

BERRIES SUPPLEMENTATION MODULATES BODY WEIGHT AND METABOLIC DETERIORATIONS IN OBESE RATSFAROUK K EL-BAZ^{1*}, HANAN F. ALY², HOWAIDA I. ABD-ALLA³¹Department of Plant Biochemistry, National Research Centre, 33 El Bohouthst (Former El Tahrirst), Dokki, Giza, P.O. 12622, Egypt.²Department of Therapeutic Chemistry, National Research Centre, 33 El Bohouthst (Former El Tahrirst), Dokki, Giza, P.O. 12622, Egypt.³Department of Chemistry of Natural Compounds, National Research Centre, 33 El Bohouth St. (Former El Tahrirst), Dokki, Giza, P.O. 12622, Egypt. Email: fa_elbaz@hotmail.com

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ABSTRACT

Objective: The aim of the present work is to investigate the potential of purple (*Morus rubra* [MR]) and white (*Morus alba* [MA]) berry supplementation to modulate body weight (BW) and metabolic deteriorations in obese Wistar male rats.

Methods: Seventy rats weighing 150.00±10 g (mean±standard deviation) were used; the rats were randomly divided into seven groups of 10 rats each. Obesity was induced by feeding animals high-fat diet (HFD), for 6 consecutive weeks then treated with the purple (MR) and white (MA) berry ethanol extracts for 6 weeks in a dose 300 mg/Kg BW. BW gain, food intake fecal fat content, food consumptions were determined. The fats and organs such as liver, kidney, and heart were also weighed. Lipid profiles included triglyceride, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, as well as liver enzymes; alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, γ -glutamyltransferase (γ -GGT), and leptin were also determined.

Results: Treatment of obese rats with white and purple berries showed significant reduction in BW reached to 23.68% with MR and 21.19% with MA berries compared to Orlistat (31.16%). Liver weight, visceral fat, and liver enzyme activities were also markedly ameliorated. In addition, lipid profile biomarkers were improved on treated obese rats with both *Morus* species. In addition, increase in fecal fat (250.73% and 256.25%, for MR and MA, respectively) was noticed while blood glucose, insulin, leptin levels, and pancreatic lipase activity was improved. In addition, supplementing obese rats with both *Morus* species extract significantly increase food intake reached 33.33% and 36.66%, for MR and MA, respectively.

Conclusion: The ameliorating effect of both *Morus* extracts may be attributed to polyphenolic rich compounds which have antioxidant properties, suppressed lipid synthesis in hepatic tissue and inhibited pancreatic lipase activity which consequently recommended using as a promising anti-obesity agents.

Keywords: Obesity, Berry, Lipid profile, Pancreatic lipase, Leptin level, Visceral fat.

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INTRODUCTION

Obesity is a serious health problem around the world and is associated with an increased risk of complications including insulin resistance, dyslipidemia, and diabetes mellitus [1]. Obesity is also coincided with metabolic alterations including nonalcoholic fatty liver disease (NAFLD) and characterized by hyperleptinemia [2].

The occurrence of NAFLD is rising in parallel with insulin resistance and the epidemic of obesity and that is joined to the diet of most global populations [3]. The liver is considered for the regulation of entire body energy homeostasis because of its central role in glucose and lipid metabolism.

High-fat diets (HFDs) have been shown to induce dysregulated gene expression of hepatic tissues which affect cholesterol, lipid, oxidative, and inflammatory pathways [4]. NAFLD is characterized by the increase of lipids and its derivatives in liver tissues and increase the potential damage by induction of fibrosis and inflammation, due to oxidative stress induction [5].

Healthy body weight (BW) is affected by the types of foods. It is recommended that increase in the consumption of fruit and vegetables, protecting the body from incidence of high weight gain and therefore, preventing NAFLD of obesity [6].

Morus, also called berry, is a well-known functional food and medicinal herb. The herb has been used as a remedy for dysentery, diuretic, expectorant, and antidiabetic in traditional medicine. The fruits of berries have been made into a variety of food products [7].

Leaves of berry have been known to possess beneficial insulin tropic properties and its antioxidant potential can yield protection against complications of diabetes mellitus [8]. A positive effect was exhibited by *Morus* tea supplement as it reduces the post-prandial hyperglycemia type-2 diabetes [9].

