Chapter

The Effect of *M. latifolia* Leaf Extract on High-Fructose Corn Syrup (HFCS)-Induced Non-alcoholic Fatty Liver Disease in Rat Models

Subha Mary Varghese and Jibu Thomas

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a condition where the content of intrahepatic triglycerides (steatosis) rises, inclusive or exclusive of inflammation and fibrosis (namely steatohepatitis). It is acknowledged all over the world as the leading cause of chronic liver disease (CLD). Mulberry, a phytonutrient-rich plant belongs to the genus *Morus*, has been widely used as one of the conventional medicinal plants due to its chemical composition and pharmacological utility. Identification of leaf extract (*M. latifolia*) revealed chlorogenic acid, rutin, quercetin, caffeic acid and coumaric acid as functional bioactive principles. Objective of the current study was to evaluate the beneficial effect of *M. latifolia* in treating HFCS-induced metabolic disorders, namely, dyslipidaemia and non-alcoholic fatty liver disease (NAFLD), in rat models. Study determined body weight, blood glucose, lipid profile, liver marker enzymes and histopathology of liver tissues. Study concluded that administration of *M. latifolia* leaf extract showed a significant decrease in body weight and the levels of lipid profile, blood glucose and liver marker enzymes in HFCS-induced rats compared to HFCS control rats. Histopathological studies confirmed the antihyperlipidaemic properties of *M. latifolia* leaf extract in reducing the hepatic fat accumulation causing regeneration of liver tissues in HFCS-fed rats.

Keywords: HFCS, NAFLD, mulberry leaf, dyslipidaemia

1. Introduction

Obesity has become a major public health problem worldwide and is of crucial concern in the field of preventive medicine. It is a medical condition where excessive lipid is accumulated in the body, which results in increased adipose tissue mass and dysregulation of lipid metabolism. The prevalence of obesity has rapidly increased in the past few decades due to changes in lifestyle factors, especially diet [1]. Obesity is closely associated with diseases such as non-alcoholic fatty liver disease (NAFLD), hypertension, hyperlipidaemia, arteriosclerosis and cancer [1–3].

Non-alcoholic fatty liver disease (NAFLD) results in a rise in content of intra-hepatic triglyceride (IHTG) (i.e. steatosis), inclusive or exclusive of inflammation and fibrosis (namely steatohepatitis). Today, NAFLD is acknowledged all over the world as the leading cause of chronic liver disease (CLD). The condition brings about an extreme accretion of hepatocytes. It comprises a pathological field varying from mild, harmless steatosis to acute, critical non-alcoholic steatohepatitis, liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [4]. NAFLD is generally linked to insulin resistance and considered as the hepatic expression of the metabolic disorder. There is a great dearth of pharmacological remedies presently for the treatment of NAFLD, with intentional changes in lifestyle such as weight loss through wholesome diet and exercise the only known means for bringing about an improvement in the status.

A predominant factor behind the sudden escalation of obesity is the use of high-fructose corn syrup in beverages. It is found that HFCS embodies >40% of caloric sweeteners which are included in foods and beverages. Furthermore, it is the primary caloric sweetener added in soft drinks in the USA, where the cases of obesity are steadily rising as a result. The drawback of HFCS is that the digestion, absorption and metabolism of fructose vary from that of glucose. Hepatic metabolism of fructose supports de novo lipogenesis. Moreover, fructose does not promote the secretion of insulin or improvement of leptin generation. As insulin and leptin serve as crucial afferent signals in the control of food consumption and body weight, it indicates that dietary fructose may be instrumental in enlarged energy intake and a rise in weight. Additionally, calorically sweetened beverages are likely to intensify caloric consumption. These given factors suggest that the heightened ingestion of HFCS and its excessive intake through various liquid refreshments are decisive in the surge of obesity in recent times [5].

Current findings show HFCS-55 consumption to modify hepatic lipid metabolism and increase the amassment of triglycerides. HFCS-55 is considered to be more lipogenic than sucrose, which greatly raises the danger of developing non-alcoholic fatty liver disease (NAFLD) and dyslipidaemia. Rats which imbibed HFCS-55 solution were found to have the highest (P = .03) hepatic total lipid and triglyceride content as well as histological evidence for fat penetration [6].

Natural bioactive substances contained in fruits and vegetables inclusive of phenolic compounds, flavonoids and anthocyanins have generated widespread interest owing to their antioxidant properties. They are being steadily looked upon as possible remedies to combat obesity and obesity-related metabolic syndrome [7–10].

