Research article

The modulating effect of ferulic acid on high fat diet induced hyperlipidemia and obesity: A dose response study in male Sprague Dawley ratsT. A. K. Mumtaz Begum¹, V. Ramamurthy²¹Research Scholar, ²Head, Postgraduate and Research Department of Biochemistry, Marudupandiyar College (Affiliated to Bharathidasan University), Thanjavur - 613 403, Tamil Nadu, India

(Received: May 2021 Revised: July 2021 Accepted: July 2021)

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ABSTRACT

Introduction and Aim: Ferulic acid (FA) is a phenolic compound predominantly found in cereals have been used in traditional Chinese medicine. Here, we studied the effect of FA on high-fat diet (HFD) induced hyperlipidemia and obesity in rats.

Materials and Methods: Hyperlipidemia was induced in male Sprague Dawley rats by feeding HFD for 14 weeks. The hypolipidemic effect was evaluated by co-administering 50, 100, 200 and 250 mg/kg body weight of FA. At the end of the experimental period, rats were sacrificed and serum/plasma levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), 3-hydroxy-3methyl-glutaryl-coenzyme A reductase (HMG CoA reductase) and adiponectin were determined. Moreover, Histopathological examination of liver and visceral adipose tissue (AT) was also carried out.

Results: HFD treatment significantly increased weight gain, body mass index, total fat pad mass, blood lipids, LDL cholesterol and serum transaminases. HFD +FA fed rats showed a significant decrease in blood lipids and an increase in antioxidant enzymes when compared to the HFD control rats. The activity of HMG CoA reductase and serum adiponectin levels were elevated in rats administered with FA. Among the 4 doses studied, 200 mg of FA/kg body weight exhibited optimum hypolipidemic activity. Histological observations in the liver and visceral AT added additional evidence for the lipid-lowering effect of FA.

Conclusion: These findings indicate that FA can act as a hypolipidemic agent, probably by modulating the activity of HMG CoA reductase and serum adiponectin levels.

Keywords: HMG CoA reductase; adiponectin; blood lipids.

INTRODUCTION

Hyperlipidemia and obesity are the major risk factors leading to the development of cardiovascular diseases, type 2 diabetes mellitus, hypertension, fatty liver diseases and metabolic syndrome. Clinically, hyperlipidemia can be defined as a heterogeneous group of disorders characterized by higher levels of TC, TG, very low-density lipoprotein (VLDL), LDL, intermediate-density lipoprotein (IDL), and lower levels of HDL. Obesity is a global epidemic disease generally characterized by an excessive increase in body fat mass. According to the World Health Organization, the prevalence of obesity is rapidly increasing worldwide, including both developed and developing countries (1).

The pathogenesis of obesity is not only influenced by common factors like genetics, environmental, and lifestyle modifications but also by several metabolic parameters like glucose, insulin, fatty acids, adipocytes, gut peptide hormones, gastric and nervous systems are involved in the regulation of appetite control. However, AT plays a key role in the pathophysiology of obesity (2).

AT is an important endocrine and fat storage organ that plays a significant role in regulating several aspects of fat metabolism. Therefore, it is necessary to study the structure, composition, and function of AT to understand the consequences of obesity. AT is a connective tissue mainly composed of adipocytes. This tissue is widely distributed throughout the body with differences in their location, structure, function, and composition. White AT (WAT) is found subcutaneously underneath the skin (sc WAT) and around intra-abdominal viscera (vis WAT). The primary function of WAT is to efficiently store lipids and release it when the energy supply is required by the body. In contrast, brown AT (BAT) is involved in heat generation, a process called thermogenesis by uncoupling mitochondrial fatty acid oxidation (3). AT also secretes hormones termed as adipokines or adipocytokines.

Adiponectin acts as a starvation signal and circulates in different multimeric forms. The trimeric form is found in human cerebrospinal fluid and the high molecular weight (HMW) multimer form is abundant in the plasma of females, whereas hexameric forms are found in the plasma of males. Adiponectin activity is

regulated by the phosphorylation of receptors AdipoR1 and AdipoR2. The former is expressed abundantly in muscle, while the latter is predominantly expressed in the liver. Deficiency of both these receptors prevents adiponectin binding and induces inflammation, oxidative stress, and insulin resistance. Plasma adiponectin levels are reduced in obese mice and increased in response to fasting, suggesting the potential role of adiponectin in adiponectin knock-out mice (4).

