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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Natural Antioxidants Effect of Mulberry Fruits (*Morus nigra* and *Morus alba* L.) On Lipids Profile and Oxidative Stress in Hypercholestrolemic Rats

Mohamed Y. Mahmoud
Department of Home Economics, Faculty of Education,
Suez Canal University, El Salam District, Ismaileya, Egypt

Abstract: Mulberry belongs to the Morus genus of the Moraceae family. There are 24 species of Morus, with at least 100 known varieties. Mulberry leaves, bark and branches have long been used in Chinese medicine. In most European countries mulberries are grown for fruit production. Mulberry are a good source of polyphenols, especially anthocyanins and micronutrients. In epidemiological and clinical studies, these constituents have been associated with improved cardiovascular risk profiles. Human intervention studies using (either fresh, or as juice, or freeze-dried), or purified anthocyanin extracts have demonstrated significant improvements in LDL oxidation, lipid peroxidation, total plasma antioxidant capacity, dyslipidemia and glucose metabolism. Benefits were seen in healthy subjects and in those with existing metabolic risk factors. The present study was performed to determine the natural antioxidants content of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) in Egypt and study the effects of different levels different levels of black and white mulberry fruits on lipid profile and oxidative stress in hypercholestrolemic rats. Forty adult male albino rats Sprague Dawley strain with an average weight of (100±120 g) randomly divided into 8 groups (each of 5 rats), Group 1: Control negative, rats fed on basal diet, Group 2: Control positive, rats fed on hypercholesterolemic diet composed of basal diet +2% cholesterol. Others groups (3-8), rats fed on hypercholestrolemic diet supplemented by different levels of black or white mulberry dry fruits (2.5, 5 and 10%) for four weeks. Results observed that rats fed on hypercholestrolemic diet without supplementation (control positive) had significant increase in malonaldhyde (MDA), nitric oxide (NO), total cholesterol, triglycerides (T.G), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) while it had significant decrease in total antioxidant capacity (TAC) and high density lipoprotein (HDL) comparing with rats fed on basal diet (control negative). Moreover, The group administrated with *Morus nigra* L. 10% had lowest value in malonaldhyde (MDA) and nitric oxide (NO) followed by the group administrated with *Morus alba* L. 10%. In addition, all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had significant increase in total antioxidant capacity (TAC). The group administrated with *Morus nigra* L. 10% had highest value in total antioxidant capacity (TAC) followed by the group administrated with *Morus alba* L. 10%. In addition, all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had significant decrease in total cholesterol, triglycerides (T.G), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). and significant increase in high density lipoprotein (HDL),comparing with rats fed on hypercholestrolemic diet (control positive). It concluded that, consumption of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) my modify the risk of hypercholesterolemia and it have more potential as a health supplement rich in natural antioxidants. Moreover, mulberries are emerging as a dietary source of multiple compounds and nutrients, including anthocyanins, flavonoids, vitamins and fiber, that reduce CVD risk. While limited epidemiological data inversely associate consumption of berries with inflammation and CVD, these conclusions need to be strengthened in future case-control or cohort studies investigating the long-term health benefits of berries in specific populations.

Key words: *Morus alba*, *Morus nigra*, antioxidants, mulberry, hypercholestrolemic, oxidative stress

INTRODUCTION

Raised serum lipid levels, particularly of cholesterol along with generation of Reactive Oxygen Species (ROS), play a key role in the development of Coronary Artery Disease (CAD) and atherosclerosis, (Ross, 1999) Coronary artery disease is a serious medical problem that affects millions of people annually throughout the world. People who are predisposed to a combination of risk factors (dietary habits, genetic susceptibility, etc.,) are more prone to develop atherosclerosis and CAD. Besides stress, sedentary habits such as use of tobacco and alcohol are reported to have an additive effect in contributing to the development of atherosclerosis and CAD, (Ashakumary, 1993) Dietary