Mulberry is a phytonutrient-rich plant which belongs to the genus *Morus* and family Moraceae. It has been widely used as one of the conventional medicinal plants for centuries due to its chemical composition and pharmacological utility [11]. Mulberry leaves can be regarded as potential sources of phytochemical compounds with verified biological properties. The latest reports suggest mulberry leaves (*Morus alba* L.) to be a rich source of polyphenolic substances, including phenolic acids and flavonoids [12]. Earlier studies have revealed mulberry leaves to possess anticholesteremic effects [13] and cardiovascular and hepatoprotective properties [14, 15]. In more recent past, Song et al. [4] evaluated the positive impact of mulberry fruit extract on NAFLD present in mice fed on a high-fat diet.

The main objective of the current study is to evaluate the beneficial effect of *Morus latifolia* leaf extract in treating HFCS-induced metabolic disorders, namely, dyslipidaemia and non-alcoholic fatty liver disease (NAFLD), in rat models.

2. Materials and methods

2.1 Collection of plant material

Twigs of *M. latifolia* var. BC 259 were collected from the germplasm collections of Sericulture Department, Tamil Nadu Agricultural University, Coimbatore. Five to seven leaves from the apex were plucked and shade dried.

2.2 Preparation of *M. latifolia* leaf extract

Mulberry leaves of BC 259 were dried overnight at 90°C, and 20 g was powdered and extracted with 70% ethanol using the Soxhlet apparatus. The extract obtained was further separated with chloroform and ethyl acetate using a separating funnel. The polyphenolic ethyl acetate layer finally acquired was evaporated using rotary evaporator and was taken for its quantification of polyphenolic constituent profile using HPLC [16].

2.3 Identification of polyphenolic compounds by HPLC and its quantification

Phenolic constituents were determined using the HPLC method described by Rodriguez et al. [17]. Chromatographic conditions include separation in HPLC (Waters, Model: 515) fitted with photodiode array detector (Model: 2998) and ODS column (250 mm × 4.6 mm, 4 μm) (Hichrom, USA). Binary elution was performed with solvents (methanol-acetic acid-water) in the proportion of 10:2:88 and 90:2:8 as mobile phases A and B, respectively. Measurements were made at 254 and 280 nm. Phenolic acids were identified based on the retention time and UV spectrum of reference standards. Reference standards at concentrations (10–40 μg/mL) were injected into the HPLC-DAD system, and the calibration curves were generated. Concentrations of the compounds were calculated from peak area according to the generated calibration curves.

2.4 Animals

Animal studies were performed with 30 male Wistar rats weighing ~200 g procured from Veterinary College, Thrissur, Kerala. Animals were acclimatized at a temperature of 25 ± 2°C with a relative humidity of 45–55% in a 12-hour light-dark cycle and were fed with standard laboratory diet (rat chow diet) where water was given ad libitum. Rats used in the study were maintained in accordance with guidelines of the Institutional Animal Ethical Committee of the University. Institutional Animal Ethical Committee's (IAEC) approval was obtained under experiment no. IAEC/KU/BT/13/02 for the present study.

2.5 Experimental design

Rats were divided into five groups with six rats each, and they were treated as follows: Group I, normal control rats (non-obesity control); Group II, high-fructose corn syrup (HFCS 20%)-induced obese rats; Group III, obese rats with standard drug, orlistat (120 mg/kg body weight); Group IV, obese rats with *Morus latifolia* leaf extract at dose of 250 mg/kg/day; and Group V, obese rats with *Morus latifolia* leaf extract at dose of 500 mg/kg/day. The treatment was continued orally for 21 days (3 weeks).

2.6 Experimental induction of obesity

All rats (four groups) except normal group were fed with HFCS (G55) dissolved in water to a final concentration of 20% through liquid diet in a feeding bottle with a capacity of 125 mL to achieve obesity, while normal control rats were fed with water. This diet was fed to rats of Group II to Group V for 21 days from the start of experiment. The obese rats (Group III) were treated with standard drug orlistat (200 mg/kg), and Groups IV and V were treated with *Morus latifolia* (BC 259) leaf extract with water at doses 250 and 500 mg/kg/day, respectively, for 21 days. The dosages were administered orally using an oral gavage.

2.7 Body weight

The body weight of the experimental animals was recorded prior to treatment and before sacrificing the animals according to the experimental schedule.

2.8 Biochemical analysis

At the end of the experimental period (21 days), after euthanizing the animals, the blood was withdrawn by cardiac puncture, and the blood samples were centrifuged (10,000 rpm/10 min at room temperature) to get the serum. The serum was transferred into the Eppendorf tubes to be stored at −20°C.