Berries are rich in polyphenolic compounds which may cause a reduction of accumulated fat in liver tissues [10]. Polyphenolic compounds are commonly metabolized in the liver and intestine, in which they recalculate in the hepatic circulation and several metabolites association with hepatic and immune cells [11].

Morus species are multifunctional plants with promising medicinal properties [12]. Many reports indicated that berries are a good source of micronutrients, stilbenes, anthocyanins, flavonoids, and benzofurans [12,13]. These compounds have shown a wide range of bioactive features including hypoglycemic, neuroprotective functions, anti-inflammatory, and antioxidative effects [10,14,15]. Therefore, the objective of the present study is to investigate the potency of purple and white berry extracts on obesity induced by HFD through measuring

different physiological biomarkers related metabolic changes including liver function enzyme activities, BW gain percentage, organ weight, food intake, visceral and fecal fats percentages, blood glucose, insulin, and leptin levels as well as pancreatic lipase activity.

MATERIALS AND METHODS

Collection of plant material

Fresh fruits of white *Morus alba* (MA) and purple *Morus rubra* (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, and 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 (EtOH) for 3 h, on an orbital shaker in the dark at room temperature. Each extract was separated by centrifugation (13,000 \times g, 10 min), the supernatant was taken, the residue was resuspended in 50 mL of the same solvent, and the mixture was again separated by centrifugation. The two resulting supernatants were then combined and concentrated under reduced pressure at 40°C till dryness to get 2.54% and 2.39% of crude EtOH extract of MA and MR, respectively. The residues were stored in the dark at -20°C .

Biological assay

Experimental animals

Male albino rats (n=50) weighted (150 \pm 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 $^{\circ}\text{C}$) 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 Adaramoye et al. [16] by feeding rat's HFD of cholesterol. 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 (ND) (1 kg of animal lard was added to 5 kg of ND). The occurrence of obesity was determined by measuring BW gain percentages, visceral and fecal fat percentages.

Doses and routes of administration

Obese rats received an oral dose of 2 mg/kg BW dissolved in distilled water of the antiobesity reference drug; Orlistat (12 mg/kg) for 6 weeks [17]. Purple and white berry ethanol extracts were administered orally for 6 weeks in a dose 300 mg/kg BW [18].

Determination of BW gain, food intake, and energy intake

BW change and food intake was measured according to the method of Akase et al. [19]. Intake of food was measured weekly on a cage basis and expressed as g of food/day. Initial BW of all animals was measured before they were fed with either ND or HFD for 12 weeks. At the end of each week, the weight gain percentage was calculated as followed:

$$\text{Weight gain\%} = \frac{\text{New weight } W_1 - \text{Initial weight } W_0}{\text{Initial weight } W_0} \times 100$$

Determination of fecal fat content

Fecal fat content of the rats was determined based on the slightly modified method described by Bligh and Dyer [20]. Briefly, feces (0.5 g) were soaked in 2 mL distilled water and homogenized completely. It was then stored at 4°C for 24 h followed by homogenization by vortex

for 1 min. Extraction of lipids from feces was executed using 7.5 mL chloroform: methanol (1:2, v:v) for 30 min, followed by the addition of 2.5 mL of chloroform, and deionized water and shaking for another 30 min. Resultant mixtures were then centrifuged (at 2000 \times g, 15 min). The lipophilic layer was isolated and dried under reduced pressure.

Food consumption

Individual BW gains were recorded before study initiation (Day 0), and weekly thereafter. Mean BW gains were calculated for each group at each interval and for the overall testing interval. During the study, food consumptions were measured weekly per cage and mean food consumptions by individual rats were calculated [15].

Experimental design

Seventy male Wistar albino rats weighing at 150.00 \pm 10 g (mean \pm standard deviation [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 ND feeding rats (ND). Group (2) and (3) are ND rats treated with 300 mg/kg BW of MR and MA extracts for 12 consecutive weeks (control ND/MR and control ND/MA, respectively). Group (4) is the HFD-treated rats for 12 weeks. Groups (5) and (6) are obese rats treated for 6 weeks with 300 mg/kg BW of ethanolic extract of purple and white berry (HFD/MR and HFD/MA, respectively). Group (7) is the obese rats treated for 6 weeks with anti-obesity standard drug Orlistat (OS) (12mg/kg BW) (HFD/OS). Health conditions of all rats were monitored daily and no adverse events were observed throughout the study. At the beginning of the experiments, the weights of all rats were recorded at 155.00 \pm 5.00 g (weight of rats after 10 days of acclimatization). All experiments and biochemical analysis were conducted using 50 rats with triplicate measurements. The permission to conduct this study was according to the ethics of NRC, Egypt.

