THERAPEUTIC IMPACT OF BERRIES (MORUS ALBA AND MORUS RUBRA) FRUIT EXTRACT IN THE REGRESSION OF HIGH-FAT DIET-INDUCED CARDIAC DYSFUNCTION IN RATS

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ABSTRACT

Objective: The aim of the present study is to investigate the effect of Morus alba (MA) and Morus rubra L. (MR) fruit extract on obesity-induced cardiac dysfunction and fibrosis in cardiac tissue.

Methods: Seventy male Wistar albino rats were randomly divided into five groups of ten rats each. MA and MR have been administered for 6 weeks in obese rats induced by high-fat diet (HFD). Adiponectin, glucagon, troponin, plasminogen activator inhibitor, cell adhesion molecules-1 (intracellular and vascular respectively), C-reactive protein, collagen type II and collagen alpha-1 (III) chain, and lipoxygenase activity were also estimated in serum of obese rats. Histopathological investigation of cardiac tissue was carried out.

Results: MA and MR treatments significantly normalized cardiac dysfunction biomarkers as well as cardiac fibrosis as examined by histopathological examination with higher percentage of improvement for MR extract.

Conclusion: Hence, it could be concluded that MA and MR extracts have useful effects on obesity-associated cardiac diseases through lipid metabolism regulation, cardiac functions and reversed cardiac fibrosis.

Keywords: Mulberry fruit, Cardiac dysfunction, Cardiac fibrosis, Obesity.

INTRODUCTION

It was found that excess free fatty acids (FFAs) can enhance the response to oxidative stress, which is mechanism implicated obesity, cardiovascular alterations, and cancers [1]. Moreover, fat mass expansion leads to infiltration of macrophage in adipose tissue and pro-inflammatory cytokine production accompanied by an anti-inflammatory cytokines suppression, which is ultimately connected with the obesity development-related comorbidities. Hence, chronic low-grade inflammation and oxidative stress occur as a result of the dysregulation of adipokines and infiltration of inflammatory cells in adipose tissue [1]. Thus, endothelial cell dysfunction occurs as a leading cause of oxidative stress and systemic inflammation, resulting in insulin resistance, diabetes, and atherosclerosis [1]. Most current drugs for obesity have adverse side effects. Hence, the approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs [2]. Mulberry leaf and fruit have been a part of traditional medicine for a long time and have been used to prevent or treat obesity, diabetes, and dyslipidemia [3]. It is a medicinally important plant belonging to genus Morus that is widely distributed in India, China, Japan, North Africa, Arabia, South Europe, etc. [4]. Several studies have also declared that mulberry leaves suppress the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) stimulated by tumor necrosis factor alpha (TNF-α) in vascular endothelial cells and regulate the inflammatory response and oxidative stress by inhibiting nitrite and thiobarbituric acid production reactive substances in blood and tissues [5]. Anthocyanins in mulberry fruit can trap free radicals and decrease the oxidation of low-density lipoprotein (LDL) [1]. In addition, mulberry fruit ameliorates inflammation induced by lipopolysaccharide (LPS) in mouse and arthritic rats [1].

The mulberry fruits (Morus alba L [MA] and Morus rubra [MR]) distributed widely in Asia as a food containing potential health benefits beyond the traditional nutrients they provide [6]. It is rich in polyphenolic compounds including rutin, quercetin, and 1-deoxynojirimycin (DNJ). Hence, it has been medicated several diseases including dyslipidemia [7], diabetes [8], fatty liver [6], and hypertension diseases [8]. Besides, the mulberry fruits induce enzymatic antioxidant in diabetic models [8]. However, few studies pay particular attention to the mechanism in which fruits of the mulberry attenuate lipogenesis, lipolysis, and fibrosis in obese models induced by HFD. Hence, the aim of the present research is to investigate whether the fruit extract of mulberry (MA and MR) could improve cardiac dysfunction and fibrotic cardiac tissue in HFD-induced obesity in rats.

MATERIALS AND METHODS

Collection of plant material

Fresh fruits of white MA and purple MR were collected in the Delta region, Egypt. The berries were selected according to uniformity of shape and color. The identification of the plant was confirmed by Therese Labib, Herbarium Section, El-Orman Botanical Garden, Giza, Egypt. The fresh fruit samples were cleaned, stored in polyethylene bags, and frozen at ~20°C till further use.