The serum collected during the post-treatment periods were analyzed with commercially available assay kits (ERBA Diagnostics, Mannheim, Germany) for determination of the levels of glucose (glucose oxidase/peroxidase (GOD/POD) method), lipid profile levels for high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG).

LDL-C was calculated using Friedewald's equation [18]:

$$LDL - C \; = \; [TC - \{HDL + (TG/5)\}]. \tag{1}$$

VLDL-C levels were calculated by subtracting the sum of HDL-C and LDL-C concentrations from TC [19].

Liver marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) present in serum were also estimated with assay kits (ERBA Diagnostics, Mannheim, Germany). After the animals were euthanized by ether overdose, they were sacrificed by cervical dislocation.

2.9 Histopathology of liver tissue

The liver was dissected and washed with ice-cold saline immediately to remove the blood. The liver was immediately transferred to the fixative of 10% formalin. The tissues were dehydrated and embedded in paraffin wax to make it firm for section cutting. A section of 6 μm was made and stained in hematoxylin and eosin dye for microscopic observations [20].

2.10 Data analysis

Data generated was presented as mean value ± standard error mean (SEM) for all experimental groups. Statistical analysis was performed using SPSS version 16.0 to conduct one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test (DMRT) to find out the significant differences among the various

treatment groups wherever possible. Values corresponding to $p < 0.05$ were considered statistically significant.

3. Results

3.1 Effect of *M. latifolia* (var. BC 259) leaf extract on serum glucose level in HFCS-fed rats

Table 1 represents the effect of *M. latifolia* leaf extract on changes in serum glucose level in HFCS-fed rats. In HFCS-fed rats, there was a significant increase in blood glucose level (178.14 mg/dL) compared to normal control rats (89.83 mg/dL). On the other hand, HFCS-fed rats treated with standard orlistat reduced the blood glucose level to an extent of 111.87 mg/dL compared to HFCS control group ($p < 0.05$). Both the concentrations of *M. latifolia* leaf extract kept the glucose level under control compared to HFCS control group (HFCS 20%) at $p < 0.01$. The degree of reduction of the blood glucose by standard drug and *M. latifolia* leaf extract at 500 mg/kg was statistically the same.

3.2 Effect of *M. latifolia* leaf extract on body weight in HFCS-fed rats

The effect of *M. latifolia* leaf extract on body weight in HFCS-fed rats is represented in **Table 2**. Normal control rats registered 182.20 g body weight at the end of the experimental period. Contrary to normal control rats, HFCS-fed rats showed drastic increase in body weight (280.74 g) compared to normal control rats. HFCS-fed rats treated with standard drug reduced the body weight by 31.54% compared to HFCS control rats. Oral administration of *M. latifolia* leaf extract at 250 and 500 mg/kg body weight reduced the body weight significantly at $p < 0.05$ compared to HFCS control rats. The percentage of decrease in body weight was 25 and 29%, respectively.

3.3 Effect of *M. latifolia* leaf extract on lipid profile in HFCS-fed rats

The serum lipid profiles of rats at the end of the experiments are illustrated in **Table 3**. Untreated HFCS-fed rats had enhanced levels of total cholesterol and triglycerides and decreased levels of HDL compared to normal control rats. A significant reduction in the levels of serum cholesterol and triglycerides was observed in HFCS-induced rats on administration with standard drug orlistat (200 mg/kg) or mulberry leaf extract at 250 or 500 mg/kg compared with HFCS control rats ($p < 0.05$). It may be noted that the effect of standard drug and *M. latifolia* leaf extract at 500 mg/kg was found to be statistically the same based on DMRT. A

Groups	Glucose (mg/dL)
Normal control rats	89.83 ± 5.91[a]
HFCS control rats	178.14 ± 11.82[d]
HFCS + orlistat (120 mg/kg)	111.87 ± 7.37[b]
MLE (250 mg/kg) + HFCS (20%)	139.45 ± 9.23[c]
MLE (500 mg/kg) + HFCS (20%)	116.43 ± 7.70[b]

n = 6 in each group; values were presented as mean ± standard deviation. The superscript letters [a–d] *in the column represent significant difference between each treatment group at 5% probability.*

Table 1.
Effect of M. latifolia leaf extract on serum glucose level in HFCS-fed rats.

Groups	Final body weight (g)
Normal control rats	182.20 ± 11.06[a]
HFCS (20%) control rats	280.74 ± 16.60[d]
HFCS + orlistat (120 mg/kg)	192.17 ± 11.29[a,b]
MLE (250 mg/kg) + HFCS (20%)	210.54 ± 12.19[c]
MLE (500 mg/kg) + HFCS (20%)	198.37 ± 11.06[b,c]

n = 6 in each group; values presented are mean ± standard deviation. The superscript letters [a–d] in the column represent significant difference between each treatment group at 5% probability.