Blood sample and collection of serum liver, kidney, heart, visceral fats, and feces

Blood samples were obtained following an overnight fasting state at the end of treatment (week 12) at 8 a.m. Samples were withdrawn from a cubital vein into blood tubes under diethyl ether anesthesia and immediately stored on ice at 4°C . The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min and they were stored until analyzing at -80°C [21]. After 12 and 18 weeks of treatment, all the rats were sacrificed. The fats and organs such liver, kidney, and heart were also weighed. Feces were collected in the final week of the study for the determination of fecal fat content.

Biochemical measurements

Various biochemical parameters were measured including lipid profiles of plasma triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) (Biodiagnostic, Egypt), liver enzymes of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), γ -glutamyltransferase (γ -GGT) (Biodiagnostic, Egypt), insulin (Mercodia Rat Insulin ELISA Kit, Uppsala, Sweden), leptin (RayBio Rat Leptin ELISA kit, Norcross, GA, USA, Cat# ELR-Leptin-001). Concentration of LDL-C was calculated by Friedwald's formula [22]. Whereas the serum, TG, and HDL-C were determined using enzymatic colorimetric methods according to Kostner [23]. ALT and AST activities enzymes were estimated according to Reitman and Frankel [24]. ALP was measured by the method of Belfield and Goldberg [25] using a diagnostic kit (Biosystems, Egypt). Serum γ -GGT activity was measured according to Persijnandvan der Slik [26] using QCA Diagnostic kits (Spain). Pancreatic lipase activity was performed according to previously described method [27].

Statistical analysis

Data are expressed as mean \pm SD of 10 rats in each group. Statistical analysis is carried out using SPSS compute program coupled with

CO-state computer program, where unshared letters are significant at $p \leq 0.05$.

RESULTS

The effects of purple and white berries were evaluated on the following.

BW gain percentage and food intake in obese rats

Significant increase was noticed in BW gain reached to 140.63% in obese rats feed HFD for 12 weeks. While, significant reduction in BW gain in HFD treated rats with MR berry (-23.68%), MA berry (-21.19%) compared to Orlistat (31.16%). Beside, food intake showed insignificant change in control rats treated either with red or white berry extracts compared to normal control untreated rats. In addition, HFD feed rats showed insignificant change in food intake compared to control one. However, obese treated rats with purple, white, and standard drug exhibited significant increase in food intake with percentage increase reached to 33.33, 36.66, and 25.55%, respectively (Table 1).

Visceral and fecal fats percentage in obese rats

Table 2 clearly demonstrated significant increase in visceral fat in obese rats (HFD) with percentage 285.80%. While significant reduction in visceral fat in obese rats treated with MR (-39.79%), and MA extract (-38.29%), was noticed compared with standard drug (-55.01%). Further, significant increase in fecal fat was recorded in obese rats with percentage 110.29%, compared to control rats. Obvious increase reached to 250.73, 256.25, and 396.32% on treated obese rats with MR, MA, and OS, respectively.

Organ weights of obese rats treated for 6 weeks

Table 3, indicated significant increase in liver weight in obese rats with percentage increase 45.16%. Treatment of obese rats with purple and

white berry extracts as well as OS as reference drug showed significant reduction in liver weight by 18.88 and 14.44 and 22.22%, respectively compared to HFD-rats liver. While insignificant change was detected in cardiac and renal tissues in obese rats compared to normal control one.

Insulin, blood glucose, leptin levels, and pancreatic lipase activity in treated obese rats

Results indicated in Table 4, showed significant increase in blood glucose, insulin, leptin levels and pancreatic lipase activity in obese rats (G4) with percentage 79.08, 52.00, 70.54 and 240.29%, respectively compared to control one. Noticeable improvement in blood glucose levels was noticed recorded 35.69, 25.60 and 45.67%, respectively, on treated obese rats with red, white and OS reference drug. Also, treatment of obese rats with red and white berry extracts demonstrated improvement in insulin level by 9.52 and 12.00%, respectively while normalization in its level was noticed with OS (38.07%). Further, lipase activity in obese rats treated with red and white berry extracts recorded improvement percentages reached to 32.55 and 50.38%, respectively, while normalization in its activity was noticed using OS. In addition, obvious improvement was recorded also for leptin level in obese rats treated with both purple and white extracts as well as standard drug (94.66, 67.96 and 218.93%, respectively).

