Anti-Obesity of Dried Mulberry Fruit Powder in Mice Fed with High-Fat Diet

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Abstract

Obesity is a global health problem often caused by a high-fat diet (HF). Certain natural products can prevent this, including mulberry fruit (*Morus alba* L.), which has been reported to be able to reduce the body weight (BW) and contain several health benefits. However, most studies have mainly looked at the effect of plant extracts, not a natural unprocessed mulberry fruit. Therefore, the present study seeks to investigate the effect of dried mulberry fruit powder (DMP) on BW, food intake, visceral fat accumulation and liver weight and lipid in mice fed with HF. C57BL/6J mice were divided into 5 groups and fed with different diets for 3 months; i) normal diet (control), ii) HF, iii) HF+10 mg/kg DMP (HF+DMP10), iv) HF+100 mg/kg DMP (HF+DMP100) and HF+5mg/kg atorvastatin (HF+ATV5). BW, food intake, visceral fat accumulation and liver lipid including total cholesterol (TC) and triglyceride (TG) were evaluated. HF consumption significantly increased BW, food intake, visceral fat accumulation, liver weight and liver TC and TG ($P < 0.001$ vs control). DMP (10 and 100 mg/kg) and atorvastatin effectively reduced BW and food intake ($P < 0.001$-0.01 vs HF) and prevented visceral fat accumulation in HF-fed mice. The liver weight was found significant lower in DMP group ($P < 0.001$ vs HF), but not in ATV5 group. Liver TC was reduced by treatment with DMP, both high and low dose, and atorvastatin ($P < 0.001$ vs HF), while the reduction of TG was observed only in the mice treated with the high dose of DMP or atorvastatin ($P < 0.001$ vs HF). In conclusion DMP, a natural unprocessed dried mulberry fruit product, possessed anti-obesity by reducing food intake and body weight. DMP also improved liver lipid profile, thus it can be developed as a food supplement for weight control and prevention against liver lipid accumulation due to HF consumption.

Keywords: *Morus alba*, Mulberry Fruit, Obesity, Food Intake, High Fat Diet

Introduction

The World Health Organization (WHO) defined obesity as an abnormal or excessive fat accumulation that may impair health. Adult with a body mass index (BMI) of 30 kg/m^2 or higher is generally considered obese (World Health Organization (WHO), 2020). The WHO also reported that the worldwide prevalence of obesity has dramatically increased and has nearly tripled since 1975. This pandemic has become a global health burden because obesity is a major risk factor for several chronic diseases, including cardiovascular diseases, diabetes, and some types of cancers (Manna & Jain, 2015; Meldrum, Morris, & Gambone, 2017; Parto & Lavie, 2017; Elagizi et al., 2018; Saliba & Maffett, 2019). It is also associated with poorer health outcomes and reduced quality of life. Although obesity is a complex disorder related to multi-factorial interactions of genetic, socioeconomic, cultural influences, consumption patterns, urban development, and lifestyle habits, it is preventable (Apovian, 2016; Gadde et al., 2018).

Obesity fundamentally results from an energy imbalance between calories consumed and calories expended, hence an increased intake of energy-dense foods (such as high fat and high sugar) and/or a decrease in physical activity can lead to obesity (Apovian, 2016; Meldrum, Morris, & Gambone, 2017). Maintaining healthy lifestyle by limiting energy intake from total fats and sugars, increasing consumption of fruits and vegetables, and engaging in regular physical activity can essentially prevent obesity (Manna & Jain, 2015; Apovian, 2016; Meldrum et al., 2017; Gadde et al., 2018). However, long-term lifestyle modification is challenging and frustrating for obese patients (Thomas et al., 2014). Pharmacological intervention, therefore, could be another option to tackle obesity. This includes various drugs targeting weight loss by increasing energy expenditure, suppressing appetite, interfering lipid metabolism, modifying intestinal microbiota composition or inhibiting pancreatic lipase to reduce lipid absorption in the intestine, nevertheless, their potential side effects could be harmful and intolerant (Krentz, Fujioka, & Hompesch, 2016; Gadde et al., 2018; Patel & Stanford, 2018). In view of this problem, there has been growing interest in the development of food supplements, and functional ingredients from natural sources with less adverse effects, for preventing and reducing obesity (Fu et al., 2016; Sun, Wu, & Chau, 2016; Karri et al., 2019; Rodrí guez-Pé rez, Segura-Carretero, & Contreras, 2019; Wharton et al., 2020). Among these are mulberry fruits and leaves, which have been reported to contain anti-obesity properties, and possess several health benefits (Lim et al., 2013; Sun, Wu, & Chau, 2016; Yuan & Zhao, 2017; Zhang et al., 2018; Mahmoubi, 2019).