Table 2.
Effect of M. latifolia leaf extract on body weight in HFCS-fed rats.

| Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control rats	142.45 ± 8.85[a]	80.29 ± 4.42[a]	55.70 ± 3.32[d]	71.79 ± 4.20[a]	16.05 ± 0.97[a]
HFCS (20%) control rats	268.57 ± 16.60[c]	182.45 ± 10.89[c]	24.14 ± 1.38[a]	207.94 ± 12.30[c]	36.49 ± 2.21[c]
HFCS + orlistat (120 mg/kg)	148.45 ± 8.85[a]	82.10 ± 3.32[a]	53.70 ± 3.15[cd]	78.33 ± 4.64 a,b	16.42 ± 1.02[a]
MLE (250 mg/kg) + HFCS (20%)	172.85 ± 10.31[b]	116.21 ± 6.96[b]	42.34 ± 2.43[b]	84.11.11 ± 5.01[b]	23.20 ± 1.46[b]
MLE (500 mg/kg) + HFCS (20%)	150.38 ± 9.05[a]	85.47 ± 5.09[a]	51.90 ± 3.05[c]	81.39 ± 4.87[b]	17.09 ± 1.04[a]

n = 6 in each group; values presented are mean ± standard deviation. The superscript letters [a–d] in the column represent significant difference between each treatment group at 5% probability.
TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Table 3.
Effect of M. latifolia (var. BC 259) leaf extract on levels of serum TC, TG, HDL, LDL and VLDL in normal and HFCS-fed rats.

Groups	ALT (U/dL)	AST (U/dL)	ALP (U/dL)
Normal control rats	35.45 ± 2.01[a]	49.54 ± 2.89[a]	101.94 ± 5.98[a]
HFCS (20%) control rats	56.77 ± 3.31[e]	112.31 ± 6.63[e]	192.76 ± 11.36[d]
HFCS + orlistat (120 mg/kg)	38.43 ± 2.24[b]	61 ± 3.6[b]	124.56 ± 7.34[b]
MLE (250 mg/kg) + HFCS (20%)	51.31 ± 3.01[d]	75 ± 4.44[c]	152.31 ± 9.00[c]
MLE (500 mg/kg) + HFCS (20%)	42.15 ± 2.48[c]	63 ± 3.73[b]	132.31 ± 7.81[b]

n = 6 in each group; values presented are mean ± standard deviation. The superscript letters [a–d] in the column represent significant difference between each treatment group at 5% probability.
AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase.

Table 4.
Effect of M. latifolia leaf extract on levels of liver marker enzymes in HFCS-fed rats.
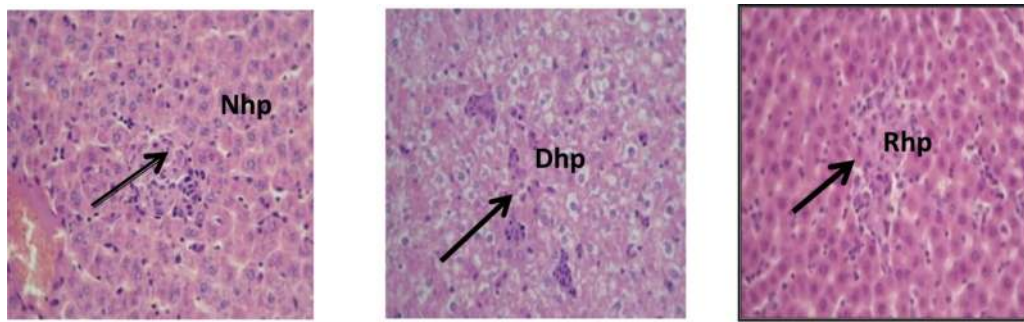
i. Normal hepatocytes ii. Damaged hepatocytes iii. Restored hepatocytes
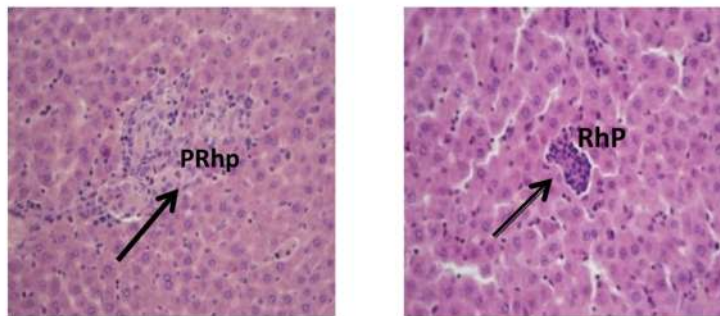
iv. Partially recovered hepatocytes   v. Recovered hepatocytes