Liver function enzymes in obese rats for 6 weeks of treatment post induction with HFD

Table 5 demonstrated insignificant change in all liver function enzyme activities in ND feed rats and treated with red and white berries as compared to control one. While obese induced rats with HFD exhibited significant increase in all liver function enzymes reached to 79.83, 41.04, 199.82 and 75.57%, for ALT, AST, γ -GGT, and ALP, respectively.

Table 1: Effects of *Morus rubra* and *Morus alba* extract on body weight gain percentage and food intake in obese rats

BW gain	Control ND	Control ND/MR	ND/MA control	HFD	HFD/MR	HFD/MA	HFD/OS
Initial body weight (g)	155.00 ^a ±4.16	150.00 ^a ±4.26	158.00 ^a ±4.90	160.00 ^a ±2.32	380.00 ^b ±4.77	387.00 ^b ±2.12	385.00 ^b ±8.88
Final body Weight (g)	195.00 ^c ±4.99	180.00 ^c ±6.34	189.00 ^c ±3.26	385.00 ^b ±3.26	290.00 ^d ±7.55	305.00 ^d ±8.00	265.00 ^e ±7.20
Body weight gain (%)	25.80 ^a ±2.88	20.00 ^b ±1.00	19.62 ^b ±0.76	140.63 ^c ±6.87	-23.68 ^d ±1.99	-21.19 ^d ±0.44	-31.16 ^e ±0.66
%improvement				453.29	462.94	424.3	
Food intake (g/week)	180.00 ^a ±6.77	179.00 ^a ±4.16	185.00 ^a ±3.65	176.00 ^a ±8.54	240.00 ^b ±6.64	246.00 ^b ±5.88	226.00 ^b ±2.00
% change	-	0.5	2.77	2.22	33.33	36.66	25.55

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with MR extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with *Morus alba* extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD fed rats treated orally with MR for 6 weeks post-induction. HFD/MA: HFD fed rats treated orally with *Morus alba* for 6 weeks post induction. FHD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with co-state computer program. Unshared letter is significant at $P \leq 0.05$. HFD: High-fat diet

Table 2: Effects of *Morus rubra* and *Morus alba* extract on of visceral and fecal fats percentage in obese rats

Parameters	Control ND	Control ND/MR	ND/MA control	HFD	HFD/MR	HFD/MA	HFD/OS
Visceral fat (%)	1.55 ^a ±0.55	1.52 ^a ±0.05	1.50 ^a ±0.06	5.98 ^b ±0.71	3.60 ^c ±0.09	3.69 ^c ±0.56	2.69 ^d ±0.30
%change		-1.93	-1.50	285.8	-39.79	-38.29	-55.01
Fecal fat (%)	2.72 ^a ±0.25	2.89 ^a ±0.21	2.80 ^a ±0.22	5.72 ^b ±0.91	9.54 ^c ±0.68	9.69 ^c ±0.66	13.50 ^d ±0.96
%change		6.25	8	110.29	250.73	256.25	396.32

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with *Morus rubra* extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with *Morus alba* extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD fed rats treated orally with *Morus rubra* for 6 weeks post induction. HFD/MA: HFD-fed rats treated orally with MA for 6 weeks post induction. FHD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with co-state computer program. Unshared letter is significant at $P \leq 0.05$. HFD: High-fat diet

Table 3: Organ weights of obese rats treated with *Morus rubra* and *Morus alba* for 6 weeks

Organ weights	Control ND	Control ND/MR	ND/MA control	HFD	HFD/MR	HFD/MA	HFD/OS
Liver weight (g)	6.20 ^a ±0.16	6.00 ^a ±0.26	5.80 ^a ±0.70	9.00 ^b ±0.36	7.30 ^c ±0.77	7.70 ^c ±2.12	7.00 ^d ±8.88
%change		-3.22	-6.45	45.16	18.88	14.44	22.22
Heart weight (g)	1.05 ^c ±0.09	1.25 ^c ±0.34	1.19 ^c ±0.20	1.22 ^c ±0.16	1.20 ^c ±0.25	1.05 ^c ±0.04	1.26 ^c ±0.20
Kidney eight (g)	2.80 ^a ±0.37	2.73 ^a ±0.26	2.85 ^a ±0.69	2.76 ^a ±0.59	2.85 ^a ±0.34	2.56 ^a ±0.38	2.67 ^a ±0.10