Preparation of extracts for bioassays

The fresh fruits of each species (200 g) were extracted separately with 700 mL of 70% aqueous ethanol for 3 h, on an orbital shaker in the dark at room temperature. Each extract was separated by centrifugation (13,000× g, 10 min), the supernatant was taken, the residue was resuspended in 50 mL of the same solvent, and the mixture was again...
Experimental animals
Male albino rats (n=70) weighing 150±10 g were obtained from the Animal House of the National Research Centre (NRC). Animals were quarantined and allowed to acclimate for 10 days before beginning experimentation. They were housed 10 per cage under temperature-controlled environment (26–29°C) with a fixed light/dark cycle with free access to water and food. All procedures of the present study were performed according to the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.

Induction of obesity in rats
Obesity was induced in rats according to the method of Adamnoy et al. [9] by feeding rats HFD of lard. Cholesterol was orally administered at a dose of 30 mg/0.3 mL olive oil/kg animal 5 times a week for 12 consecutive weeks. Lard fat was mixed with normal diet (1 kg of animal lard was added to 5 kg of normal diet). The occurrence of obesity was determined by measuring body weight gain percentages, visceral, and fecal fat percentages.

Doses and routes of administration
Obese rats received an oral dose of 2 mg/kg body weight dissolved in distilled water of the anti-obesity reference drug, orlistat (OR) (12 mg/kg) for 6 weeks [10]. Purple and white berry ethanol extracts were administered orally for 6 weeks in a dose of 300 mg/kg body weight [11].

Biochemical measurements
Various biochemical parameters were measured including adiponectin. Measurement of serum adiponectin levels gives us important information on the role of adiponectin in the regulation of glucose and/or lipid metabolism. The rat adiponectin ELIZA kit was used for quantitative determination of adiponectin in rat serum. Glucagon enzyme immunoassay (EIA) kit is an in vitro quantitative assay for detecting glucagon peptide based on the principle of competitive EIA. Troponin rats’ cardiac troponin I was measured in serum using ELIZA kit. Plasminogen activator inhibitor (PAI-1), intracellular and vascular cell adhesion molecules (ICAM and VCAM), were qualitatively determined in serum of rats by ELIZA. Rat C-reactive protein (CRP) was quantitative measured in rat serum using ELIZA. Rat collagen type II (Col II) and collagen alpha-1(III) chain (Col 3A1) were measured in rat serum by ELIZA kit. Lipoxynegase activity (LOX) was measured in serum using fluorometric method.

Experimental design
Seventy male Wistar albino rats (15-16 weeks old) weighing at 150±10 g (mean ±SD) were used. Weight of rats was on the day received from supplier. After adaptation period to the environment, the rats were randomly divided into seven groups (n=10/group) as follows: Group (1) is the normal diet (ND). Groups (2) and (3) are ND treated with 300 mg/kg/BW of MA and MR extracts for 12 consecutive weeks (control ND/MA and control ND/MR, respectively). Group (4) is the HFD-treated rats for 12 consecutive weeks. Groups (5) and (6) are obese rats treated for 6 weeks with 300 mg/kg body weight of ethanolic extract of white and purple berry (HFD/MA and HFD/MR, respectively). Group (7) is the obese rats treated for 6 weeks with anti-obesity standard drug OR (12 mg/kg body weight) (HFD/OR). Health conditions of all rats were monitored daily, and no adverse events were observed throughout the study. At the beginning of the experiment, the weight of all rats was recorded at (150±10.0 g) (weight of rats after acclimatization). All experiments and biochemical analysis were conducted using 70 rats with triplicate measurements. The permission to conduct this study was according to the Ethics of NRC, Egypt.

DISCUSSION
The current results present higher adiponectin serum levels, in HFD rats. This finding is in a parallel with the results of Davidson et al. [14,15] who reported an increase in adiponectin plasma levels post 24 and 32 weeks of HF diet supplementation. These could be explained on the basis of; adiponectin is renowned, by its sensitizing action of insulin. However, obesity may produce a fail on adiponectin signaling (resistance of diponectin). However, Marques et al. [16] showed that adiponectin plays a role in the decrease of insulin sensitivity happened by HFD and illustrated that hyperglycemia initiated by the HFD was associated with linked by a decrease in gene expression of adiponectin in the adipose tissue while did not cause a reduction in the serum levels of adiponectin, speculating that there is a compensative influence of the other depot of fat on serum levels of adiponectin. Previously, it
was proposed that the expression of the adiponectin receptors may be affected by the high insulin levels due to HFD [17].

Further, the present results declared a significant reduction in glucagon levels in the rats with HFD as compared to control rats, and also inhibits gastric emptying, food intake, and glucagon secretion [18]. Moreover, obesity may be developed in many species of rodents connected with different metabolic syndrome, including glucose intolerance, insulin resistance, and dyslipidemia [19]. The cause why a rodent connected with different metabolic syndrome, including glucose intolerance, insulin resistance, and dyslipidemia [18].