modifications, physical exercise, abstinence from tobacco and alcohol and changes in lifestyle have been proposed to reduce the incidence of CAD and other cardiac maladies by the medical fraternity all over the world. Phytosterols and natural antioxidants have also been shown to be effective in reducing lipid profiles and also mitigate peroxidative modification of lipoproteins and atherosclerosis, (Ikeda, 1998). Free radicals are atoms or molecules containing unpaired electrons. The chain reaction caused by free radicals can lead to cross-linking of atomic structures. In cases where the free radical induced chain reaction involves base pair molecules in a strand of DNA, the DNA can become cross-linked. DNA cross-linking can in turn lead to various effects of aging, especially cancer. Other cross linking can occur between fat and protein molecules, which leads to wrinkles. Free radicals can oxidize LDL and this is a key event in the formation of plaque in arteries, leading to heart disease and stroke. These are examples of how the free-radical theory of aging has been used to neatly "explain" the origin of many chronic diseases, (Richter, 1988) Phenol compounds, as antioxidants, may affect in various ways: direct reaction with free radicals, scavenging of free radicals, growing dismutation of free radicals to the compounds with much lower reactivity, chelatation of pro-oxidant metals (mainly iron), delaying or strengthening many enzymes. Fresh fruit extracts are an excellent source of polyphenolic compounds which exhibit antioxidant activity (Ozgen *et al*., 2009). The mulberry belongs to the *Morus* genus of the Moraceae family. There are 24 species of Morus, with at least 100 known varieties (Orban, 2010). Mulberry leaves, bark and branches have long been used in Chinese medicine (Zhishen, 1999). In most European countries mulberries are grown for fruit production, (Ercisli, 2004; Gerasopoulos, 1997). Plants of the genus *Morus* are known to be a rich source of flavonoids including quercetin 3-(-malonyl glucoside), rutin, isoquercitin (Katsube *et al*., 2006), cyanidin 3 rutinoside and cyanidin 3-glucoside (Chen *et al*., 2006; Kang *et al*., 2006). Different researchers investigated certain properties of whole mulberry fruit, leaves, bark, root and extracts of part of mulberry trees. Few species of mulberry were evaluated for their edible fruits. Previously, some authors (Ozgen *et al*., 2009; Gerasopoulos, 1997; Gunes, 2004; Ercisli, 2007; Ercisli, 2008). studied the quality, nutritional potentials and chemical composition of some of the *Morus* species. Lee *et al*. (2004) and Du *et al*. (2008) found that mulberries have cyanidin-based anthocyanins. In recent studies, Naderi *et al*. (2004) found that extract of *M. nigra* fruits have protective action against peroxidative damage to biomembranes and biomolecules. Memon *et al*. (2010) studied antioxidant properties of various extracts of mulberry leaves. Gecgel *et al*. (2011) investigated phytochemical properties and fatty acid composition of black mulberry seed oil. As mulberry can grow in a wide range of climatic, topographical and soil conditions, this may affect the chemical composition and nutritional status of plant. The present study was performed to determine the natural antioxidants content of black and white mulberry fruits in Egypt and it's effects on lipids profile and oxidative stress in hypercholestrolemic rats.

MATERIALS AND METHODS

Preparation of materials: Black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) were obtained from local market Cairo, Egypt. The fruits were stored at -20°C, dried in freezdryer until lyophilization. Lyophilished samples are ground into fine powder with mortar and pestle.

Chemical determination

Extraction of phenolic compounds: The total phenolic compounds (TP) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) were extracted using methanol solvent at solvent to samples ratio of 10:1. Extraction was carried out using a shaking incubator at room temperature for 24 h followed by filtration through whatman No.1 filter paper. The residue was re-extracted in the same manner and the two filtrates were combined (Sobhy *et al*., 2009).

Estimation of total phenolics: Total phenolic content of methanol extract was determined by the Folin-Ciocalteu micro-method (Slinkard and Singleton, 1977). Briefly, 20 µL of extract solution were mixed with 1.16 mL distilled water and 100 µL of Folin-Ciocalteu reagent, followed by addition of 300 µL of Na_2CO_3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve:

$$A = 0.98C+9.925\times10^{-3},\ R^2 = 0.9996$$

where, A is the absorbance and C is concentration as gallic acid equivalents (µg/g).