HMG CoA reductase is the rate-regulating enzyme found in the liver, which is responsible for cholesterol biosynthesis. This enzyme is the target for pharmacological drug like statin, rimonabant, and sibutramine. Hypolipidemic drugs are usually tested for their inhibitory effect on the activity of the HMG CoA reductase enzyme.

Living cells are highly equipped with enzymatic and non-enzymatic antioxidant pathways against reactive oxygen species (ROS) that are generated during oxidative metabolism. ROS causes oxidative stress and may damage bio-membranes, lipids, lipoproteins, and nucleic acids. Oxidative stress caused by ROS is associated with the pathogenesis of obesity and oxidation of LDL plays a critical role in causing cardiovascular diseases (5). Furthermore, oxidative damage is aggravated by the decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which act as free radical scavengers.

Effective treatments for hyperlipidemia include the statin group of drugs, nicotinic acid, bile acid resins, and fibrates, but these drugs produce adverse side effects like hyperuricemia, myositis, gastric irritation, flushing, dry skin, and abnormal liver function. Therefore, it is a national priority to control the onset of hyperlipidemia and obesity by identifying non-toxic natural food-based therapeutic drugs.

FA (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound that belongs to the family of hydroxy-cinnamic acid derivatives. FA was first isolated from *Ferula foetida* in 1866 and was named from the botanical name of the plant. Barley, oats, rice, and wheat are the major sources of FA. Other sources are vegetables, fruits, flowers, coffee, and cereals. FA is a known powerful antioxidant, anti-inflammatory, antihypertensive and antidiabetic agent (6). Moreover, recent studies have revealed that FA acts as an adjuvant in cancer therapy. FA is also used for the treatment of skin aging, photo aging, hyperpigmentation, seborrheic, and acne. Researchers also suggest that FA has antimicrobial, antifungal, and antiviral effects (7). However, limited investigations have been carried out on the hypolipidemic activity of FA. Thus, the present study aimed to evaluate and highlight the potential of FA against hyperlipidemia induced experimental rats.

MATERIALS AND METHODS

Chemicals and reagents

ELISA kits for the determination of serum adiponectin were purchased from Abcam, USA. FA and all other chemicals used for the analysis were of analytical grade bought from Merck, India through authorized dealers.

Composition of HFD

HFD was prepared and fed to the experimental animals as recommended by Nasimento *et al.*, (8). The ingredients which were used to prepare the HFD are ground labina, roasted peanuts, casein, corn oil, and french-fried potatoes at 439, 218, 129, 61, and 153 g/kg body weight (b. wt) respectively. They were ground and then mixed with vitamins and minerals (1-1.2 g per 100g of HFD). The mixture was then made into pellets and dried in a ventilated drying oven at $55\pm5^{\circ}\text{C}$.

Animals and husbandry

Thirty-six male Sprague Dawley rats weighing 175-200g were individually housed in polyethylene cages for 7 days under controlled conditions ($22\pm2^{\circ}\text{C}/\text{RH } 50\pm5\%$) in a 12:12 h L:D cycle and fed a standard rat chow pellet diet containing an adequate quantity of vitamins and minerals. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) (XXIII/VELS/PCOL/14/2000/CPCSE/IAEC/07.02.2020).

Experimental design

After the acclimatization period of 1 week, rats were divided into 6 groups. Group 1 (control) and 2 were fed a normal diet. Group 3-7 received HFD for 14 weeks. In addition, groups 4 to 7 were co-administered with 50, 100, 200, and 250 mg of FA/kg b. wt and group 2 rats with 200 mg/kg b. wt FA from the 3rd week onward. During the experimental period, b. wt and body mass index (BMI) of the animals were recorded weekly for 14 weeks. BMI was calculated using the formula: $\text{BMI} = \text{b. wt (g)} / \text{length}^2 (\text{nose-anus (cm}^2\text{)})$. At the end of the experimental period, all the rats were anaesthetised using diethyl ether and euthanized by cervical decapitation. Immediately blood was collected, and the plasma/serum was separated and stored at 4°C until analysis. AT was isolated and weighed from the epididymal, visceral, and retroperitoneal fat pad. The sum of the entire fat pad mass was called total fat pad mass (TFP). The organs such as the liver and heart were dissected out and washed in ice-cold saline and used for various biochemical experiments.