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with *Morus rubra* extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with *Morus alba* extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD fed rats treated orally with MR for 6 weeks post-induction. HFD/MA: HFD-fed rats treated orally with *Morus alba* for 6 weeks post-induction. FHD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with co-state computer program. Unshared letter is significant at $P \leq 0.05$. HFD: High-fat diet

On the other hand, obese rats treated with MR and MA extracts showed more or less similar results with higher percentages of improvement for MR than MA (as indicated in the Table 1), compared with standard anti-obese drug.

Lipid profile in obese rats for 6 weeks of treatment post induction with HFD

Table 6 declared insignificant change in lipid profile in ND-fed rats and treated with MR and MA extract compared with normal control untreated rats. While, HFD rats recorded significant increase in all lipid profile biomarkers with percentages 90.65, 99.73, and 53.64%, respectively, for TC, TG, LDL-C. While, HDL-C showed significant reduction (43.87%), compared to control rats. Meanwhile, obese rats treated with purple MR and white MA berry extracts showed marked normalization in TC, HDL-C, TG, LDL-C compared to obese untreated rats with higher percentages of improvement for MR (43.44, 38.16, 64.78, and 27.27%, respectively), compared to OS anti-obesity standard drug which recorded 76.00, 30.14, 95.92, and 36.36%, respectively.

DISCUSSION

The present results declared that, significant increase in BW gain percentage (140.63%) in HFD fed rats, while significant reduction in the percentages of BW gain in obese rats treated with purple (-23.68%)

and white berry (-21.19%) extracts compared to OS (-31.16%). However, food intake in HFD fed rats didn't differed significantly as compared to control. On the other hand, food intake showed significant increase in obese rats treated with purple, white and standard drug compared to HFD fed rats with percentages increase reached to 33.33, 36.66, and 25.55%, respectively. In connection with the present results, Salih *et al.* [9] suggested that the increase feed intake may enhance energy balance or caloric storage, hence increase BW in rats. However, it has been reported that BW gain may occur independent of increased feed intake [28].

The improved effect of berry may be related to the activation of glycolysis in the liver and up-regulation of genes related to cholesterol metabolism [13]. *Morus* species possess many bioactive compounds, such as deoxymycine, flavonol glycosides, and anthocyanins that may suppress the elevation of postprandial blood glucose and secretion of insulin [13,29]. These compounds may also regulate hepatic gluconeogenesis illustrating an antihyperglycemic effect of *Morus* species.

With flavonoids as major constituents, the plant leaves possess various biological activities, including antioxidant, antimicrobial, skin-whitening, cytotoxic, antidiabetic, glucosidase inhibition, anti-hyperlipidemic, anti-atherosclerotic, anti-obesity, cardioprotective, and

Table 4: Effect of insulin, blood glucose, leptin levels, and pancreatic lipase activity in obese rats treated with *Morus rubra* and *Morus alba* extracts

Bioch. Parameters	Control ND	Control ND/MR	ND/MA control	HFD	HFD/MR	HFD/MA	HFD/OS
Glucose (mg/dl)	83.20 ^a ±6.16	86.00 ^a ±7.26	85.70 ^a ±6.70	149.00 ^b ±3.36	119.30 ^c ±2.77	127.70 ^c ±2.12	111.00 ^c ±8.88
% change	-	3.36	3	79.08	43.38	53.48	33.41
%improvement					35.69	25.6	45.67
Insulin (U/I/mL)	7.25 ^c ±0.19	7.00 ^c ±0.44	7.19 ^c ±0.10	11.02 ^d ±0.16	10.33 ^d ±0.25	10.15 ^d ±0.34	8.26 ^c ±0.60
% change	-	3.44	0.83	52	42.48	40	13.93
%improvement					9.52	12	38.07
Lipase (U/L)	12.90 ^a ±2.22	12.70 ^a ±1.26	12.85 ^a ±1.69	22.00 ^b ±1.59	17.80 ^c ±1.34	15.50 ^c ±0.38	12.67 ^d ±0.10
% change	-	1.55	0.38	70.54	37.98	20.16	1.78
%improvement					32.55	50.38	72.32
Leptin (ng/mL)	4.12 ^a ±0.02	4.33 ^a ±0.06	4.00 ^a ±0.04	14.02 ^b ±0.06	10.12 ^c ±0.50	11.22 ^c ±0.15	5.00 ^d ±0.45
% change	-	5.09	2.9	240.29	145.63	172.33	21.36
%improvement					94.66	67.96	218.93