Determination of total flavonoids: Total flavonoids content were determined using the method of Ordon ez *et al*. (2006). of sample solution. A volume of 0.5 mL of 2% $AlCl_3$ in ethanol solution was added to 0.5 mL of methanolic extract. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonols content were calculated as quercetin (mg/g) using the following equation based on the calibration curve:

$$y = 0.0255x, R^2 = 0.9812$$

where, x was the absorbance and y was the quercetin equivalent (µg/g).

Experimental animal design: Forty adult male albino rats Sprague Dawley strain with an average weight of (100±120 g) obtained from the Experimental Animal House of Helwan, Egypt. The rats were housed in stainless steel cages with wire mesh bottoms and maintained in temperature and humidity control with 12 h light/dark cycle. All rats were allowed to free access drinking of water and basal diet for seven days adjustment to the laboratory environment. Then rats were randomly divided into 8 groups (each of 5 rats) as follow:

Group (1): Control negative (C-); rats fed on basal diet, the standard casein diet was prepared according to Reeves *et al.* (1993), protein (13%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), choline (0.2%), cellulose (5%) and the remainder was starch
Group (2): Control positive (C+); rats fed on hypercholesterolemic diet composed of basal diet+2 % cholesterol
Group (3): Rats fed on hypercholesterolemic diet+ *Morus alba* 2.5%
Group (4): Rats fed on hypercholesterolemic diet+ *Morus alba* 5%
Group (5): Rats fed on hypercholesterolemic diet+ *Morus alba* 10%
Group (6): Rats fed on hypercholesterolemic diet+ *Morus nigra* 2.5%
Group (7): Rats fed on hypercholesterolemic diet+ *Morus nigra* 5%
Group (8): Rats fed on hypercholesterolemic diet+ *Morus nigra* 10%

Each diet was prepared with equal in nutritional value to control casein diet. Food consumption was recorded every other day and body weight was recorded weekly throughout the feeding period which lasted for four weeks. At the end of the experiment rats (4 weeks) fasted overnight and Blood samples were collected from the aortic vein into clean dry centrifuge tubes and were stored at room temperature for 15 min, put into a refrigerator for 2 h, then centrifuged for 10 min at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wasserman tubes by using a Pasteur pipette and kept frozen at (-20°C) till analysis, while organs was removed then washed in saline and weighed after dried with filter paper.

Biological Determination: Determination of food intake, body weight gain and feed efficiency ratio: Food Intake (FI) was calculated every other day, The biological value of the different diets was assessed by the determination of its effect on Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) at the end of the experimental period using the following formulas:

BWG = Final body weight-Initial body weight
FER = BWG (g)/Food consumed (g)

Analytical methods of blood serum
Determination of total cholesterol: Serum cholesterol was determined according to the enzymatic method described by Allain *et al.* (1974).

Determination of triglycerides: The triglycerides in serum were colorimetrically determined according to (Wahlefeld, 1974).

Determination of high density lipoprotein (HDL) cholesterol: The HDL-c was determined according to (Albers *et al.*, 1983).

Determination of very low density lipoprotein (VLDL) cholesterol: The concentration of VLDL-c was estimated according to the Fridewald's equation (Fridewald *et al.*, 1972):

VLDL-c = triglycerides/5

Determination of low density lipoprotein (LDL) cholesterol: According to (Fridewald *et al.*, 1972), low density lipoprotein cholesterol can be calculated as follows:

LDL-c = Total cholesterol-(HDL-c)-(VLDL-c)

Determination of malonaldhyde (MDA): Serum MDA level, as a marker of lipid peroxidation and oxidative stress was measured through reaction with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a pink colored complex. Then, its fluorescence intensity was measured at 547 nm with excitation at 525 nm by a spectrofluorimeter (Del *et al.*, 2003).

Determination of nitric oxide (NO): Serum nitric oxide was determined indirectly by the measurement of stable decomposition product (NO_2-), employing the Griess reaction according to the modified method of Mathew *et al.* (1996).

Determination of total antioxidant capacity (TAC): Measurement of TAC in serum was performed by colorimetric method with commercial kits (RANDOX kits) on an automatic analyzer, USA.

Statistical analysis: The obtained data were statistically analyzed according to (SAS, 1996).