Figure 1.
(i–v) Histopathology of the fatty liver changes in HFCS-fed rats after treatment with M. latifolia leaf extract. (i) Photomicrographs of liver tissues of untreated control rats showing normal hepatocytes. (ii) HFCS-fed rats show distorted architecture with severe fatty liver changes. (iii) Orlistat-treated rats show complete restoration of liver tissues with normal hepatocytes. (iv) M. latifolia (250 mg/kg)-treated rats show partial restoration of liver tissues with moderate fatty liver changes. (v) M. latifolia (500 mg/kg)-treated rats show maximal restoration of liver tissues with minimal fatty liver changes. Magnification at 40×.

significant improvement in HDL cholesterol was noticed in rats treated with standard drug or rats treated with mulberry leaf extract at 250 or 500 mg/kg.

3.4 Effect of *M. latifolia* leaf extract on levels of liver marker enzymes

Normal control rats registered values of 35.45 U/dL for ALT, 49.54 U/dL for AST and 101.94 U/dL for ALP (**Table 4**). Untreated HFCS-fed rats showed significantly increased levels of these enzymes compared to normal control rats ($p < 0.05$). When the test animals were treated either with orlistat or mulberry leaf extract at 250 or 500 mg/kg, there was a significant reduction in the production of ALT, AST and ALP levels.

3.5 Histopathological evaluation of liver treated with *M. latifolia* leaf extract

Photomicrographs of liver tissues of normal control rats and HFCS-fed rats after treatment with *M. latifolia* are depicted in **Figure 1(i–v)**.

4. Discussion

The consumption of fructose has seen a huge surge in the last few years, specifically in its fundamental form, high-fructose corn syrup (HFCS, 10–53%

glucose and 42–90% fructose). HFCS is utilized as a sweetener in processed foods, baked goods, condiments, soft drinks, candy, dairy products and concentrated fruit juices. It is seen to be a key factor in various diseases afflicting mankind, such as obesity, diabetes and cancer [21]. HFCS (55), which comprises 55% fructose and 45% glucose, is considered to be more lipogenic than sucrose, thus posing a great risk in the development of non-alcoholic fatty liver disease (NAFLD) and dyslipidaemia.

In contrast to glucose, fructose does not facilitate the secretion of insulin or improvement of leptin generation. As insulin and leptin serve as crucial afferent signals in the control of food consumption and body weight, it indicates that dietary fructose may be instrumental in enlarged energy intake and a rise in weight [5]. In the present study, administration of HFCS effectively induced obesity in rats as evidenced by a significant increase in body weight possibly due to the mechanism mentioned above. Supplementation of mulberry leaf extract showed a tendency towards reduction of body weight caused by HFCS. Bocarsly and his co-workers [22] reported an increase in the body weight of rats consuming HFCS, and their results coincide with data obtained in the present study.

The metabolism of fructose in the liver results in the formation of circulating triglyceride in the blood stream which will eventually lead to insulin resistance and hyperglycaemia. In the present study, HFCS has increased the serum glucose level by the stated mechanism. The administration of *Morus latifolia* leaf extract decreased the serum glucose due to the positive effect of polyphenolic extract of mulberry on glucose homoeostasis. Song et al. [4] suggested that mulberry fruit ethanol extract administration significantly improved insulin sensitivity in high-fat-fed mice.

In the present endeavor, the levels of triglycerides and total cholesterol were enhanced in HFCS-fed rats compared to normal control rats. The data obtained from the current study indicated that the model was successful in inducing hyperlipidaemia in rats. The latest findings show HFCS-55 intake to adversely affect hepatic lipid metabolism and increase the build-up of triglycerides [6]. Over the experimental period, treatment with *M. latifolia* leaf extract ameliorated the abnormalities in lipid profile as indicated by the significant ($p < 0.05$) decrease in serum triglycerides, cholesterol and LDL in HFCS-fed rats compared with untreated HFCS control rats. The degree of decrease of TC and TG levels and LDL induced by mulberry leaf extract (500 mg/kg body weight) suggested that mulberry leaf extract had a potent lipid lowering effect in hyperlipidaemia rats. Compared to normal control group, there was a significant decrease in HDL in HFCS control rats. In this study, we found that administration of *M. latifolia* leaf extract raised HDL levels in HFCS-fed animals. This rise in HDL may be the result of antiobesity effect caused by the inhibition of dyslipidaemia, hepatosteatosis and oxidative stress in obese rats [23, 24].