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with *Morus rubra* extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with *Morus alba* extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD fed rats treated orally with *Morus rubra* for 6 weeks post-induction. HFD/MA: HFD fed rats treated orally with *Morus alba* for 6 weeks post-induction. HFD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with costate computer program. Unshared letter is significant at P≤0.05. HFD: High-fat diet

Table 5: Effect of purple and white berries on liver function enzymes in obese rats for 6 weeks of treatment post induction with HFD

Bioch. Parameters	Control ND	Control ND/MR	Control ND/MA	HFD	HFD/MR	HFD/MA	HFD/OS
ALT (U/I)	49.00 ^a ±0.22	43.00 ^a ±3.11	46.00 ^a ±2.80	88.12 ^b ±3.23	60.10 ^c ±3.23	63.10 ^c ±4.00	53.80 ^c ±4.78
% change	-	-12.24	-6.12	79.83	22.65	28.77	9.79
% improvement					57.18	51.06	70.04
AST (U/I)	44.10 ^a ±3.21	40.00 ^a ±1.67	42.00 ^a ±1.54	62.20 ^b ±2.14	53.89 ^c ±2.75	52.44 ^c ±5.10	52.42 ^c ±1.90
% change	-	-9.20	-4.76	41.04	22.19	18.91	18.87
% improvement					18.84	22.13	22.17
γ-GTT (U/I)	22.00 ^a ±0.77	19.00 ^a ±0.87	19.54 ^a ±0.32	65.96 ^b ±4.16	35.00 ^c ±2	37.90 ^c ±2.61	25.06 ^a ±0.67
% change	-	-13.63	-11.18	199.82	59.09	72.27	13.91
% improvement					140.73	127.54	185.90
ALP (U/I)	80.90 ^a ±5.33	78.00 ^a ±2.77	80.00 ^a ±3.00	142.04 ^b ±8.55	100.00 ^c ±6.00	103.00 ^c ±3.76	88.00 ^a ±3.15
% change	-	-3.58	1.11	75.57	23.60	27.31	8.77
% improvement					51.95	48.25	66.79

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with *Morus rubra* extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with MA extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD fed rats treated orally with *Morus rubra* for 6 weeks post-induction. HFD/MA: HFD fed rats treated orally with MA for 6 weeks post-induction. HFD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with co-state computer program. Unshared letter is significant at P≤0.05. HFD: High-fat diet, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, γ-GTT: γ-glutamyltransferase, ALP: Alkaline phosphatase

Table 6: Effect of *Morus rubra* and *Morus alba* on lipid profile in obese rats for 6 weeks of treatment post induction with HF

Bioch. Parameters	Control ND	Control ND/MR	Control ND/MA	HFD	HFD/MR	HFD/MA	HFD/OS
TC (mg/dl)	122.00 ^a ±6.76	120.00 ^a ±3.65	120.00 ^a ±2.90	232.6 ^b ±4.45	179.6 ^c ±8.15	189.00 ^c ±6.4	139.88 ^c ±6.00
% change		1.64	1.64	90.65	47.21	54.91	14.66
% improvement					43.44	35.74	76.00
HDL-C (mg/dl)	35.90 ^a ±1.20	37.00 ^a ±3.00	36.50 ^a ±1.87	20.15 ^c ±9.10	33.85 ^a ±0.10	29.85 ^c ±8.10	30.97 ^d ±2.10
% change		3.06	1.67	43.87	5.71	16.85	13.73
% improvement					38.16	27.02	30.14
TG (mg/dl)	96.33 ^a ±2.44	90.00 ^a ±4.22	93.00 ^a ±1.22	192.40 ^b ±7.8	130.00 ^c ±7.0	132.90 ^c ±6.8	100.00 ^a ±3.00
% change		-6.57	-3.46	99.73	34.95	37.96	3.81
% improvement					64.78	61.77	95.92
LDL-C (mg/dl)	10.00 ^a ±2.88	96.00 ^a ±3.22	98.00 ^a ±4.00	169.00 ^b ±2.00	39.00 ^c ±4.99	39.87 ^c ±6.00	129.00 ^a ±9.00
% change		12.73	10.91	53.64	26.36	27.15	17.28
% improvement					27.27	26.48	36.36