RESULTS AND DISCUSSION

Many reports have revealed that the physiological function of natural foods can be attributed to the antioxidative capacity of their phenolic components. (Ness, 1997; Halliwell, 1999). Table 1 shows the extraction yield of methanol solvents, total phenolic, flavonoids content, as well radical scavenging activity of white and black mulberry species fruits grown in Egypt (*Morus nigra* and *Morus alba* L.). The yield of extractable components, expressed as (w/w) (mg/g dry materials) was ranged from 187.22±3.08 (mg/g) *Morus alba* L. to 230.08±2.76 (mg/g) *Morus nigra* L. The mulberry extracts were a rich source of phenolics, with the high level of these compounds detected in dry fruits extracts of *Morus nigra* (169.08±4.12mg/g) and the low level was detected in dry fruits extracts of *Morus alba* (129.14±1.38 mg/g). Also, high flavonoids shown extracts of *Morus nigra* (16.67±0.185 mg/g). The lowest flavonoids shown extracts of *Morus alba* (12.89±0.18 mg/g).

The results are agreement with some authors (Ozgen *et al*., 2009; Ercisli, 2007, 2008; Lin, 2007) studied the phenolic content of *Morus* species. Total phenolic content of *Morus nigra* L. were 17.66-34.88 in Turkey (Ozgen, 2009), 14.22 in Turkey (Ercisli, 2007), 19.43-22.23 in Turkey (Ercisli, 2008) and 8.80 in Pakistan (Imran, 2010) (mg phenolic content/g fresh fruits material), while the total phenolic content of *Morus alba* L. were 15.16 mg in Taiwan (Lin, 2007), 1.81 mg in Turkey (Ercisli, 2007) and 16.50 mg in Pakistan (Imran, 2010) as (mg phenolic content/g fresh fruits material). Dietary supplementation with fruits rich in antioxidants is associated with inhibition of atherogenic modifications to LDL, macrophage foam cell formation and atherosclerosis. The present study was performed to determine the natural antioxidants content of black and white mulberry fruits in Egypt and it's different levels effects on lipids profile and oxidative stress in hypercholestrolemic rats. Feed intake, body weight gain, feed efficiency ratio, organs to body weight ratios, total cholesterol, triglycerides, low density lipoprotein LDL, high density lipoprotein HDL, very low density lipoprotein VLDL, malonaldhyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC).

From the data in Table 2, it could be observed that, rats fed on hypercholestrolemic diet without supplementation (control positive) had significant decrease in feed intake, body weight gain ratio and feed efficiency ratio comparing with rats fed on basal diet (control negative). Moreover, all hypercholestrolemic rats administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had significant difference values in feed consumption, while all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had significant increase in body weight gain ratio and markedly increase in feed efficiency ratio comparing with control positive group.

From the data in Table 3, it could be observed that, rats fed on hypercholestrolemic diet without supplementation (control positive) had significant increase in organs/ body weight gain ratio (liver, heart, spleen and kidney) comparing with rats fed on basal diet (control negative). Moreover, all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had markedly decrease in liver, heart, spleen and kidney/ body weight gain ratio comparing with control positive group.

Data in Table 4, it could be observed that rats fed on hypercholestrolemic diet without supplementation (control positive) had significant increase in malonaldhyde (MDA) and nitric oxide (NO) and significant decrease in total antioxidant capacity (TAC) comparing with rats fed on basal diet (control negative). Moreover, hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black mulberry fruits (*Morus nigra* L.*)* and (5 and 10%) white mulberry

Table 1: The yield methanol extraction, total phenolic and flavonoids contents of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.)

Fruits	*Morus nigra* L.	*Morus alba* L.
Extraction yield	230.08±2.76	187.22±3.08
Total phenolic	169.08±4.12	129.14±1.38
Flavonoids	16.67±0.185	12.89±0.18

Results calculated as mg/g dry weight basis

Table 2: Effect of different levels of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) on feed intake, body weight gain and feed efficiency ratio

