and Et-CAF reverted (300 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 Et-CAF suppressed the ACC1, CyP7A1, HMGR, and SREBP-2 expression compared to the hyperlipidemic group. (Fig. 6)
Fig. 6 Effect of Et-CAF on the cholesterol regulatory protein expression in the liver. Hyperlipidemia was induced in rats by Triton WR-1339, and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. The protein expression levels of ACC1, CyP7A1, SREBP-2, HMGR, was determined by western blot analysis. The expression levels were normalized by actin. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared with the hyperlipidemic group (with statistical difference of ###p < 0.001), and the hyperlipidemic group were compared with the statin/ET-CAF treated group (with statistical difference of ###p < 0.001, **p < 0.01, *p < 0.05 and NS - not significant).
4.7 Effect of Et-CAF 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 expression levels of ABCA1 in the liver of the hyperlipidemic rats, and protein expression was increased with Et-CAF (300 mg/kg/b. wt) administration as compared with the hyperlipidemic group (Fig. 7)

*Fig. 7 Protein expression levels of cholesterol efflux genes in liver.* Hyperlipidemia was induced in rats by Triton WR-1339 and the anti-hyperlipidemic effect was analyzed with drug statin and various doses of Et-CAF. The protein expression levels of ABCA1 was determined after normalizing with actin using western blot analysis. The experiments were performed in triplicates, and the values (mean ± SD) were representative of six independent experiments. The control group were compared to the hyperlipidemic group, (with statistical difference of ***p < 0.001), and the hyperlipidemic group were compared with the statin/ET-CAF treated group (with statistical difference of **p < 0.01, *p < 0.05 and NS - not significant).
4.8  Effect of Et-CAF ethanolic extract on liver histopathology of triton induced hyperlipidemic condition

In triton induced hyperlipidemic rats, liver tissue was significantly larger than those in the control group compared to triton treated group, Fig. 8(a) and 3(b). However, the data clearly showed that lipid droplet liver of hyperlipidemic rats was significantly increased and decreased markedly after treatment with statin and Et-CAF treatment Fig.8 (c) HC + statin (d) HC+ Et-CAF +100mg/kg/b.wt. e) HC+ Et-CAF +200mg/kg/b.wt. f) HC+ Et-CAF +300mg/kg/b.wt.

Fig.8 Effect of Et-CAF 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 Et-CAF (100mg/kg/b.wt.); (e) Hyperlipidemic rats treated with Et-CAF (200mg/kg/b.wt.) f) Hyperlipidemic rats treated with Et-CAF (300mg/kg/b.wt.) were stained with and the different groups compared. The red arrows indicated the fat droplets in the liver sections.
Dysfunctional lipid metabolism is the important causative factor for cardiovascular diseases. The commercial anti-hyperlipidemic drug that is being used currently is reported to have a side effect. However, the traditional practitioners have used plant material to treat hyperlipidemic conditions, and it was considered worthwhile to investigate the claim in experimentally induced hyperlipidemia male Wistar rats. In our current study, an alternative drug from CAF (ethanol extract) has been tested for restoring the lipid levels to near control levels in the hyperlipidemic animals. The Triton-WR 1339 suppresses the lipases and blocks the lipoprotein uptake in the extra hepatic tissues, resulting in increased lipid level in the blood (Kellner et al., 1951). The sterol regulatory element binding proteins (SREBPs) are transcription factors that regulate the transcription of genes and are involved in cholesterol homeostasis, TAG, and fatty acid synthesis (Horton et al., 1998). Acetyl CoA carboxylase -1 (ACC-1) and fatty acid synthase (FAS), are the key enzymes involved in the denovo fatty acid synthesis and are regulated by SREBP-1c (Dentin et al., 2005; Kim et al., 2010). The expression of SREBP-1c was increased in the liver of the hyperlipidemic rats (Horton et al., 1998), and in our hyperlipidemic rats also we observed an increased expression of SREBP-1c which was reverted to near control levels with the statin/Et-CAF treatment. The 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGCS), 3-hydroxy-3-methylglutaryl coenzyme reductase (HMGCR), farnesyl dipiphosphate synthase (FDS), and squalenesynthase are regulated by SREBP2 (Cintra et al., 2012). In the hyperlipidemic animals, the SREBP2 levels (both protein and mRNA expression) were reduced and were reverted to near normal levels with Et-CAF treatment. When compared to Triton WR-1339 induced hyperlipidemic group the Et-CAF treatment reduced serum triacylglycerol (44.7%), cholesterol (34.11%), LDL (60%) and VLDL (44.7%), whereas a significant increase of HDL (85%) was observed (Fig.1). Alteration of lipid, lipoprotein levels (Fig.1A & E) and the mRNA expression
of key enzymes of cholesterol synthesis were observed during the hyperlipidemic animals and were restored to near control levels with atorvastatin or Et-CAF treatment (Fig. 3A& C). In the current study, there was a marked increase in hepatic mRNA expression and protein expression of SREBPs in hyperlipidemic rats and a concomitant decrease in animals receiving atorvastatin or Et-CAF. It was reported that during both the alcoholic and non-alcoholic fatty liver disease, the SREBP-1c levels were high (Brown MS and Goldstein JL 1997). Et-CAF treatment reduced the expression of SREBP-1c (Fig.2A&B) ACC-1, SREBP2, thus reducing the activation of HMGR and ACC-1, thereby inhibiting the synthesis of total cholesterol, TAG, and fatty acid in the liver. The CyP7A1 encoded cholesterol 7α-hydroxylase converts’s cholesterol to bile acids. The bile acids play a critical role in the maintenance of mammalian cholesterol and administration of Et-CAF 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 ABCAl decreases serum HDL level, resulting in lipid accumulation. In cardiovascular diseases, serum HDL level decreases and precipitation of intracellular cholesterol in peripheral cells was noticed, and there was a defect of ABCAI gene (Clee et al., 2000). The Triton WR-1339 induced rats depicted a down-regulation of ABCAI in the liver, and the level was reverted to normal (Fig.5&7) with the administration of Et–CAF (300 mg/kg/b.wt.).The efflux of cholesterol was positively correlated with the levels of ABCAI (Fig.5& 7).

To conclude, hyperlipidemic rats with Et-CAF treatment depicted reduced the lipid levels. The treatment with Et-CAF reduced serum lipids, LDL and VLDL and increased HDL levels as observed with hyperlipidemic animals with statin treatment. The expression of SREBP1, ACC-1, SREBP-2, HMGR, HMGS, CyP7A1, and ABCAI all reverted as in statin treated animals and similar to those in the control
animals. Thus Et-CAF aids as a potential anti-hyperlipidemic drug and can be exploited commercially in future

SUMMARY