Yang et al. [25] found that administration of freeze-dried mulberry fruit powder resulted in a significant decrease in the serum levels of TC, TG and LDL and the hepatic levels of TG and TC and an increase in HDL in high-fat-fed rats. Chang et al. [26] illustrated from their studies that mulberry leaf polyphenol extract (MLPE) improves obesity by inducing adipocyte apoptosis and inhibiting pre-adipocyte differentiation and hepatic lipogenesis. They found quercetin and caffeic acids to be the main ingredients of MLPE which inhibit the differentiation of 3T3-L1 pre-adipocytes. Orally administering MLPE significantly reduced body weight gain and lipid accumulation in the liver and serum/hepatic triglyceride and total cholesterol levels compared with those in high-fat diet (HFD) group. Song et al. [4] demonstrated the effect of ethanolic extract of mulberry fruit in reducing HFD-induced obesity and hepatic steatosis in mice.

The protective effect of mulberry ethanol extract was associated with the induction of fatty acid oxidation and decreased fatty acid and cholesterol biosynthesis. Our results are also at par? with Andallu et al. [27]. Results obtained from the present study substantiated the earlier findings as well [13, 28, 29].

From the current study, the major polyphenolic compounds identified in *M. latifolia* were chlorogenic acid, caffeic acid, coumaric acid, rutin and quercetin. Studies have indicated that rutin could significantly reduce the levels of oleic acid-induced lipid accumulation through reducing lipogenesis and oxidative stress in hepatic carcinoma cells [30]. Previous studies have also shown that rutin may possess antiobesity effects in decreasing body weight, improving serum lipid profiles and regulating lipid metabolism [31]. Similarly, quercetin has been reported to have antiobesity effects in promoting hepatic lipolysis [32], inhibiting adipocyte differentiation, suppressing adipocytes adipogenesis, decreasing body weight [33] and improving serum lipid profiles [28]. Taher et al. [34] reported that caffeic acid isolated from *Arctium lappa* has antihyperlipidaemic effect in hyperlipidaemic rat models. Caffeic acid was reported to have antiobesity effects in decreasing body weight [35], attenuating fatty liver [36], promoting hepatic lipolysis [37] and regulating lipid metabolism [35].

Antihyperlipidaemic activity of p-coumaric acid in diabetic rats was also reported [38]. Based on these reports, we infer that crude plant extract contributing to the antiobesity effect would be due to the multiple bioactive polyphenolic compounds present in *M. latifolia* which had played a major beneficial role in treating the abnormalities in lipid metabolism.

In the present study, the increased level of liver marker enzymes (AST, ALT and ALP) was reduced and attained near-normal values when the animal groups were administrated with mulberry leaf extract. Similar results were obtained by Song and his co-workers [4] who reported that serum ALT and AST levels were reduced when the rats were fed with mulberry leaf ethanolic extract. High-fructose diets have induced fatty liver in rats [39]. NAFLD is characterized by lipid deposition in the liver, that is, steatosis [40]. Meli et al. [41] established that HFD induced hepatic steatosis in mice and rats. In addition, Chien et al. [42] reported that HFD feeding significantly increased liver weight, hepatic triglycerides and total cholesterol accumulation and hepatic vacuoles.

In this study, histological analysis of livers revealed that mulberry leaf extract administration reduced attenuated hepatic fat accumulation, even causing a regeneration of liver tissues. This has also been corroborated with reduced triglycerides and total cholesterols and serum ALT and AST levels in the mulberry leaf extract administrated group of rats. Identical results were reported earlier by Song et al. [4] with special reference to histopathological studies.

In conclusion, the results established that mulberry leaf extract amelioration improved hepatic steatosis which is due to lowered serum TC and TG levels. The study further suggested that mulberry leaf extract could be a natural dietary choice in the management of metabolic disorders such as obesity and fatty liver disease probably due to the presence of bioactive constituents, and it provides valuable input in adding to the current knowledge of the human nutrition and health.

