Effect of Low Fat Diet with or Without Some Types of Berries Fruit on Rats Suffering from Hypercholesterolemia and Diabetes

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Abstract: This study aimed to investigate the effect of low fat hypercholesterolemic diet with or without white mulberry, black mulberry and blueberries fruits on rats suffering from hypercholesterolemia and diabetes. In addition to determined the chemical composition and phenolic compounds. Fifty four male albino rats (Sprague Dawley) were divided into two main groups. The first main group (6 rats) was fed basal diet, as negative control. Thesecond main group (48 rats) fed 6 weeks on hypercholesterolemic diet containing casein 24%, soybean oil 25%, cholesterol 1%, choline chloride 0.4%, salt mixture 10%, vitamin mixture 2%, cellulose 8% and the remainder corn starch, to induce hypercholesterolemia, after this period, total cholesterol and triglycerides were determined in normal and hypercholesterolemic groups to insure the induction of hypercholesterolemia. Then the second main group was injected with alloxane (150 mg / kg body weight) to induce hyperglycemia. After four days, serum glucose was determined in the first and second main groupsto ensure the induction. The second main group divided into 8 subgroups, as a following: Subgroup (1) fed on hypercholesterolemic diet (positive control group)[a]. Subgroup (2) fed on hypercholesterolemic diet containing half amount of soybean oil 12.5% and used as low fat hypercholesterolemic diet (LFHD) (positive control group)[b]. Subgroup (3 and 4,5,6,7 and 8) fed on low fat diet as described in subgroup(2), but containing 2.5 and 5% white mulberry, respectively. Subgroup (5 and 6) fed on low fat diet as described in subgroup (2), but containing 2.5 and 5% black mulberry, respectively and Subgroup (7 and 8) fed on low fat diet as described in subgroup (2), but containing 2.5 and 5% blueberries, respectively. The highest decrease in BWG% and liver and kidney weights/ body weight% recorded for hypercholesterolemic and diabetic group which treated with 5% blue berries. The best results in lipid profile recorded for the group which treated with 5% blue berries, followed by the groups which treated with 5% black mulberry and 5% blue berries, respectively. The highest improvement in serum glucose and liver enzymes recorded for the group which treated with LFHD containing 5% blue berries, followed by the groups which were fed on LFHD containing 2.5% blue berries and 5% black mulberry, respectively. The results revealed that, blue berries and black mulberry have a stronger effect in improving kidney function than white mulberry.White mulberry, black mulberry and blueberries fruits improved the nutritional and biochemical parameters on rats suffering from hypercholesterolemia and diabetes.

Keywords:berries fruits **-** lipid profile – glucose - liver enzymes - kidney function.

Introduction

Seventeen million people die annually worldwide due to cardiovascular diseases involving stroke and coronary heart disease **(Townsend *et al.*, 2016)** and **(Ma and Lee, 2016)**. Hyperlipidemia resulted from a high-fat diet represents one of primary risk factors towards cardiovascular disease**(Chobanian, 1991)**. The defects of secretion of insulin led to diabetes, characterized by hyperglycemia **(Kalofoutis*et al.*, 2007)**. In contrast, **Wolpert*et al.* (2013)** and **Turley *et al.* (1998)** demonstrated that when saturated fat was replaced with carbohydrates from vegetables and fruits, total and low-density lipoprotein cholesterol was reduced, featuring only a slight impact on high-density lipoprotein cholesterol and triglyceride.

There are many problems with hypolipidemic drugs, such as their side effects and high cost.Therefore, research works have shown a growing emphasis on the efficacy of natural alternative medicines to lower blood lipids**(Yang *et al.*, 2010)**.Natural products have long been an abundant supplier of compounds with biological activity. Such substances exist in fruits as well as vegetables and have attracted grown interest due to their antioxidant properties in addition to being considered as a possible approach to reduce risk of specific kinds of diseases like metabolic syndrome **(Ma and Zhang, 2017)** and **(Veeresham, 2012)**.

In addition to fruits, deep-colored vegetables represent good suppliers of phenolics such as anthocyans, carotenoids and flavonoids **(Lin and Tang 2007)**.Fruits, as well as vegetables, comprise several antioxidant compounds such as thiols, carotenoids, vitamins like ascorbic acid, phenolics and tocopherols (**Rangkadilok*et al.*, 2007).** Suchantioxidant constituents have ability for delaying or lipid oxidation inhibition through precluding oxidizing chain reactions (initiation or propagation) and can be used for free radicalsscavenging **(Othman *et al.*, 2007)**.

In tropical, subtropical as well as temperate settings, mulberry fruit shows high adaptability in America, Europe, Asia and Africa**(Ercisli and Orhan, 2008)** and **(Orhan and Ercisli, 2010)**. Mulberry fruits (berries) comprise numerous phytochemicals with antioxidant, anti-inflammatory in addition to antimicrobial features. The best significant species of *Moraceae* family utilized towards their edible berries include *Morusnigra, Morus alba*, *Morusindica*, *Morusrubra*, in addition to *Moruslaevigata***(Ozgen*et al.*, 2009)**.