Tissue homogenate preparation

The isolated liver and heart tissues were washed with ice-cold saline and homogenized in 0.1M Tris HCl buffer (pH 7.4) at a low temperature. The collected

supernatant was used to determine protein and antioxidant enzymes such as GPx, SOD, and CAT.

Biochemical parameters

(i) Determination of serum adiponectin

ELISA kit (ab108784) was used for the quantitative determination of serum adiponectin. The assay was performed as per the instructions of the kit manual.

(ii) The activity of HMG CoA reductase

HMG CoA reductase activity was assayed indirectly by assessing the ratio of HMG CoA to mevalonate in the liver as described by Philip and Shapiro (9). The specific activity of the enzyme was expressed as the ratio of HMG CoA to mevalonate. The lower ratio indicates higher enzyme activity and vice versa.

(iii) Estimation of protein

The protein present in tissue homogenate was determined according to the method of Bradford (10). It was used to calculate the specific activities of enzymes.

(iv) Estimation of blood lipids and liver marker enzymes

Zak's method (11) was used for the determination of TC. TG was calculated by the method of Van Handel and Ziversmit (12). HDL cholesterol was measured by following precipitation with heparin and manganese chloride (13). The precipitate was sedimented using centrifugation for 30 minutes at 1000g. An aliquot of the clear supernatant was extracted with isopropanol; the extract was treated with a zeolite containing mixture to remove interfering substances and concentration of cholesterol was then determined. The estimation of LDL was done by a dual precipitation method (14). The plasma was incubated with sodium dodecyl sulphate for 2 h and then centrifuged at 10,000 g for 10 minutes at 4°C. VLDL will be aggregated as a pellicle at the top. The supernatant containing a mixture of HDL and LDL were expressed in terms of cholesterol. The activity of liver marker enzymes such as alanine transaminases (ALT) and aspartate transaminases (AST) were also determined (15).

(v) Estimation of GPx and antioxidant enzymes

The activity of GPx was assayed by the method of Flohe and Gunzler. A known amount of enzyme

preparation was allowed to react with hydrogen peroxide (H_2O_2) and glutathione (GSH) for a specified time. The GSH content remaining after the reaction was measured by Ellman's reaction. The activity of GPx was expressed as nm of glutathione oxidized / min/ mg protein (16). SOD activity was determined according to the method of Kakkar *et al.*, (17). Inhibiting the formation of nitro blue- tetrazolium to blue coloured formazan in the presence of phenazine methosulfate and NADH was measured at 540 nm using n-butanol as blank. The decomposition of H_2O_2 in the presence of CAT was measured at 240 nm (18). CAT activity was defined as the amount of enzyme required to decompose 1 μ M of H_2O_2 consumed / min/ mg protein.

Histopathological examinations

Liver and visceral AT tissue was fixed in 10% (v/v) formalin for histopathological studies. It was processed through a graded series of alcohol, cleared in methyl benzoate, paraffin methyl benzoate and paraffin-embedded for light microscopic study. Paraffin-embedded tissue sections cut at 5 μ M thickness were prepared and then stained with hematoxylin and eosin. The morphological assessment was done under a light microscope at 400x magnification (19).

Statistical analysis

Data were analysed by the statistics software package (SPSS for Windows v.10). The statistical significance of mean values between different groups was determined by applying one-way ANOVA with post hoc Bonferroni test and P value < 0.05 was considered significant.

RESULTS

Effect of FA on weight gain, BMI and TFP

The weight gain, BMI, and TFP of all experimental rats are shown in Table 1. Group 3 rats fed with HFD showed a significant increase in weight gain, BMI, and TFP ($P=0.000$) when compared to Group 1 control rats. FA co-administered rats showed a significant decrease in body weight, BMI, and TFP. The effect was more significant in Group 6 rats which received 200 mg/kg body weight of FA.

