CHAPTER 6

EFFECT OF *M. LATIFOLIA* LEAF EXTRACT ON HIGH FRUCTOSE CORN SYRUP (HFCS) INDUCED OBESITY IN RAT MODELS

6.1 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 (Kopelman 2000). Obesity is closely associated with diseases such as non-alcoholic fatty liver disease (NAFLD), hypertension, hyperlipidemia, arteriosclerosis and cancer (Kopelman 2000; Tilg and Moschen 2006; Wellen and Hotamisligil 2005).

NAFLD results in a rise in content of intrahepatic triglyceride (IHTG) (that is, steatosis), inclusive or exclusive of inflammation and fibrosis (namely, steatohepatitis). Today, it is acknowledged all over the world as the leading cause of chronic liver disease. 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 (Song et al. 2016). 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 at present for its treatment.
Intentional changes in lifestyle, such as weight reduction through wholesome diet and exercise, are the only known means for bringing about an improvement in the condition.

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 varies from that of glucose. Hepatic metabolism of fructose supports \textit{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 (Bray et al. 2004).

HFCS-55 is considered to be more lipogenic than sucrose, which greatly raises the danger of developing NAFLD and dyslipidemia. 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 (Mock et al. 2017).

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.
Figure 6.1 Proposed mechanism of action of mulberry leaf extract against HFCS induced obesity
Earlier studies have revealed mulberry leaves to possess anticholesteremic effects (Chan et al. 2013), and cardiovascular and hepatoprotective properties (Chan et al. 2010; Yang et al. 2012). In more recent times, Song et al. (2016) 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 was to evaluate the beneficial effect of *M. latifolia* leaf extract in treating HFCS induced metabolic disorders, namely, dyslipidemia and non-alcoholic fatty liver disease in rat models. The proposed mechanism of action of mulberry leaf extract against HFCS induced obesity is shown in Figure 6.1.

### 6.2 MATERIALS AND METHODS

#### 6.2.1 Collection of plant material

Twigs of *M. latifolia* var. BC259 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.

#### 6.2.2 Preparation of polyphenols from mulberry leaf

Dried samples were ground into powder and utilised for extraction of bioactive molecules using 70% ethanol in a Soxhlet apparatus for three cycles. After three cycles, the mixture was filtered and the extract was kept separately. Following this, 70% ethanol was added to the filtered plant material and extraction
was repeated for three cycles. After extraction, the mixture was filtered and the extracts were pooled together. Pooled samples were further separated with chloroform followed by ethyl acetate separation. The extract was vacuum dried and lyophilized. Obtained \textit{M. latifolia} var. BC259 leaf extract was stored under refrigerated conditions until it was used. Polyphenolic extract thus obtained was used for animal studies (Jibu Thomas et al. 2006).

6.2.3 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 \textit{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 (IAEC) approval was obtained under experiment No. IAEC/KU/BT/13/02 for the present study.

6.2.4 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 \textit{Morus latifolia} leaf extract at dose of 250 mg/kg/day; and, Group V: Obese rats with \textit{Morus latifolia} leaf extract at dose of 500 mg/kg/day. The treatment was continued orally for 21 days (3 weeks).
6.2.5 Experimental induction of obesity

All rats except normal group were fed with HFCS (G55) dissolved in water to a final concentration of 20% through liquid diet in a feeding bottle of capacity 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 Group IV and V were treated with Morus latifolia leaf extract with water at doses 250 mg/kg/day and 500 mg/kg/day respectively for 21 days. The dosages were administered orally using an oral gavage.

6.2.6 Body weight

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

6.2.7 Biochemical analysis

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

The serum collected during the post treatment periods, were analysed 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 (Friedewald et al. 1972) as shown in Equation (6.1).
VLDL-C levels were calculated by subtracting the sum of HDL-C and LDL-C concentrations from TC (Morita et al. 1997).

\[
\text{LDL-C} = [\text{TC} - \{\text{HDL} + (\text{TG}/5)\}]\quad \text{Equation (6.1)}
\]

Liver marker enzymes such as AST, ALT and 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.

**6.2.8 Histopathology of liver tissue**

The liver was dissected and washed with ice-cold saline immediately to remove blood. 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. Section of 6 microns was made and stained in hematoxylin and eosin dye for microscopic observations (Bancroft and Stevens 1982).