ND: Normal diet, ND/MR: Rats feed normal diet and treated orally with *Morus rubra* extract for 6 weeks. ND/MA: Rats feed normal diet and treated orally with *Morus alba* extract for 6 weeks. HFD: Rats feed with HFD for 12 weeks. HFD/MR: HFD-fed rats treated orally with *Morus rubra* for 6 weeks post-induction. HFD/MA: HFD-fed rats treated orally with *Morus alba* for 6 weeks post-induction. HFD/OS: Rats feed HFD and treated orally for 4 weeks with Orlistat standard drug. Statistical analysis is carried out using SPSS computer program combined with co-state computer program. Unshared letter is significant at P≤0.05. HFD: High-fat diet, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, TC: Total cholesterol

cognitive enhancement activities. Rich in anthocyanins and alkaloids, mulberry fruits have pharmacological properties, such as antioxidant, anti-diabetic, anti-atherosclerotic, anti-obesity, and hepatoprotective activities.

The plant extract may be effective for improving obesity-related metabolic stressors by the lower BW gain and epididymal adipocyte-sized despite the difference in food intake. The action of polyphenolic compounds could prevent ROS damage, and protect cells from free radicals [9]. Oxidative stress is considered as a critical mechanism of NAFLD. Moreover, the present results demonstrated that increase in visceral fat (285.80%) of HFD rats, while fecal fats increased by 110.29%, compared to control rats. However, obese rats treated with purple and white berry extracts revealed significant reduction in visceral fats reached to 39.79 and 38.29%, respectively compared to OS (55.01%). While, fecal fat demonstrated significant increase by percentages 250.73, 256.25, and 396.32% on treated obese rats with purple, white, and OS reference drug. In addition, the present data indicated significant increase in liver weight (45.16%). However, liver weight exhibited significant decrease post-berry extracts therapy as well as OS comparing to HFD-fed rats with percentages 18.88, 14.44, and 22.22%, respectively. However, kidney and heart tissue did not show a significant difference between groups. These comprehensive data could be explained based on increased liver TG depots which may cause further fat accumulation in hyperlipidemic mice leading to steatosis of macro- and microvesicular. The rats treated by each *Morus* species may exhibit reduction in fat accumulation of liver as well as inhibition in adipocyte hypertrophy.

The current results revealed significant increase glucose, insulin, leptin levels, and lipase activity in HFD-fed rats compared to normal control one. While, amelioration in these biomarkers levels was noticed tendency berry extracts treatment. It was found that hypoxia of adipocyte, due to hypertrophy of adipose tissue, is a leading cause of obesity and can cause necrosis of adipocyte [30]. The later may cause macrophage infiltration and secretion of proinflammatory cytokine. It was suggested that tumor necrosis factor- α and interleukin (IL)-6 inhibit the pathway of cascade signaling of insulin by preventing insulin receptors phosphorylation [31]. IL-1 β also stimulates insulin resistance in adipocytes and its expression is upregulated in the adipose tissue of obese and insulin-resistant mice [32].

On the other hand, the increment in pancreatic lipase in HFD-fed rats may be due to its involvement in the TG absorption from the small intestine to the enterocytes and if somehow this initial movement of TG from the small intestinal lumen is blocked, hyperlipidemia can be prevented [27]. While, the improvement in its activity post berry therapy to HFD-fed rats may be related to berry can work as pancreatic

lipase inhibitors whereby the digestion and absorption of fats can be suppressed while it increased the fecal lipid content in mice [30].

In addition, HFDs are considered to cause chronic inflammation in the hypothalamus and passivate leptin signaling, which are mechanisms mediating sustained appetite enhancement [33]. Fat accumulation and obesity are major underlying causes of chronic inflammation, due to the inherent ability of adipocytes to secrete adipokines which is an inflammatory mediator, and hormones like leptin [34]. The ameliorative effect of leptin level in obese rats treated with berry extracts may be related to polyphenolic compounds from berries which have been shown to decrease fat accumulation and suppress pancreatic lipase [35]. Therefore, berry extracts could be used against quite several diseases, since many of the biological actions of berry extracts have been attributed to their antioxidant properties.