Treatments	Feed efficiency ratio Mean±SD	Body weight gain (%) Mean±SD	Body weight gain (g) Mean±SD	Feed intake (g/day) Mean±SD
Control (-)	0.11±0.03^{c,b,d}	80.66±9.01^{a}	36.33±5.06^{a}	14.50±1.00^{a}
Control (+)	0.06±0.02^{e,d}	37.00±3.00^{c}	24.00±3..00^{b}	10.69±0.99^{b}
Morus alba 2.5%	0.14±0.03^{c,b}	45.00±7.53^{b}	32.45±4.05^{a}	11.09±1.00^{c,b}
Morus alba 5%	0.18±0.11^{b}	61.33±8.01^{a}	41.73±5.91^{a}	10.96±1.00^{b}
Morus alba 10%	0.27±0.03^{a}	64.50±5.97^{a}	47.75±4.34^{a}	9.14±1.63^{d}
Morus nigra 2.5%	0.06±0. 02^{e,d}	38.33±2.30^{c}	20.33±3.78^{b}	8.46±1.00^{d}
Morus nigra 5%	0.06±0.03^{e,d}	48.33±3.78^{e,b}	20.00±5.56^{b}	9.19±1.00^{e,d}
Morus nigra 10%	0.05±0.02^{d}	41.33±3.51^{c}	28.33±4.50^{b}	7.70±1.00^{d}

Values in the same column sharing the same superscript letters are not statistically significant

Table 3: Effect of different levels of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) on organs / body weight ratio

Treatments	Kidney Mean±SD	Spleen Mean±SD	Heart Mean±SD	Liver Mean±SD
Control (-)	0.51±0.064^{b,c}	0.22±0.033^{c}	0.26±0.123^{a}	1.95±0.20^{a,b}
Control (+)	0.55±0.036^{a}	0.35±0.066^{a}	0.35±0.114^{a}	2.55±0.14^{a}
Morus alba 2.5%	0.54±0.105^{b,c}	0.26±0.044^{a,b,c}	0.32±0.012^{a}	2.37±0.125^{a,b}
Morus alba 5%	0.51±0.104^{b,c}	0.23±0.054^{b,c}	0.30±0.115^{a}	2.31±0.110^{a,b}
Morus alba 10%	0.50±0.054^{c}	0.32±0.036^{a,b}	0.29±0.015^{a}	2.40±0.108^{a,b}
Morus nigra 2.5%	0.58±0.041^{a,b}	0.34±0.076^{a}	0.34±0.055^{a}	2.14±0.214^{a,b}
Morus nigra 5%	0.49±0.033^{c}	0.36±0.110^{a}	0.29±0.032^{a}	1.92±0.317^{b}
Morus nigra 10%	0.53±0.031^{b,c}	0.31±0.011^{a,b,c}	0.32±0.047^{a}	2.10±0.215^{a,b}

Values in the same column sharing the same superscript letters are not statistically significant

Table 4: Effect of different levels of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) on malonaldhyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC)

Treatments	TAC (mg/dl) Mean±SD	NO (mg/dl) Mean±SD	MDA (mg/dL) Mean±SD
Control (-)	2.20±0.80^{a}	1.37±0.63^{e}	1.65±0.10^{d}
Control (+)	0.76±0.09^{c}	5.61±16.48^{a}	4.11±2.42^{a}
Morus alba 2.5%	1.33±0.035^{a,b}	4.21±0.86^{a,b}	3.11±0.53^{a,b}
Morus alba 5%	1.39±0.06^{a,b}	3.42±0.98^{a,b,c}	2.33±0.79^{b,c}
Morus alba 10%	1.97±0.08^{b}	3.24±1.09^{c,d}	2.17±0.71^{c,d}
Morus nigra 2.5%	1.18±0.25^{a,b}	3.01±1.42^{b,c}	3.13±0.06^{a}
Morus nigra 5%	1.40±0.23^{a,b}	3.42±1.22^{c,d}	2.68±0.31^{b,c}
Morus nigra 10%	2.20±0.01^{a}	2.24±1.48^{d,e}	1.86±0.99^{c,d}