Table 1: Effect of various concentrations of FA on weight gain, BMI, and TFP mass in control and experimental rats

Groups	Net weight gain (g) (Final-initial)	BMI (g/cm^2)	TFP (g)
Group 1(Control)	154.4 $\pm$ 20.99	0.58 $\pm$ 0.06	14.3 $\pm$ 1.70
Group 2 (FA Control)	153.5 $\pm$ 20.57 ^{NS}	0.57 $\pm$ 0.08 ^{NS}	13.3 $\pm$ 1.53 ^{NS}
Group 3(HFD)	265 $\pm$ 38.16*	1.67 $\pm$ 0.24*	26.2 $\pm$ 3.51*
Group 4 (HFD + FA 50 mg/kg b.wt)	197.3 $\pm$ 25.96*	1.37 $\pm$ 0.20*	22.7 $\pm$ 2.49*
Group 5 (HFD + FA 100 mg/kg b.wt)	192.5 $\pm$ 25.75*	1.24 $\pm$ 0.19*	19.7 $\pm$ 2.21*
Group 6 (HFD + FA 200 mg/kg b.wt)	179.4 $\pm$ 21.53*	0.93 $\pm$ 0.11*	18.2 $\pm$ 1.86*
Group 7 (HFD + FA 250 mg/kg b.wt)	181.4 $\pm$ 22.67*	0.98 $\pm$ 0.12*	17.8 $\pm$ 1.72*

[Values are expressed as mean $\pm$ S.D. for six animals in each group. Statistical significance was calculated by comparing Control vs. FA control, Control vs. HFD, HFD vs. HFD + FA. * $P=0.000$, NS = Non significant]

Effect of FA on blood lipids

Table 2, displays the plasma levels of TC, TG, HDL, and LDL. There was a significant ($P=0.000$) increase in TC, TG, LDL, and a non-significant decrease in the HDL level in Group 3 rats than in Group 1 rats. FA co-

administration increased the HDL level significantly ($P=0.000$) and decreased the level of TC, TG, and LDL in Group 3 rats. The lipid-lowering effect was more significant in Group 6 and Group 7 rats which received 200 and 250 mg/kg body weight of FA.

Table 2: Effect of various concentrations of FA on TC, TG, LDL, and HDL in the plasma of control and experimental rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1(Control)	65.23 $\pm$ 8.87	92.55 $\pm$ 9.35	25.6 $\pm$ 3.05	18.7 $\pm$ 2.17
Group 2 (FA Control)	63.07 $\pm$ 8.45 ^{NS}	90.6 $\pm$ 12.41 ^{NS}	26.8 $\pm$ 3.08 ^{NS}	17.1 $\pm$ 1.83 ^{NS}
Group 3 (HFD)	128.11 $\pm$ 18.45*	132.1 $\pm$ 19.42*	15.9 $\pm$ 2.13*	33.3 $\pm$ 3.53*
Group 4 (HFD + FA 50mg/kg b. wt)	119.13 $\pm$ 17.27*	124.5 $\pm$ 17.43*	18.6 $\pm$ 2.23*	29.7 $\pm$ 3.26*
Group 5(HFD +FA100mg/kg b. wt)	107.19 $\pm$ 12.26 [#]	117.3 $\pm$ 12.05*	31.4 $\pm$ 3.03 [#]	27.3 $\pm$ 3.39*
Group 6 (HFD + FA 200 mg/kg b. wt)	97.43 $\pm$ 11.70 [#]	104.3 $\pm$ 12.83*	20.4 $\pm$ 2.08 [#]	21.4 $\pm$ 3.13*
Group 7 (HFD + FA 250 mg/kg b. wt)	93.23 $\pm$ 10.50*	110.7 $\pm$ 11.87*	19.3 $\pm$ 2.01*	20.8 $\pm$ 2.57*

[Values are expressed as mean $\pm$ S.D. for six animals in each group. Statistical significance was calculated by comparing Control vs. FA control, Control vs. HFD, HFD vs. HFD + FA. * $P=0.000$, # $P=0.001$, NS = Non significant].