**6.2.9 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 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.
6.3 RESULTS

6.3.1 Effect of *M. latifolia* leaf extract on serum glucose level in HFCS fed rats

Table 6.1 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) when 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 when compared to HFCS control group (p<0.05). Both the concentrations of *M. latifolia* leaf extract kept the glucose level under control when compared to HFCS control group (HFCS 20%) at p<0.01. The degree of reduction of blood glucose by standard drug and *M. latifolia* leaf extract at 500 mg/kg were statistically same.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dL)</th>
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<tbody>
<tr>
<td>Normal control rats</td>
<td>89.83 ± 5.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS control rats</td>
<td>178.14 ± 11.82&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS + Orlistat (120 mg/kg)</td>
<td>111.87 ± 7.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (250 mg/kg) + HFCS (20%)</td>
<td>139.45 ± 9.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (500 mg/kg) + HFCS (20%)</td>
<td>116.43 ± 7.70&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

n= 6 in each group; values were presented as mean ± standard deviation. The superscript letters <sup>a-d</sup> in the column represents significant difference between each treatment groups at 5% probability.
6.3.2 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 6.2. Normal control rats registered 182.20 g bodyweight at the end of the experimental period. Contrary to normal control rats, HFCS fed rats showed drastic increase in body weight (280.74 g) when compared to normal control rats. HFCS fed rats treated with standard drug reduced the bodyweight by 31.54% when compared to HFCS control rats. Oral administration of *M. latifolia* leaf extract at 250 mg/kg and 500 mg/kg body weight reduced the bodyweight significantly at p<0.05 when compared to HFCS control rats. The percentage of decrease in body weight was 25% and 29% respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final body weight (g)</th>
</tr>
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<tbody>
<tr>
<td>Normal control rats</td>
<td>182.20 ± 11.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS (20%) control rats</td>
<td>280.74 ± 16.60&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS + Orlistat (120 mg/kg)</td>
<td>192.17 ± 11.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (250 mg/kg) + HFCS (20%)</td>
<td>210.54 ± 12.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (500 mg/kg) + HFCS (20%)</td>
<td>198.37 ± 11.06&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n= 6 in each group; values presented are mean ± standard deviation. The superscript letters <sup>a-d</sup> in the column represents significant difference between each treatment groups at 5% probability.
6.3.3 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 6.3. Untreated HFCS fed rats had enhanced levels of total cholesterol and triglycerides and decreased levels of HDL when 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 mg/kg or 500 mg/kg when 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 same based on DMRT test. A significant improvement in HDL cholesterol was noticed in rats treated with standard drug or rats treated with mulberry leaf extract at 250 mg/kg or 500 mg/kg.
Table 6.3 Effect of *M. latifolia* leaf extract on levels of serum TC, TG, HDL, LDL and VLDL in HFCS fed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control rats</td>
<td>142.45 ± 8.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.29 ± 4.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.70 ± 3.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71.79 ± 4.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.05 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS (20%) control rats</td>
<td>268.57 ± 16.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>182.45 ± 10.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.14 ± 1.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.94 ± 12.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.49 ± 2.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS + Orlistat (120 mg/kg)</td>
<td>148.45 ± 8.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.10 ± 3.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.70 ± 3.15&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>78.33 ± 4.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.42 ± 1.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (250 mg/kg) + HFCS (20%)</td>
<td>172.85 ± 10.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.21 ± 6.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.34 ± 2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.11 ± 5.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.20 ± 1.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (500 mg/kg) + HFCS (20%)</td>
<td>150.38 ± 9.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.47 ± 5.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.90 ± 3.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.39 ± 4.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.09 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n= 6 in each group; values presented are mean ± standard deviation. The superscript letters<sup>a-d</sup> in the column represents significant difference between each treatment groups at 5% probability.

TC- Total Cholesterol; TG- Triglycerides; HDL- High density lipoprotein; LDL- Low density lipoprotein; VLDL- Very low density lipoprotein
6.3.4 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 6.4). Untreated HFCS fed rats showed significantly increased levels of these enzymes when compared to normal control rats (*p*<0.05). When the test animals were treated either with orlistat or mulberry leaf extract at 250 mg/kg or 500 mg/kg, there was a significant reduction in the production of ALT, AST and ALP levels.