Values in the same column sharing the same superscript letters are not statistically significant

fruits *(Morus alba* L.*)* had significant decrease in malonaldhyde (MDA) and nitric oxide (NO), while hypercholestrolemic group administrated with 2.5% white mulberry fruits *(Morus alba* L.*)* had non-significant decrease in malonaldhyde (MDA) and nitric oxide (NO) comparing with control positive group. The group administrated with *Morus nigra* L. 10% had lowest value in malonaldhyde (MDA) and nitric oxide (NO) followed by the group administrated with *Morus alba* L. 10%. In addition, all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) had significant increase in total antioxidant capacity (TAC). The group administrated with *Morus nigra* L. 10% had highest value in total antioxidant capacity (TAC) followed by the group administrated with *Morus alba* L. 10%. The results may be due to oxidative stress and inflammation that play a pivotal role in the initiation and progression of atherosclerosis and CVD (Libby, 2007; Real *et al*., 2009). Several lines of evidence indicate a role for berry anthocyanins in significantly decreasing oxidative damage and inflammation in cellular and animal models of CVD. Youdim *et al*. (2000) have reported the incorporation of elderberry anthocyanins by endothelial cells, following a 4-h incubation at a concentration of 1 mg/mL. In addition to the cellular bioavailability, elderberry anthocyanins significantly decreased cytotoxicity caused by chemical inducers of oxidative stress (Youdim *et al*., 2000). Anthocyanins from blackberry extract were shown to protect against peroxynitrite-induced oxidative damage in human umbilical vein endothelial cells (Serraino *et al*., 2003). Mulberry anthocyanins have also exhibited antioxidative and antiatherogenic affects, by inhibiting oxidation of LDL and formation of foamcells, respectively, in an *in vitro* model of atherosclerosis (Liu *et al*., 2008). Anthocyanins from berries commonly consumed in the United States, such as blueberries and cranberries, have been reported to reduce TNF-α induced up regulation of inflammatory mediators in human microvascular endothelial cells (Youdim *et al*., 2002). In an 8-week study, DeFuria *et al*. have shown the attenuation of inflammatory gene expressions in male C57Bl/6j mice fed a high-fat diet supplemented with blueberry powder versus the unsupplemented group. This study also showed the protective effects of blueberries against insulin resistance and hyperglycemia, thus reducing the risk factors for CVD (DeFuria *et al*., 2009). In a rat model of prediabetes and hyperlipidemia, Jurgonski *et al*. (2008) further demonstrated decreased activities of inñtestinal mucosal disaccharidases (maltase and sucrose) following dietary supplementation with choke berry fruit extract for 4 weeks. These animal and in vitro data show the potential of berries to ameliorate inflammation, glucose and lipid abnormalities that contribute to CVD. Nitric oxide (NO), when formed through activation of inducible nitric oxide synthase (iNOS), has proinflammatory effects, leading to increased vascular permeability, induction of inflammatory cytokines and the formation of peroxynitrite, a strong oxidizing agent (Stampler, 1992). Pergola *et al*. (2006) have reported inhibitory effects of the anthocyanin fraction of blackberry extract on NO biosynthesis in the murine monocyte/macrophage J774 cell line stimulated with lipopolysaccharide. The study also reported that

Table 5: Effect of different levels of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) on total cholesterol, triglycerides (T.G), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL)

Treatments	LDL (mg/dL) Mean±SD	V LDL (mg/dL) Mean±SD	HDL (mg/dL) Mean±SD	T.G (mg/dL) Mean±SD	Cholesterol (mg/dL) Mean±SD
Control (-)	26.73±0.09^{c}	23.26±0.09^{b}	64.33±0.09^{a}	116.33±1.26^{c}	114.33±5.13^{c}
Control (+)	80.60±0.05^{a}	31.00±0.08^{a}	39.33±0.09^{c}	155.00±1.73^{a}	154.00±0.5^{a}
Morus alba 2.5%	70.67±0.09^{a}	27.73±0.03^{a,b}	40.66±0.05^{b}	138.67±1.48^{a,b}	140.00±2.42^{b}
Morus alba 5%	60.60±0.06^{b}	26.40±0.06^{a,b}	49.00±0.06^{a,b}	132.00±0.86^{b}	136.00±0.79^{b}
Morus alba 10%	45.35±0.01^{b,c}	24.73±0.02^{a,b}	53.25±0.08^{a,b}	123.65±1.49^{b,c}	123.33±2.71^{b,c}
Morus nigra 2.5%	65.20±0.25^{b}	25.46±0.05^{a,b}	46.33±0.20^{a,b}	127.33±0.98^{b,c}	137.00±2.06^{b}
Morus nigra 5%	60.67±0.06^{b}	24.66±0.20^{a,b}	49.33±0.03^{a,b}	123.33±1.42^{b,c}	127.67±0.31^{b}
Morus nigra 10%	31.93±0.01^{c}	24.00±0.01^{b}	58.66±0.01^{a}	120.00±1.22^{b,c}	114.60±0.99^{c}