Effect of FA on HMG CoA reductase in liver tissue and serum adiponectin

Table 3, represents the activity of HMG CoA reductase in the liver tissue and serum adiponectin level of all experimental rats. HMG CoA reductase activity was assayed indirectly by assessing the ratio of HMG CoA to mevalonate in hepatic tissue. The

lower ratio indicates higher enzyme activity and vice versa. We observed a significant elevation in the activity of HMG CoA reductase and a significant decrease in the serum adiponectin level in Group 3 HFD fed rats. Treatment with FA markedly reduced the activity of HMG CoA reductase and elevated the serum adiponectin level in group 4, 5, 6 and 7 rats.

Table 3: Effect of various concentration of FA on the activities of HMG CoA Reductase in liver tissue and serum adiponectin level of control and experimental rats

Groups	HMG CoA reductase (Ratio of HMG CoA to Mevalonate) ***	Adiponectin (μ g/ml)
Group 1(Control)	4.25 $\pm$ 0.5	6.39 $\pm$ 0.72
Group 2 (FA Control)	4.0 $\pm$ 0.52 ^{NS}	6.32 $\pm$ 0.92 ^{NS}
Group 3(HFD)	2.66 $\pm$ 0.29*	3.25 $\pm$ 0.42*
Group 4 (HFD + FA 50 mg/kg b. wt)	3.42	4.37
Group 5 (HFD +FA 100 mg/kg b. wt)	5.75 $\pm$ 0.48 ^{NS}	4.93 $\pm$ 0.49
Group 6 (HFD +FA 200 mg/kg b. wt)	6.3 $\pm$ 0.81*	5.34 $\pm$ 0.64*
Group 8 (HFD +FA 250 mg/kg b. wt)	7.0 $\pm$ 0.91*	5.02 $\pm$ 0.53*

[Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. FA control, Control vs. HFD, HFD vs. HFD + FA. *** Lower ratio indicates higher enzyme activity and vice versa. Values were expressed as * $P=0.000$, NS = Non significant].

Effect of FA on antioxidant parameters

Activities of GPx, SOD, and CAT in the liver and heart tissues of experimental rats are depicted in Table 4. Their activities decreased significantly in HFD

induced Group 3 rats when compared to the control rats. An improved antioxidant status was observed in FA co-administered rats mainly at the concentration of 200 and 250 mg/kg b. wt.

Table 4: Effect of various concentrations of FA on the level of GPx, CAT, and SOD in the liver and heart tissue of experimental rats

Groups	GPx		CAT		SOD	
	nM/ min/mg protein		μ m/min/mg protein		μ m/min/mg protein	
	Heart	Liver	Heart	Liver	Heart	Liver
Group 1 (Control)	22.6 $\pm$ 3.25	16.5 $\pm$ 1.782	8.7 $\pm$ 1.061	4.93 $\pm$ 0.592	2.7 $\pm$ 0.27	4.21 $\pm$ 0.42
Group 2 (FA control)	20.9 $\pm$ 2.36 ^{NS}	16.3 $\pm$ 1.92 ^{NS}	7.9 $\pm$ 0.98 ^{NS}	3.67 $\pm$ 0.45 ^{NS}	2.44 $\pm$ 0.290 ^{NS}	3.7 $\pm$ 0.425 ^{NS}
Group 3 (HFD)	18.1 $\pm$ 2.06 ^S	13.6 $\pm$ 1.66 [#]	5.90 $\pm$ 0.75 *	1.3 $\pm$ 0.162 *	1.93 $\pm$ 0.218*	2.4 $\pm$ 0.273*
	18.48 $\pm$ 2.78	15.7 $\pm$ 1.76*	6.8 $\pm$ 0.82*	2.56 $\pm$ 0.52*	2.13 $\pm$ 0.32 *	3.08 $\pm$ 0.43 *
Group 5 (HFD + FA 100 mg/kg b.wt)	19.45 $\pm$ 2.78	16.4 $\pm$ 1.76*	7.2 $\pm$ 0.82*	3.52 $\pm$ 0.52*	2.5 $\pm$ 0.32 *	4.08 $\pm$ 0.43 *
Group 6 (HFD + FA 200 mg/kg b.wt)	21.5 $\pm$ 3.01	17.6 $\pm$ 2.41*	8.9 $\pm$ 1.41 *	4.8 $\pm$ 0.60 *	3.3 $\pm$ 0.366 *	5.00 $\pm$ 0.585 *
Group 7 (HFD + FA 250 mg/kg b.wt)	22.8 $\pm$ 3.67*	18.5 $\pm$ 2.97*	7.12 $\pm$ 0.85 ^N _s	4.12 $\pm$ 0.54*	2.76 $\pm$ 0.33*	4.85 $\pm$ 0.48*