Author details

Subha Mary Varghese and Jibu Thomas*
Department of Biotechnology, School of Agriculture and Biosciences, Karunya
Institute of Technology and Sciences, Coimbatore, Tamilnadu, India

*Address all correspondence to: jibuthomas.t@gmail.com

IntechOpen

References

[1] Kopelman PG. Obesity as a medical problem. Nature. 2000;**404**:635-643

[2] Tilg H, Moschen AR. Evolution of inflammation in non-alcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology. 2010;**52**(5):1836-1846

[3] Wellen KE, Hotamisligil GS. Inflammation, stress and diabetes. Journal of Clinical Investigation. 2005;**115**:1111-1119

[4] Song H, Lai J, Tang Q, Zheng X. Mulberry ethanol extract attenuates hepatic steatosis and insulin resistance in high-fat diet-fed mice. Nutrition Research. 2016;**36**(7):710-718

[5] Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. The American Journal of Clinical Nutrition. 2004;**79**(4):537-543

[6] Mock K, Lateef S, Benedito VA, Tou JC. High-fructose corn syrup-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation. The Journal of Nutritional Biochemistry. 2017;**39**:32-39

[7] Valenti L, Riso P, Mazzocchi A, Porrini M, Fargion S, Agostini C. Dietary anthocyanins as nutritional therapy for nonalcoholic fatty liver disease. Oxidative Medicine and Cellular Longevity. 2013;**2013**:145421

[8] Gossard AA, Lindor KD. Current therapies for nonalcoholic fatty liver disease. Drug Today. 2011;**47**:915-922

[9] Dembinska-Kiec A, Mykkanen O, Kiec-Wilk B, Mykkanen H. Antioxidant phytochemicals against type 2 diabetes. British Journal of Nutrition. 2008;**99E**(Suppl. 1):ES109-ES117

[10] Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, et al. Novel insights of dietary polyphenols and obesity. Journal of Nutritional Biochemistry. 2014;**25**:1-18

[11] Ramesh HL, Sivaram V, Murthy VNY. Antioxidant and medicinal properties of mulberry. World Journal of Pharmaceutical Research. 2014;**3**(6):320-343

[12] Memon AA, Memon N, Luthria DL, Bhanger MI, Pitafi AA. Phenolic acids profiling and antioxidant potential of Mulberry (*Morus laevigata* W., *Morus nigra* L., *Morus alba* L.) leaves and fruits grown in Pakistan. Polish Journal of Food and Nutrition Sciences. 2010;**60**(1):25-32

[13] Chan KC, Yang MY, Lin MC, Lee YJ, Chang WC, Wang CJ. Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in cultures aortic vascular smooth muscle cells. Journal of Agricultural and Food Chemistry. 2013;**61**:2780-2788

[14] Chan KC, Ho HH, Peng CH, Lan KP, Lin MC, Chen HM, et al. Polyphenol-rich extract from mulberry leaf inhibits vascular smooth muscle cell proliferation involving upregulation of p53 and inhibition of cyclin-dependent kinase. Journal of Agricultural and Food Chemistry. 2010;**58**:2536-2542

[15] Yang MY, Chan KC, Lee YJ, Chang XZ, Wu CH, Wang CJ. Mulberry leaf polyphenol extract induced apoptosis involving regulation of adenosine monophosphate-activated protein kinase/fatty acid synthase in a p53-negative hepatocellular carcinoma cell. Journal of Agricultural and Food Chemistry. 2012;**60**:6891-6898

[16] Thomas J, Raj Kumar R, Mandal AKA. Metabolite profiling and characterization of somaclonal variants

in tea (*Camellia spp.*) for identifying productive and quality accession. Phytochemistry. 2006;**67**(11):1136-1142

[17] Rodriguez-Delgado MA, Malovana S, Perez JP, Borges T, Garcia-Montelongo FJ. Separation of phenolic compounds by high performance chromatography with absorbance and flurimetric detection. Journal of Chromatography. 2001;**912**:249-257

[18] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;**18**(6):499-502

[19] Morita T, Oh-hashi A, Takei K, Ikai M, Kasaoka S, Kiriyama S. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. Journal of Nutrition. 1997;**127**(3):470-477

[20] Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. London, UK: Longman Group; 1982

[21] Charrez B, Qiao L, Hebbard L. The role of fructose in metabolism and cancer. Hormone Molecular Biology and Clinical Investigation. 2015;**22**(2):79-89

[22] Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels. Pharmacology, Biochemistry and Behaviour. 2010;**97**(1):101-106

[23] Kals J, Kampus P, Kals M, Zilmer K, Kullisaar T, Teesalu R, et al. Impact of oxidative stress on arterial elasticity in patients with atherosclerosis. American Journal of Hypertension. 2006;**19**:902-908

[24] Smith SC Jr, Jackson R, Pearson TA, Fuster V, Yusuf S, Faergeman O. Principles for national and regional guidelines on cardiovascular disease prevention: A scientific statement from the World Heart and Stroke Forum. Circulation. 2004;**109**:3112-3121