Statistical analysis is carried out using SPSS computer program, combined with costate computer program, where unshared letter is statistically significant at $P \leq 0.05$. MA: Morus alba, MR: Morus rubra, HFD: High-fat diet, OR: Orlistat, CRP: C-reactive protein, ICAM: Intracellular adhesion molecule-1, VCAM: Vascular cell adhesion molecule-1, PAI: Plasminogen activator inhibitor.

**Table 1: Effects of MA and MR on adiponectin, glucagon, troponin, and PAI-1 in obese rats and therapeutic groups**

| Markers          | Control | Control/MA | Control/MR | HFD | HFD/MA | HFD/MR | HFD/OR |
|------------------|---------|------------|------------|-----|--------|--------|--------|
| Adiponectin (ng/ml) | 15.00±0.34 | 15.34±1.00 | 15.67±1.23 | 27.56±0.92 | 21.32±0.65 | 19.11±0.44 | 20.00±0.65 |
| % change          | 2.27    | 4.47       | 83.73      | 42.13 | 24.78  | 27.40  | 33.33  |
| % improvement     |         |            |            | 41.60 | 56.33  | 50.40  |        |
| Glucagon (Pg/ml)  | 12.20±0.90 | 12.90±0.12 | 12.65±0.10 | 5.53±0.87 | 7.12±0.67 | 7.14±0.99 | 7.15±1.09 |
| % change          | -       | 3.69       | 3.68       | 54.67 | 35.90  | 41.48  | 41.39  |
| % improvement     |         |            |            | 18.77 | 13.20  | 12.28  |        |
| Troponin I (Pg/ml) | 40.00±4.32 | 40.54±2.90 | 41.65±4.22 | 90.22±5.11 | 60.51±5.33 | 54.26±7.80 | 67.45±6.78 |
| % change          | -       | 1.35       | 4.13       | 125.55 | 51.28  | 35.65  | 68.63  |
| % improvement     |         |            |            | 74.28 | 89.70  | 56.92  |        |
| PAI-1 (Pg/ml)     | 12.80±0.90 | 12.00±1.12 | 12.35±1.43 | 23.00±2.11 | 17.24±1.33 | 15.52±1.33 | 16.00±1.25 |
| % change          | 6.25    | 3.52       | 79.69      | 34.69 | 21.25  | 25.00  |        |
| % improvement     |         |            |            | 45.00 | 58.43  | 54.68  |        |

**Table 2: Effects of MA and MR on ICAM, VCAM, and CRP in obese and therapeutic groups**

| Markers          | Control/ND | Control/MA | Control/MR | HFD | HFD/MA | HFD/MR | HFD/OR |
|------------------|------------|------------|------------|-----|--------|--------|--------|
| ICAM (ng/ml)     | 2.02±11.24 | 2.07±7.80  | 2.01±9.11  | 5.90±13.00 | 4.51±15.00 | 42.36±18.66 | 38.24±12.55 |
| % change          | 1.44       | 4.82       | 168.04     | 89.05 | 92.48  | 73.73  |        |
| % improvement     |            |            |            | 78.98 | 75.35  | 94.3   |        |
| VCAM (Pg/ml)     | 1.10±0.09  | 1.05±0.12  | 1.0±0.23   | 3.97±0.99 | 2.90±0.21 | 2.51±0.77 | 2.33±0.29 |
| % change          | 4.55       | 9.1        | 251.82     | 163.65 | 128.18 | 111.82 |        |
| % improvement     |            |            |            | 88.18 | 123.64 | 140    |        |
| CRP (Pg/ml)      | 9.15±0.89  | 9.00±0.15  | 9.23±0.29  | 18.76±1.23 | 14.05±1.26 | 13.20±0.96 | 12.90±0.69 |
| % change          | -         | 1.64       | 99.73      | 105.03 | 53.55  | 44.26  | 40.98  |
| % improvement     |            |            |            | 51.48 | 60.76  | 64.26  |        |