[Data were analysed by one way ANOVA followed by *post hoc* Bonferroni test. Statistical significance was calculated by comparing Control vs. FA control, Control vs. HFD, HFD vs. HFD + FA. $^{\$}P = .011$, $^{\#}P = 0.006$, $^*P = 0.000$, NS = Non significant].

Effect of FA on liver marker enzymes

Fig. 1, shows an elevation in the activity of liver marker enzymes (AST and ALT) of Group 3 rats than

in Group 1 rats. Administration of FA reverted the levels of these markers in a dose-dependent manner up to the dose of 250 mg/kg b. wt.

Fig. 1: Effect of various concentrations of FA on ALT and AST activity levels of control and experimental rats.

[Values as mean $\pm$ SD for 6 animals in each group. Data were analysed by one way ANOVA followed by *post hoc* Bonferroni test. Statistical significance was calculated by comparing Control vs. FA control, Control vs. HFD, HFD vs. HFD + FA. $^*P = 0.000$, NS = Non significant]

Histopathology of liver and visceral AT

Fig. 2 and 3, illustrates the photomicrographs of the liver and visceral AT from all the experimental groups, respectively. Liver sections from HFD fed rats (Group 3) showed fat deposition, inflammation, cell necrosis, and accumulation of fat droplets (2C). Moreover,

photomicrographs of visceral AT showed hypertrophied adipocytes in Group 3 rats. Tissue architecture of liver (2E and F) and visceral AT (3E and F) of HFD induced rats co-administered with FA at 200 and 250 mg/kg body weight was found to be improved.

Fig. 2: Histopathological changes in the liver (H&E x 100) [Photomicrographs of liver showing normal tissue architectures in control rats (A) and no significant pathological alteration in FA control rats (B); A high degree of steatosis, inflammation and fat droplets in rats fed with HFD(C); (D and E) showing very mild steatosis and inflammation and fat accumulation; (F) showing normal and improved vesicles of hepatocytes; and (G) showing normal hepatic cells with very mild fatty regeneration]

Fig. 3: Histopathological changes in visceral adipose tissue (H&E x 100). [Photomicrograph of adipocytes from (A and B) control rats and rats treated with FA alone shows undisturbed structure.; (C) sections from rats administered with HFD showing large adipocytes; (D and E) showing moderately decreased adipocyte (F) HFD+FA 200mg/kg body weight showing small and large adipocytes; (G) HFD+FA 250mg/kg body weight showing a mild decrease in the size of adipocytes.

DISCUSSION

Feeding of HFD for the animal models of hyperlipidemia used in this study is a well-accepted design that resembles human obesity. BMI is a measure of relative weight based on weight in kilograms divided by height in metre². Pankaj et al., stated that HFD induced in laboratory rats possibly increased the body weight, BMI, and TFP when compared to the rats fed with a normal diet (20). In our research, FA was found to decrease the body weight, BMI, and TFP significantly at the dosage of 200 mg/kg body weight.

The HFD used in the present study showed an increase in the plasma TC, TG, and LDL levels. This is because of the metabolic products of HFD which serves as a precursor for endogenous synthesis of lipids thus contributing to elevated blood lipid levels. Another reason for the increase in the blood lipid levels in group 3 rats may be due to the increase in the *de novo* synthesis of lipids in the liver and exchange with plasma lipids or both. Furthermore, elevated plasma LDL levels in HFD fed rats might be due to impairment in the activity of LDL receptors, which usually clear LDL from the plasma. Therefore, a rise in the plasma concentration of LDL is highly dependent on the ratio of formation and clearance from the circulation. The lipid-lowering effect of FA is well evidenced by the decreased level of LDL and increased level of HDL. These results are also consistent with Naowaboot *et al.*, who showed that FA has anti-atherogenic properties (21).