Mulberry (*Morus alba* L.), belonging to genus Morus in the Moraceae family, has been widely planted in several regions including Europe, America, Africa and Asia (Yuan & Zhao, 2017; Zhang et al., 2018). A number of biological activities of this plant have been reported such as anti-oxidation (Yang, Yang, & Zheng, 2010; Arfan et al., 2012; Leyva-Jimé nez et al., 2020), anti-diabetes (Jiao et al., 2017; Min et al., 2020), anti-dyslipidemia (Yang, Yang, & Zheng, 2010; Lee et al., 2020), as well as anti-obesity (Lim et al., 2013; Mahmoubi, 2019). Regarding anti-obesity properties, it has been reported that mulberry leaf extract ameliorated obesity by reducing fat accumulation, hepatic lipogenesis, fibrosis, and oxidative stress, but had no effect on body weight in HF-fed mice (Ann, Eo, & Lim, 2015; Lee et al., 2019), while combined treatment of mulberry leaf and fruit extract reduced body weight and obesity-related inflammation, and oxidative stress in HF-induced obese mice (Lim et al., 2013). In line with these findings, several studies clearly support the beneficial effect of mulberry on obesity (Jó zefczuk et al., 2017; He et al., 2018; Mahmoubi, 2019; Metwally, Rashad, & Mahmoud, 2019; Leyva-Jimé nez et al., 2020).

Although the anti-obesity properties of mulberry have been previously demonstrated, most investigators have mainly tested the plant extract. The extraction methods used might vary from one study to another, hence resulting in the different final bioactive components of the extract. In addition, mulberry can grow in a wide range of climates and soil types, and different habitats can affect the chemical composition and nutritional status of the plants, leading to the differences in pharmacological actions of different genotypes of *M. alba* (Song et al., 2009; Yuan & Zhao, 2017; Leyva-Jimé nez et al., 2020). The fresh or unprocessed mulberry fruit may partly have more advantage over the extract, in that it contains the original nutrient composition, and avoids any contamination from the solvent used in the extraction process. This study aims to evaluate the effect of mulberry fruit grown in Thailand on HF-fed mice. The dried mulberry fruit powder (DMP) was used in our study in order to test its anti-obesity efficacy, and to test the health benefits of the natural unprocessed form of mulberry fruit, which can be easily and safely consumed as a food supplement. The body weight, food intake, visceral fat

accumulation and liver weight and lipid levels, including total cholesterol (TC) and triglyceride (TG) were evaluated.

Methods and Materials

Mulberry Fruit Sample Preparation

DMP was provided by the Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. Mature mulberry (*M. alba* L.) fruits were collected from Nakhon Pathom province, Thailand. The fresh mulberry fruits were dried using a freeze dryer and ground with a roller grinding machine to obtain DMP. The obtained sample was kept at -20° C, and protected from light, until use. The quality control of DMP was carried out by monitoring the total anthocyanin content in DMP, using the pH differential method (Chaovanalikit & Wrolstad, 2004). The DMP sample contained total anthocyanin of 1.46 g/100 g dry weight.

Animal and Experimental Protocol

C57BL/6J female mice (18-20 g) were purchased from the Nomura Siam International Co. Ltd., Bangkok, Thailand. The animal experimental protocol was ethically approved by Naresuan University Animal Care and Use Committee (NUACUC, Naresuan University, Phitsanulok, Thailand; ethic protocol number NU-AE 610727) for the care and use of animals for scientific purposes. All animals were acclimatized for 1 week in plastic cages at 22±1° C with a 12-12 hour light-dark cycle and allowed access to food and water *ad libitum* at the Centre for Animal Research, Naresuan University, Phitsanulok, Thailand. Before the experiment, they were randomly divided into 5 groups, each were fed with a different diet for 3 months; i) control (normal diet), ii) high fat diet (HF), iii) HF+10 mg/kg DMP (HF+DMP10), iv) HF+100 mg/kg DMP (HF+DMP100), and v) HF+5 mg/kg atorvastatin (Lipitor, Pfizer pharmaceuticals LLC, Freiburg, Germany) (HF+ATV5). Composition of normal and HF diet, obtained from Harlan Teklad Laboratory, Madison, Wisconsin, USA, was shown in Table 1. HF+DMP10, HF+DMP100 and HF+ATV5 groups were daily fed with HF and concomitantly orally administered 10, 100 mg/kg DMP and 5 mg/kg atorvastatin, respectively, via the intragastric route. Water was used instead in control and HF groups. The body weight of all animals and food intake was monitored throughout the experimental period. At the end of the experiment, following overnight fasting (12-14 hour), mice were sacrificed by exsanguination after deep anesthetization with intraperitoneal injection of 50-70 mg/kg sodium thiopental, then abdominal visceral fat and liver were collected for corresponding experiments.