**Table 3: Effects of MA and MR on Col II, Col 3A1, and LXO in obese rats and therapeutic groups**

| Parameters       | Control/ND | Control/MA | Control/MR | HFD | HFD/MA | HFD/MR | HFD/OR |
|------------------|------------|------------|------------|-----|--------|--------|--------|
| Col II (ng/ml)   | 20.30±1.28 | 19.00±1.20 | 19.80±1.50 | 45.00±3.70 | 26.31±1.64 | 25.33±2.10 | 27.21±1.78 |
| % change         | -          | 6.4        | 2.46       | 121.67 | 29.61  | 24.78  | 34.04  |
| % improvement    |            |            |            | 92.07 | 96.89  | 87.64  |        |
| Col 3A1 (ng/ml)  | 12.29±1.01 | 12.00±1.20 | 12.55±1.10 | 30.00±1.90 | 15.00±0.99 | 12.66±1.10 | 19.82±2.00 |
| % change         | -          | 2.35       | 2.12       | 144.11 | 22.05  | 3.01   | 53.94  |
| % improvement    |            |            |            | 122.05 | 141.09 | 90.15  |        |
| LXO              | 0.50±0.03  | 0.55±0.04  | 0.50±0.02  | 1.12±0.07 | 0.80±0.05 | 0.70±0.03 | 0.72±0.06 |
| % change         | 10         | 0          | 124        | 60     | 40     | 44     |        |
| % improvement    | -          |            |            | 64     | 84     | 80     |        |

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obese mice. Cardiac hypertrophy is a very early consequence of diet-induced obesity, apparent early and accompanied by substantial cardiac dysfunction [21]. Troponin I may be elevated due to HFD-promoted...
The results of the present research showed a significant increase in both collagen type fractions 11 and 111 in serum of obese rats. No information was found in the literature as a possible explanation for this finding. Dey et al. [27] inferred that the reduction in collagen fraction 1 and related to the decrease in the synthesis and/or an increase in degradation of collagen 1. Besides, adipose tissue secretes several substances that are implicated in the myocardial collagen regulation, such as leptin, which is released by adipocytes and is manufactured by various tissues including the heart [28]. A clear relation between obesity and heart failure exists and a significant role for leptin, the product of the obese gene, has been suggested. One aspect of myocardial remodeling which characterizes heart failure is a disruption in the balance of extracellular matrix synthesis and degradation. Leptin regulates cardiomyocyte hypertrophy and adjusts output of the different components to the extracellular matrix of the myocardium that act on cardiac fibroblasts. It is possible that increased activity of both matrix metalloproteinase 2 (MMP-2) and MMP-9 is responsible for the reduction in myocardial collagen type I. In controversy with the present results, Silva et al. [27] showed that myocardial collagen type III did not change in obesity.

In parallel with our results, Carroll et al. [29] explained that a HFD for a 12-week period causes fibrosis in coronary vessels, as well as accumulation of collagen in the cardiac interstitium. The mechanisms underlying the accumulation of collagen in animals remain unknown; Leopoldo et al. [30] proposed that the higher levels of collagen are associated with anomaly in insulin metabolism. Insulin growth factor induces transforming growth factor-beta-1, which directly stimulates collagen expression. However, given that obesity also has been linked with cardiac fibrosis through elevation of some factors implicated in the development of cardiac fibrosis such as cytokines, endothelin, and renin-angiotensin-aldosterone [30]. The same authors added that obesity promotes pathological myocardial fibrosis and damage to the myocardial ultrastructure, as indicated in the present study (Photomicrograph 4).

The current results also indicated a significant increase in Lox in obese rats; Chakrabarti et al. [31] proposed that low-grade inflammation is a leading cause of obesity and promotes type 2 diabetes and cardiovascular disease in obese individuals. The 12- and 5-LOX (12-LOX and 5-LOX) enzymes have been connected with inflammatory alterations, causing atherosclerosis development. On the other hand, treatment of obese rats with MAF and MRF extracts exhibited marked improvement in aforementioned biomarkers, especially with MRF extract which demonstrated higher percentages of improvement than standard drug. These findings may be explained by mulberry leaf extract (MLE) exhibited its useful effect on lipid profiles regulation and the atherogenic index, joined with reduction of fat accumulation in the liver [8]. Moreover, MLE decreased fibrosis of hepatic tissue as examined by collagen gene expressions. In addition, medication with MLE improved oxidative stress and metabolic abnormalities in obese subjects induced by HFD, due to its strong antioxidant [32], anti-obesity, and anti-inflammatory effects [1,33]. In addition, Ann et al. [8] declared that mulberry leaf has different biological effects including free radicals scavenging, oxidation inhibition, and atherogenic risk-reducing activities, related to several polyphenolic compounds such as DNJ-1 and resveratrol. These compounds were demonstrated to exhibit anti-obesity activities by blocking preadipocytes differentiation [8] and activating β-oxidation system [8]. Previously, DNJ-rich MLE has been effectively used to ameliorate lipid profiles in human subjects [34] and to inhibit accumulation of lipid through stimulation of β-oxidation [8]. In addition, resveratrol is characteristic by its ability to reduce reactive oxygen species (ROS) and activate oxidation of fatty acid [35]. Further, medication with MLE
considerably modulated the HFD-induced accumulation of hepatic lipid through the suppression in lipogenesis process and the enhancement in lipolysis in HFD-induced non alcoholic fatty liver disease (NAFLD). Accumulation of hepatic lipid is also regulated by gene expression induction implicated in energy expenditure and oxidation of fatty acids through increasing degradation of lipid and energy metabolism [8].