Regarding the plasma concentration of TG, HFD was found to influence TG composition, structure, and chain length of fatty acids which are considered as important determinants of atherogenicity. The present findings are in agreement with Salazar et al., who had also proved that plasma TG level increased significantly after treating rats with HFD which was later suppressed by FA (22). From the present outcomes, it can be concluded that FA effectively reduced the level of TG in HFD+FA treated rats.

HMG CoA reductase is an important enzyme in cholesterol biosynthesis which catalyses the conversion of HMG CoA to mevalonate. The lower ratio indicates higher enzyme activity and vice versa. An increase in HMG CoA reductase: mevalonate ratio was observed in HFD fed rats indicating higher HMG CoA reductase activity when compared to those fed with HFD+FA. There may be two reasons for the higher activity of HMG CoA reductase in group 3 rats. Firstly, diet enriched with saturated fatty acids increases the availability of acetyl CoA. Secondly, a high amount of cholesterol and saturated fatty acids is associated with the down regulation of LDL receptors. These results are further supported by elevated levels of blood cholesterol in HFD control. Hence, it is evident that FA can inhibit cholesterol biosynthesis via competitive inhibition of HMG CoA reductase.

Adiponectin is the first identified AT peptide being negatively correlated with hyperlipidaemia and obesity. Moreover, adiponectin is exclusively secreted from AT into the bloodstream and also abundant in

plasma. Thus, the level of adiponectin has been postulated to be inversely proportional to BMI. Our results show that increased adiponectin levels in FA treated rats might have been responsible for reducing hyperlipidaemia associated with HFD.

Oxidative stress is commonly associated with the development of obesity-related co-morbidities. Particularly, the cellular defence mechanism and their influence against many diseases such as coronary heart disease, metabolic syndrome, and diabetes mellitus. In hyperlipidemia, many factors contribute to oxidative stress including irregular production of adipokines generating ROS, increased rate of free radical formation from enzymatic sources within cells causing inflammation. In obesity, oxidation of LDL leads to damage in the conformation of lipoprotein, the oxidized LDL in turn is recognized by monocytes or macrophages of the arterial wall leading to the development of atherosclerotic plaques. In the present study, HFD contains saturated fat and this could have led to the excess formation of free radicals.

Under normal conditions, cells exhibit an effective enzymatic antioxidant pathway against ROS. Initially, the dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) which is catalysed by SOD, and later, the conversion of H_2O_2 to H_2O by GPx or CAT. The decrease in the activities of SOD, GPx, and CAT in the present study is supported by the findings of Marseglia *et al.*, who stated that hyperlipidemia is associated with decreased antioxidant status (23). Hence, FA co-administration restored the level of SOD, CAT, and GPx in HFD fed rats. The excellent scavenging property of FA is due to the phenolic ring structure that gives strong resonance stability and can accept the electron more easily from free radicals (24). Besides, the antioxidant property of FA was found to be more effective at a dosage of 200 mg/kg body weight.

In our study, HFD promoted the serum transaminases (AST and ALT) levels. As a consequence, changes in the liver are well-supported by the histopathological examinations. On the other hand, HFD significantly affected the development of adipocytes of visceral AT of group 3 rats showed clear hyperplasia and hypertrophy which are the characteristic feature of increased fat storage. On the contrary, the histological changes induced by HFD in the liver and AT of experimental rats were alleviated by FA proving its hepatoprotective and hypolipidemic activity.

CONCLUSION

To conclude, the results of our study proved that FA possesses anti-atherogenic, antioxidant, anti-inflammatory, and anti-obesity effects in rats fed with HFD. Among the four doses studied, 200 mg/kg body weight of FA exhibited optimum lipid-lowering activity against HFD induced hyperlipidemia and obesity.

ACKNOWLEDGMENT

We would like to thank for the help rendered by Dr. Palanivel, Department of Pathology, Tamil Nadu Veterinary and Animal Sciences University chennai-600007 for histopathology reports. We would like to extend our gratitude to Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai - 600117 for providing ethical clearance.

CONFLICT OF INTEREST

There is no conflict of interest among us.

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