Table 1 The composition of diets used in the experiment

Ingredient (% by weight)	Diets	
	Normal (T2918CSD)	High fat (TD02028)
Protein	18.6	17.3
Carbohydrate	44.2	48.5
Fat	6.2	21.2
Cholesterol	-	0.2
Energy (kcal/gram)	3.1	4.5

Animal diets were obtained from Harlan Teklad Laboratory, Madison, Wisconsin, USA.

Visceral Fat Accumulation Measurement

Visceral fat was collected by separating it from the abdominal cavity and then weighed. The quantity of visceral fat was expressed as percentage of body weight (g/100 g BW).

Liver Lipid Profile Analysis

Liver was collected and then subjected to lipid profile analysis by the following procedure. One gram of liver was homogenized in chloroform and methanol at the ratio of 2:1, then the sample was centrifuged at 4° C, 500 rpm for 10 min. The supernatant was collected and mixed with 0.9% NaCl, followed by centrifugation at 4° C, 500 rpm for 10 min. Then the supernatant was collected again and undergone nitrogen purge in order to remove all the solvent. The semi-solid lipid remnants were solubilized with 2% triton X and levels of TC, and TG were measured using an enzymatic colorimetric test according to manufacturer protocols (Human diagnostic company, Wiesbaden, Germany).

Statistical Analysis

All data were expressed as mean standard error of mean (SEM) of n animals. The statistical significance between groups was evaluated using student t-test and/or ANOVA followed by the Tukey-Kramer post hoc test. Values of $P < 0.05$ were considered statistically significant.

Results

Effect of DMP on Body Weight and Food Intake

The body weight and food intake of mice were daily monitored and evaluated monthly as shown in Table 2 and 3, respectively. In the HF group, the body weight was significantly higher than in the control group throughout the experimental period ($P < 0.001$ vs control, Table 2). On the other hand, the DMP-fed groups, both 10 and 100 mg/kg, presented a lower body weight than the HF group (Table 2), and these values were clearly restored back to normal. Efficacy of low and high dose of DMP in reducing body weight was generally similar, except for month 1, where the high dose appeared to be more effective than the low dose. Treatment with atorvastatin at the dose of 5 mg/kg also reduced body weight, but the significant effect was observed at month 3 of the experiment ($P < 0.001$ vs HF).

Table 2 Effect of DMP on body weight of mice

	Body weight (g)				
Month	**Control**	**HF**	**HF+DMP10**	**HF+DMP100**	**HF+ATV5**
0	20.24± 0.64	20.25± 0.33	20.49± 0.29	20.26± 0.26	20.44± 0.26
1	20.65± 0.14	23.64± 0.47**	22.46± 0.01*	21.46± 0.31$^{\#\#}$	22.33± 0.21*
2	21.60± 0.22	24.75± 0.22**	21.77± 0.29$^{\#\#}$	22.03± 0.61$^{\#\#}$	23.03± 0.33
3	22.24± 0.30	24.84± 0.27**	22.82± 0.29$^{\#\#}$	23.25± 0.06$^{\#}$	22.35± 0.28$^{\#\#\#}$

$^{*}P < 0.05$, $^{**}P < 0.001$ vs Control in the same month

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs HF in the same month

Control, normal diet; HF, high-fat diet; HF+DMP10, high-fat diet+10 mg/kg dried mulberry fruit powder; HF+DMP100, high-fat diet+100 mg/kg dried mulberry fruit powder; HF+ATV5, high-fat diet+5 mg/kg atorvastatin. Values are presented as mean± SEM (n = 5).

Besides increased body weight, the HF group also showed an increase (~1.7-2 fold) in food intake (P < 0.001 vs control, Table 3), which was significantly reduced by treatment with either DMP or atorvastatin (P < 0.001 vs HF). At month 1, high dose of DMP and atorvastatin were more effective than low dose of DMP in decreasing food intake.