Park et al. [36] illustrated that the mice with HFD were recognized by increasing hepatic fibrosis biomarkers such as α-smooth muscle and type 1 collagen. Huang et al. [37] demonstrated that hepatic fibrosis and development of NAFLD are induced by differentiation of adipocyte and oxidative stress. The current study could give a clue for the first time on the beneficial effect of MA and MR extracts supplementation on cardiac dysfunction and fibrosis by attenuating ROS, vascular function, lipid accumulation, inflammation, and reduced collagen in the HFD-induced obesity.

In accordance with our results, Lim et al. [1] declared that mulberry fruit improves blood lipid profiles and lipid metabolism in hyperlipidemic rats. The same author added that excessive FAs and saturated fatty acids from adipose tissue lead to fat accumulation in the liver and other tissues, resulting in an increased inflammatory reaction. Besides, fat accumulation in the liver increases LDL over production together with inflammatory cytokines, such as IL-6 and CRP. Our results show that serum levels of CRP increased in HFD group but selectively decreased by both MFE treatments. Treatments with both MLE and MFE were effectively against obesity and its related inflammation and oxidative stress [1].

The presence of DNJ in the extract of mulberry leaf serves in normalization of serum adiponentin level and enhances AMP-activated protein kinase. These in turn activate β-oxidation of fatty acids which inhibit hepatic lipid accumulation. Supplementation of MA fruits powder to rats recorded marked reduction in triglyceride, total cholesterol, LDL, and athemogenic index. Furthermore, powder of mulberry leaf can protect the cardiac function by attenuating oxidative stress, cellular infiltration, cardiac fibrosis and myocyte apoptosis. MA root extracts were shown to have anti-inflammatory efficacy [38]. MA butanol extract significantly decreased LPS-stimulated production of PGF2, TNF-α, and cyclooxygenase 2 (COX-2) expression in RAW264.7 macrophages [39], while methanol extract of MA has different compounds with inductive nitric oxide synthase (iNOS) inhibitory activity which can correlate with its anti-inflammatory activities [38]. Morus bombycis extract exhibited anti-inflammatory and inhibitory activities on collagen-induced arthritis. Further, MA root extract contains a large amount of cudinaflavone B which is a prenylated flavonoid and causes noticeable inhibition in inflammatory mediators in some in vitro models. It was a powerful TNF-α inhibitor by preventing the NF-kB translocation from the cytoplasm to the nucleus. The NF-kB inhibition activity leads to an inhibition in the gene expressions of COX-2 [40,41]. Resveratrol purified from MA inhibits a secretion of IL-8 by blocking phosphorylation of MAPK and activation of NF-kappa B in LPS-induced human monocyotic cell line THP-1 [38,42]. Sanguenos C and D, isolated from MA, inhibit production of NO through induction of iNOS and nuclear factor activation in LPS-induced RAW264.7 cells indicating its anti-inflammatory efficiency [38]. The ameliorative effects of both berries are also documented at the cellular level in the present study, which declared no histopathological alterations compared to standard drug (Photomicrographs 5 and 7).

CONCLUSION
MA and MR extracts modulate obesity-induced cardiac dysfunction through inhibition of lipogenesis, fibrosis, and enhancement of lipolysis in obesity induced by HFD. Furthermore, MA and MR regulated vascular function, inflammatory markers, and lipid accumulation which considered as risk factors for protection and/or remediation of obesity-associated cardiac dysfunction. The present findings could provide an insight into the strategy development to protect and handle obesity in the future.

AUTHOR’S CONTRIBUTIONS
Farouk K. El-Baz: Plan of work, writing, revision, and corresponding author. Hanaa F. Aly: Experimental animals, statistical analysis, and writing. Howaida I. Abd-Alla: Biochemical measurements and writing. Dalia F. Biomy: Sample preparation, preparation of extract for bioassays, and histological investigation.

CONFLICTS OF INTEREST
Authors declare no conflict of interest.

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