Values in the same column sharing the same superscript letters are not statistically significant

blackberry anthocyanin extract inhibited inducible iNOS protein expression, thereby decreasing the inflammatory response in macrophages and inhibiting the formation of foam cells (Pergola *et al*., 2006). While increased iNOS expression leads to the proinflammatory effects of NO, generation of NO by endothelial nitric oxide synthase (eNOS) plays a crucial role in maintaining cardiovascular homeostasis by favorably modulating blood pressure and reducing endothelial dysfunction. Xu *et al*. (2004) and Lazze *et al*. (2006) have reported the up regulation of eNOS by cyanidin-3-glucoside in bovine artery endothelial cells and increased protein levels of eNOS by anthocyanin treatment (cyanidin and delphinidin) in human umbilical vein endothelial cells (Xu *et al*., 2004; Lazzè *et al*., 2006).

Data in Table 5, it could be observed that rats fed on hypercholestrolemic diet without supplementation (control positive) had significant increase in total cholesterol, triglycerides (T.G), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). and significant decrease in high density lipoprotein (HDL),comparing with rats fed on basal diet (control negative). It has been shown by other investigators that an increase in dietary cholesterol intake in animals led to hypercholesterolemia (Zulet *et al*., 1999; Kishida *et al*., 2002; Czerwinski *et al*., 2004) as evidenced by significant increases in plasma total cholesterol, LDL-C,VLDL-C, triglycerides, plasma peroxides, liver peroxides and LDL:HDL ratio, concomitant with significant declines in plasma HDL-C and serum paraoxonase enzyme. These results are in close agreement with a previous study carried out in animals fed with different amounts of fats, in which the hypercholestrolemic effect was also attributed to saturated fatty acids occurring in coconut oil (Zulet, *et al*., 1999). The high levels of LDL-C found in hypercholestrolemic rats, may be attributed to a down regulation in LDL receptors by cholesterol and saturated fatty acids included in the diet (Mustad *et al*., 1997).

From the same table, all hypercholestrolemic groups administrated with different levels (2.5, 5 and 10%) of black and white mulberry fruits (*Morus nigra* L. and *Morus alba* L.) had significant decrease in total cholesterol, triglycerides (T.G), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). and significant increase in high density lipoprotein (HDL), comparing with rats fed on hypercholestrolemic diet (control positive). Berry anthocyanins have also been shown to affect lipid metabolism in cellular and animal models of dyslipidemia. Administration of choke berry juice for 30 days in rats fed a standard or 4% cholesterol-containing diet, showed the anti-hyperlipidemic effects of choke berry juice in the cholesterol-fed group (Valcheva-Kuzmanova *et al*., 2007). Purified anthocyanins from blueberries and strawberries added to drinking water were shown to prevent the development of dyslipidemia and obesity in mice fed a high-fat diet for a period of 90 days (Prior *et al*., 2009). Anthocyanin treatment of human umbilical vein endothelial cells was further demonstrated to regulate cholesterol distribution by interfering with the recruitment of tumor necrosis factor receptor-associated factors (TRAF)-2 in lipid rafts, thereby inhibiting CD40-induced proinflammatory signaling (Xia *et al*., 2007).

Conclusion: It suggested that, consumption of black and white mulberry fruits (*Morus nigra* and *Morus alba* L.) may modify the risk of hypercholesterolemia and it has more potential as a health supplement rich in natural antioxidants. Moreover, mulberries are emerging as a dietary source of multiple compounds and nutrients, including anthocyanins, flavonoids, vitamins and fiber, that reduce CVD risk. While limited epidemiological data inversely associate consumption of berries with inflammation and CVD, these conclusions need to be strengthened in future case-control or cohort studies investigating the long-term health benefits of berries in specific populations.

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