Table 3 Effect of DMP on food intake of mice

Food intake (g/day)					
Month	**Control**	**HF**	**HF+DMP10**	**HF+DMP100**	**HF+ATV5**
1	2.66± 0.06	5.31± 0.09**	4.20± 0.26$^{**,\#}$	3.03± 0.18$^{\#,\$\$}$	3.38± 0.17$^{*,\#,\$}$
2	2.92± 0.06	5.11± 0.19**	2.96± 0.23$^{\#}$	3.38± 0.09$^{\#}$	3.45± 0.16$^{\#}$
3	2.77± 0.07	5.24± 0.22**	3.56± 0.22$^{*,\#}$	3.42± 0.13$^{\#}$	3.84± 0.13$^{**,\#}$

$^{*}P$ < 0.05, $^{**}P$ < 0.001 vs Control in the same month

$^{\#}P$ < 0.001 vs HF in the same month

$^{\$}P$ < 0.05, $^{\$\$}P$ < 0.001 vs HF+DMP10 in the same month

Control, normal diet; HF, high-fat diet; HF+DMP10, high-fat diet+10 mg/kg dried mulberry fruit powder; HF+DMP100, high-fat diet+100 mg/kg dried mulberry fruit powder; HF+ATV5, high-fat diet+5 mg/kg atorvastatin. Values are presented as mean± SEM (n = 5).

Effect of DMP on Visceral Fat Accumulation and Liver Weight

HF consumption dramatically increased visceral fat accumulation by ~1.8 fold (P < 0.001 vs control, Table 4), which was significantly reduced upon the treatment with high dose of DMP (100 mg/kg, P < 0.01 vs HF) or atorvastatin (P < 0.05 vs HF). Similarly, liver weight was also remarkably increased in HF group (P < 0.001 vs control). DMP, both high and low dose, but not atorvastatin, effectively reduced liver weight (P < 0.01 vs HF).

Table 4 Effect of DMP on visceral fat and liver weight of mice

Organ weight (g/100 g body weight)					
Parameter	**Control**	**HF**	**HF+DMP10**	**HF+DMP100**	**HF+ATV5**
Visceral fat	1.79± 0.12	3.23± 0.13**	2.64± 0.17*	2.56± 0.10$^{*,\#\#}$	2.54± 0.16$^{*,\#}$
Liver	4.23± 0.16	6.49± 0.10**	5.81± 0.14$^{**,\#\#}$	5.67± 0.12$^{**,\#\#}$	6.16± 0.06**

$^{*}P$ < 0.05, $^{**}P$ < 0.001 vs Control

$^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 vs HF

Control, normal diet; HF, high-fat diet; HF+DMP10, high-fat diet+10 mg/kg dried mulberry fruit powder; HF+DMP100, high-fat diet+100 mg/kg dried mulberry fruit powder; HF+ATV5, high-fat diet+5 mg/kg atorvastatin. Values are presented as mean± SEM (n = 5).

Effect of DMP on Liver Lipid Profile

Both liver TC and TG were significantly increased in HF group (P < 0.001 vs control, Table 5). The liver TC was reduced by treatment with DMP, both low and high dose, and atorvastatin (P < 0.001 vs HF). The reduction of TG was observed only in the mice treated with high dose of DMP (P < 0.001 vs HF), and this was more pronounced in atorvastatin group (P < 0.001 vs HF, DMP10 and DMP100).

Table 5 Effect of DMP on liver lipid profile of mice

Liver Lipid Profile (mg/50 mg tissue)					
Parameter	**Control**	**HF**	**HF+DMP10**	**HF+DMP100**	**HF+ATV5**
TC	22.61± 0.79	117.46± 4.04***	93.91± 2.37$^{***,\#}$	87.17± 3.16$^{***,\#}$	89.81± 1.07$^{***,\#}$
TG	109.59± 2.45	153.26± 4.13***	142.22± 2.11$^{***,\$}$	127.14± 3.61$^{*,\#,\$}$	89.20± 0.64$^{**,\#}$

$^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$ vs Control

$^{\#}P < 0.001$ vs HF

$^{\$}P < 0.001$ vs HF+ATV5

Control, normal diet; HF, high-fat diet; HF+DMP10, high-fat diet+10 mg/kg dried mulberry fruit powder; HF+DMP100, high-fat diet+100 mg/kg dried mulberry fruit powder; HF+ATV5, high-fat diet+5 mg/kg atorvastatin. Values are presented as mean± SEM (n = 5).

Discussion

The present study demonstrates that dried mulberry fruit powder (DMP) reduces body weight, food intake and visceral fat accumulation in HF-fed mice. It also decreases liver weight and improves liver lipid profile.