[25] Yang XL, Yang L, Zheng HY. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food and Chemical Toxicology. 2010;**48**:2374-2379

[26] Chang YC, Yang MY, Chen SC, Wang CJ. Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. Journal of Functional Foods. 2016;**21**:249-262

[27] Andallu B, Vinay Kumar AV, Varadacharyulu NC. Lipid abnormalities in streptozotocin-diabetes: Amelioration by *Morus indica* L. Cv Suguna leaves. International Journal of Diabetes in Developing Countries. 2009;**29**(3):123-128

[28] Enkhmaa B, Shiwaku K, Katsube T, Kitajima K, Anurad E, Yamasaki M, et al. Mulberry (*Morus alba* L.) leaves and their major flavonol quercetin-3-(6-malonylglucoside) attenuate atherosclerotic lesion development in LDL receptor-deficient mice. Journal of Nutrition. 2005;**135**:729-734

[29] Yang Y, Zhang Z, Li S, Ye X, Li X, He K. Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. Fitoterapia. 2014;**92**:133-147

[30] Wu CH, Lin MC, Wang HC, Yang MY, Jou MJ, Wang CJ. Rutin inhibits oleic acid induced lipid accumulation via reducing lipogenesis and oxidative stress in hepatocarcinoma cells. Journal of Food Science. 2011;**76**(2):T65-T72

[31] Sikder K, Kesh SB, Das N, Manna K, Dey S. The high antioxidative power

of quercetin (aglycone flavonoid) and its glycone (rutin) avert high cholesterol diet induced hepatotoxicity and inflammation in Swiss albino mice. Food and Function. 2014;**5**:1294-1303

[32] Sun X, Yamasaki M, Katsube T, Shiwaku K. Effects of quercetin derivatives from mulberry leaves: Improved gene expression related hepatic lipid and glucose metabolism in short-term high-fat fed mice. Nutrition Research and Practice. 2015;**9**:137-143

[33] Moon J, Do HJ, Kim OY, Shin MJ. Antiobesity effects of quercetin-rich onion peel extract on the differentiation of 3T3-L1 preadipocytes and the adipogenesis in high fat-fed rats. Food and Chemical Toxicology: An International Journal published for the British Industrial Biological Research Association. 2013;**58**:347-354

[34] Taher MA, Abdul-Hussain DA, Hasan HF, Fahmi ZM, Luaibi OK, Ali MG. Hypolipidemic effect of caffeic acid isolated from *Arctium lappa* cultivated in Iraq, in hyperlipidemic rat model. Iraqi Journal of Pharmaceutical Sciences. 2015;**24**(1):18-24

[35] Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, et al. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association. 2010;**48**:937-943

[36] Yang MY, Chan KC, Lee YT, Chang XZ, Wu CH, Wang CJ. *Sechium edule* shoot extracts and active components improve obesity and a fatty liver that involved reducing hepatic lipogenesis and adipogenesis in high-fat diet-fed rats. Journal of Agricultural and Food Chemistry. 2015;**63**(18):4587-4596

[37] Liao CC, Ou TT, Huang HP, Wang CJ. The inhibition of oleic acid induced hepatic lipogenesis and the promotion of lipolysis by caffeic acid via up-regulation of AMP-activated kinase. Journal of the Science of Food and Agriculture. 2014;**94**:1154-1162

[38] Amalan V, Vijayakumar N, Indumathi D, Ramakrishnan A. Antidiabetic and antihyperlipidemic activity of p-coumaric acid in diabetic rats, role of pancreatic GLUT 2: *In vivo* approach. Biomedicine and Pharmacotherapy. 2016;**84**:230-236

[39] Tuovinen CG, Bender AE. Some metabolic effects of prolonged feeding of starch, sucrose, fructose and carbohydrate-free diet in the rat. Annals of Nutrition and Metabolism. 1975;**19**(3-4):161-172

[40] Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi R, Lenzi M, et al. Nonalcoholic fatty liver disease—A feature of the metabolic syndrome. Diabetes. 2001;**50**:1844-1850

[41] Meli R, Raso GM, Irace C, Simeoli R, Di Pascale A, Paciello O, et al. High-fat diet induces liver steatosis and early dysregulation of iron metabolism in rats. PLoS One. 2013;**8**:e66570

[42] Chien MY, Ku YH, Chang JM, Yang CM, Chen CH. Effects of herbal mixture extracts on obesity in rats fed a high-fat diet. Journal of Food and Drug Analysis. 2016;**24**(3):594-601