Table 6.4 Effect of *M. latifolia* leaf extract on levels of liver marker enzymes in HFCS fed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/dL)</th>
<th>AST (U/dL)</th>
<th>ALP (U/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control rats</td>
<td>35.45 ± 2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.54 ± 2.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.94 ± 5.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS (20%) control rats</td>
<td>56.77 ± 3.31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>112.31 ± 6.63&lt;sup&gt;e&lt;/sup&gt;</td>
<td>192.76 ± 11.36&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFCS + Orlistat (120 mg/kg)</td>
<td>38.43 ± 2.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.56 ± 7.34&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (250 mg/kg) + HFCS (20%)</td>
<td>51.31 ± 3.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>75 ± 4.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.31 ± 9.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLE (500 mg/kg) + HFCS (20%)</td>
<td>42.15 ± 2.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63 ± 3.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>132.31 ± 7.81&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

n= 6 in each group; values presented are mean ± standard deviation. The superscript letters <sup>a-d</sup> in the column represents significant difference between each treatment groups at 5% probability.

AST- aspartate transaminase; ALT- alanine transaminase; ALP- alkaline phosphatase
6.3.5 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 6.2 (i-v).

![Histopathology](image)

i. Normal hepatocytes  
ii. Damaged hepatocytes  
iii. Restored hepatocytes  
iv. Partially recovered hepatocytes  
v. Recovered hepatocytes

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 x

**Figure 6.2 (i-v) Histopathology of the fatty liver changes in HFCS fed rats after treatment with *M. latifolia* leaf extract**
6.4 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 (Charrez et al. 2015). 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 dyslipidemia.

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 (Bray et al. 2004). 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. Borcarsly and his co-workers (2010) 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 liver results in the formation of circulating triglyceride in the blood stream which will eventually lead to insulin resistance and hyperglycemia. 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 homeostasis. Song et al. (2016) suggested that mulberry fruit
ethanol extract administration significantly improved insulin sensitivity in high-fat fed mice.

In the present endeavour, the levels of triglycerides and total cholesterol were enhanced in HFCS fed rats when compared to normal control rats. The data obtained from the current study indicated that the model was successful in inducing hyperlipidemia in rats. Latest findings show HFCS-55 intake to adversely affect hepatic lipid metabolism and increase the build-up of triglycerides (Mock et al. 2017). Over the experimental period, treatment with *M. latifolia* leaf extract ameliorated the abnormalities in lipid profile as indicated by significant (p<0.05) decrease in serum triglycerides, cholesterol, 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 hyperlipidemia 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 dyslipidemia, hepatosteatosis and oxidative stress in obese rats (Kals et al. 2006; Smith et al. 2004).

Yang et al. (2010) 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. (2016) illustrated from their studies that mulberry leaf polyphenol extract (MLPE) improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. They found quercetin and caffeic acid 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. (2016) 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. (2009). Results obtained from the present study substantiated the earlier findings as well (Chan et al. 2013; Enkhmaa et al. 2005; Yang et al. 2014).

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 (Wu et al. 2011). Previous studies have also shown that rutin may possess antiobesity effects in decreasing bodyweight, improving serum lipid profiles and regulating lipid metabolism (Sikder et al. 2014). Similarly, quercetin has been reported to have antiobesity effects in promoting hepatic lipolysis (Sun et al. 2015), inhibiting adipocyte differentiation, suppressing adipocytes adipogenesis, decreasing body weight (Moon et al. 2013) and improving serum lipid profiles (Enkhmaa et al. 2005). Taher et al. (2015) reported that caffeic acid isolated from *Arctium lappa* has antihyperlipidemic effect in hyperlipidemic rat models. Caffeic acid was reported to have antiobesity effects in decreasing body weight (Cho et al. 2010), attenuating fatty liver (Yang et al. 2015), promoting hepatic lipolysis (Liao et al. 2014) and by regulating lipid metabolism (Cho et al. 2010).

Antihyperlipidemic activity of p-coumaric acid in diabetic rats was also reported (Amalan et al. 2016). 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 (2016) who reported that serum ALT and AST levels reduced when the rats were fed with mulberry leaf ethanolic extract. High fructose diets have induced fatty liver in rats (Tuovinen and Bender 1975). NAFLD is characterized by lipid deposition in the liver, that is, steatosis (Marchesini et al. 2001). Meli et al. (2013) established that HFD induced hepatic steatosis in mice and rats. In addition, Chien et al. (2016) 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 cholesterol and serum ALT and AST levels in the mulberry leaf extract administrated group of rats. Identical results were reported earlier by Song et al. (2016) 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 human nutrition and health.