In our study, HF-fed mice were characterized by significant increases in body weight, food intake, visceral fat accumulation, liver weight and liver lipid levels including TC and TG compared with the control. These abnormalities were often observed by several other studies using a similar experimental animal model (Lim et al., 2013; Mahmoud, 2013; Ann, Eo, & Lim, 2015; Lee et al., 2019). Administration of DMP (10 and 100 mg/kg) successfully restored body weight of HF-fed mice back to normal, which is consistent with the anti-obesity properties of mulberry reported in most previous studies (Lim et al., 2013; He et al., 2018; Li et al., 2019; Leyva-Jimé nez et al., 2020). Nevertheless, there is a variation in the effect of mulberry on body weight, for example, some investigators reported that mulberry leaf extract had no effect on body weight, but could ameliorate obesity by reducing fat accumulation, hepatic lipogenesis, fibrosis, and oxidative stress in HF-fed mice (Ann, Eo, & Lim, 2015; Lee et al., 2019), while others, similar to our finding, showed that mulberry fruit extract, mulberry leaf extract from some *M. alba* genotypes or the combination of fruit and leaf extract or powder could effectively reduce body weight and also provided other health benefits (Lim et al., 2013; Metwally, Rashad, & Mahmoud, 2019; Li et al., 2019; Leyva-Jimé nez et al., 2020). This discrepancy could be due to the differences in genotype of *M. alba*, extraction process, and parts of the plant used in the studies.

The underlying mechanisms of action of DMP-induced weight loss are likely due to the combined effects of several phytochemicals found in *M. alba,* such as anthocyanins and polysaccharides (Azzini, Giacometti, & Russo, 2017; Lee et al., 2017; He et al., 2018). Anthocyanins, a subfamily of flavonoids, act by modulating glucose, lipid and amino acid metabolic pathways in various targets such as liver, skeletal muscle, adipose tissues and pancreas, leading to the increase in fatty acid oxidation, the improvement of insulin sensitivity and glucose uptake, the reduction of oxidative stress, inflammation, and fatty acids, and the inhibition of cholesterol biosynthesis, resulting in a decrease in body weight and fat accumulation (Azzini, Giacometti, & Russo, 2017). Not only the phenolic compounds, but the polysaccharides isolated from mulberry fruits, also have been shown to stimulate lipolysis and inhibit pre-adipocyte proliferation, thus reducing fat cell numbers and adipose mass (Choi et al., 2016; Chen et al., 2017). This agrees with our result showing that DMP, with a dose-dependent effect, decreased visceral fat accumulation and body weight in HF-fed mice. In addition, the reduced body

weight is well correlated with diminished food intake in DMP treated mice, suggesting that the DMP anti-obesity mechanism may also involve appetite suppression, as previously reported (Yimam et al., 2016; 2019)

Our DMP showed beneficial action in preventing the liver enlargement induced by HF. This effect is associated with the reduction of liver TC and TG by DMP treatment. As mentioned earlier, DMP mechanism of action is likely due to its active ingredients, such as anthocyanins, which have been shown to modulate several metabolic pathways of various organ targets including liver. At the cellular level, anthocyanin has been shown to activate AMP-activated protein kinase (AMPK), which phosphorylates the sterol response element-binding protein-1c (SREBP1c), resulting in inhibition of de novo lipogenesis (Guo et al., 2012). Anthocyanin also stimulated peroxisome proliferator-activated receptor alpha (PPARα), the major lipid oxidation regulator in liver, causing the increase in fatty acid oxidation, hence reducing hepatic lipid concentration (Jia et al., 2013).

The present study used atorvastatin as a positive control. It is a lipid-lowering drug, acting via the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis, resulting in the effective suppression of cholesterol production (Istvan, 2002; Ward, Watts, & Eckel, 2019). Similar to our test product, DMP, atorvastatin also showed positive effects on HF-fed mice including the reduction of body weight, food intake, visceral fat accumulation and liver TC and TG. However, unlike DMP, atorvastatin was unable to restore the increased liver weight caused by HF consumption. This may imply a hepatotoxicity in atorvastatin, as it has been reported that statins could induce hepatocellular injury, although the mechanisms are still unclear (Ward, Watts, & Eckel, 2019). This is of interest since it suggests that DMP is apparently safe and could help prevent the liver abnormality caused by HF consumption.

Conclusion and Suggestions

Taken together, our results indicate that DMP carries anti-obesity properties and has a beneficial effect in the prevention of liver lipid accumulation. This is among the few studies that have tested the biological activity of natural unprocessed mulberry fruit, which could potentially be cost-effectively and safely developed as a dietary supplement in local markets. There is, however, a need to further characterize the nutrients and chemical composition of DMP, as well as to evaluate the protective effect of DMP on chronic diseases associated with obesity such as cardiovascular diseases, dyslipidemia and diabetes. Full range of liver lipid profile including TC, TG, LDL and HDL as well as liver histology should also be evaluated. Clinical trials designed to investigate the safety and health benefits of DMP are also required.

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