The anti-obesity effect of purple berry may be involving the inhibition of pancreatic lipase activity and adipogenesis *via* the down-regulation of lipid accumulation. A quite vast range of polyphenolic compounds are found in berries and their intake on human health, performance, and disease are of valuable impact [36].

The results of the current work indicated significant increase in activities of liver function AST, ALT, ALP and γ -GGT in obese rats. While, treatment of obese rats with purple and white berries showed more or less similar results in modulating liver enzyme activities. In these concerns, Katsube *et al.* [29] speculated that oxidative stress is increased in obesity *via* nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, and NADPH oxidase is a major source of reactive oxygen species (ROS) in various organs [37]. It has also been demonstrated that increased oxidative stress in the liver precedes high-fat diet, induced obesity, and insulin resistance [38]. Liver plays a central role in energy balance and keep insulin sensitivity, while the disturbance of its function by nutritional overload such as HFD feeding may result in steatosis, inflammation, and insulin resistance [38]. In the current study, the anti-obesity of MR could be related to the polyphenolic content such as flavonoids and anthocyanins that improve glucose metabolism through relaxation of oxidative stress in the liver derived from its antioxidant activities [39].

Long-term exposure to an HFD can cause NAFLD, a condition wherein large droplets of fat deposited in hepatocytes *via* the process of steatosis. Excessive cholesterol accumulation disrupts membrane fluidity, promotes cellular dysfunction, and thereby results in the progression fatty liver [40].

Our previous work reported the antiproliferative and radical scavenging effect of berries fruits [12]. In the present study, the supplementation of each *Morus* species to obese rats may increase expression of

antioxidant enzymes to remove excess ROS production by the action of their bioactive secondary metabolites [41].

Fat accumulation in the liver increases LDL overproduction together with inflammatory cytokines lead to increase liver enzyme activities. On the other hand, the hepatoprotective activity of mulberry leaves on rat liver injury in diabetic rats or against varied hepatotoxic antirheumatic and analgesic drugs was reported Tag [42]. The possible ameliorative mechanism of the white and purple berries may be through free radical scavenger and antioxidant activity of its constituents, especially flavonoids which could prevent liver dysfunction.

The present study clearly indicated a significant increase in lipid profile TC, TG, and LDL-C, while, marked reduction in HDL-C in obese rats. The treatment of obese rats with purple and white berry extracts showed noticeable reduction in lipid profile, while elevation in HDL-C comparing with HFD-fed rats. The activity of the white berry (MA) may be attributed to quercetin 3-(6-malonylglucoside), the quantitatively major antioxidant flavonol glycoside [29].

Berry is a natural dietary supplement containing 1-deoxyynojirimycin and other α -glucosidase inhibitors in high concentration. These compounds hold some interesting mechanism of action regarding their hypoglycemic effect [13]. Moreover, the expression of LDL-R, a receptor mediating the endocytosis of cholesterol-rich LDL, may be enhanced in the berry extracts, which was in line with the decreased plasma LDL-c levels [30]. HMG-CoA reductase, the rate-controlling enzyme in the pathway of cholesterol synthesis, also may be decreased in the berry extracts treated obese groups [30]. In a good agreement with the present results, Gaidhu *et al.* [43] found the expression levels of adipose TG lipase and hormone-sensitive lipase were both increased in the subcutaneous adipose tissue of HFD-induced obese mice. Flavanols and flavonols, condensed tannins (proanthocyanidins), hydrolysable tannins (ellagitannins and gallotannins), stilbenes, anthocyanins, and phenolic acids were reported as the most commonly found polyphenols in *Morus* fruits [13]. Polyphenols content may reduce plasma TG, TC, and LDL-C in hyperlipidemic rats [15,44].

CONCLUSION

It could be concluded that the supplementation of obese rats with berry extracts which are rich with polyphenolic compounds, has antiaccumulation action of lipids and consequently anti-obesity properties. In addition, due to the antioxidant properties of berry extracts could be recommended for utilization as nutraceuticals against quite different human diseases. Consumption of berries may help to prevent obesity and obesity-related metabolic complications.

AUTHOR CONTRIBUTION

Farouk K El-Baz: Plan of work, writing revision, corresponding author. Hanan F. Aly: Experimental animals, Statistical analysis, writing. Howaida I. Abd-alla: Biochemical measurements, writing.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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