Mulberry fruits possess diverse biological activities, including liver protection from damage, strengthening joints, facilitating urine discharge and lowering blood pressure and holding laxative as well as hypoglycaemic**(Zhishen*et al.*, 1999)**; antioxidant **(Hassimotto*et al.*, 2005)**; antimicrobial **(Takasugi*et al.*, 1979)**; anti-inflammatory **(Kim and Park, 2006)**and neuroprotective impacts **(Kang *et al.*, 2006)**. Such activities can be attributed to phenolics, flavonoids **(Ercisli and Orhan, 2007)** and **(Lin and Tang, 2007)** in addition to anthocyanins**(Lee *et al.*, 2004)**.

Therefore,the current research was carried out to assess low-fat hypercholesterolemic diet influences with or without white mulberry, black mulberry and blueberries fruits on rats suffering from hypercholesterolemia and diabetes.

Materials and Methods

Materials

- Casein, all minerals, vitamins, cellulose, choline chloride, cholesterol and alloxan were obtained from Al-Gomhoria Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt.
- White and black mulberry*(Morus albaand Morusaustralispoir)* were obtained from Agricultural Research Center, while blueberries (*Vacciniumcorymbosum*L*)* were supplied by local market, Cairo, Egypt.
- Normal male albino rats (54) Sprague Dawley Strain weighing (150 ± 5 g) were supplied by Helwan farm, Cairo, Egypt.
- Corn oil, corn starch and vegetable ghee were supplied by local market.

Methods

Dried white mulberry, black mulberry and blueberry

Slied white mulberry, black mulberry and blueberry samples were dried in solar energy for 2 days at about 50-60 ˚C.The dried samples were finely powdered by using a coffee grinder and stored in polyethylene bags.

Chemical analysis of white mulberry, black mulberry and blueberry

Water, total protein, total lipid, ash and fiber were analyzed in dried white mulberry, black mulberry and blueberry, according the methods outlined in **(A.O.A.C. 2000)**. While phenolic compounds and the extraction and identification of anthocyanins in fresh white mulberry, black mulberry and blueberry estimated according to the method described by **Crozier*et al.*, (1997)** and **A.O.A.C. (1990)**.

Biological Part

Fifty four male albino rats Sprague Dawley weighed (150 ± 5 g) were housed in wire cages in a animal house at 22 ± 2^{o} C . The animals were kept under normal healthy conditions and fed on basal diet for one week for adaptation period. Basal diet consisted of 14% casein (protein >80%), corn oil 4%, choline chloride 0.4%, vitamin mixture 1%, salt mixture 3.5%, fiber 5% and the remainder corn starch **(Reeves *et al.*, 1993)**, water was provided ad libitum. After the adaptation period, the rats were divided into two main groups. The first main group (n = 6) was fed basal diet, as negative control. The second main group (n = 48) fed 6 weeks on hypercholesterolemic diet containing (casein 24%, soybean oil 25%, cholesterol 1%, choline chloride 0.4%, salt mixture 10%, vitamin mixture 2%, cellulose 8% and the remainder corn starch, to induce hypercholesterolemia **(Matos*et al.*, 2005)**, after this period, total cholesterol and triglycerides were determined in normal and hypercholesterolemic groups to insure the induction of hypercholesterolemia. Total cholesterol and triglycerides were (78.702 ± 4.109 mg/dl and 40.332 ± 1.875 mg/dl) and (176.559 ± 7.421 mg/dl and 90.413 ± 3.320 mg/dl) for the first and second main groups, respectively. Then the second main group was injected with alloxane (150 mg / kg body weight) to induce hyperglycemia after fasting overnight **(Buko *et al.*, 1996)**. After four days, serum glucose was determined in the first and second main groupsto ensure the induction. Serum glucose was (82.488 ± 5.533 mg/dl in healthy rats fed on basal diet vs. 185.407 ± 4.702 mg/dl) in the second main group, respectively.

The second main group divided into 8 subgroups, as a following: Subgroup 1 fed on hypercholesterolemic diet (positive control group)[a]. *Subgroup* 2*:* fed on hypercholesterolemic diet containing half amount of soybean oil "12.5%" and used as "low fat hypercholesterolemic diet LFHD" (positive control group)[b]. Subgroup 3 and 4 fed on low fat diet as described in subgroup 2, but containing 2.5 and 5% white mulberry, respectively. Subgroup 5 and 6 fed on low fat diet as described in subgroup 2, but containing 2.5 and 5% black mulberry, respectively and the last two subgroups fed on low fat diet as described in subgroup 2, but containing 2.5 and 5% blueberries, respectively.

Diets consumed and body weights were recorded every week during the experimental period (6 week). After this period, the rats were fasted overnight, anaesthetized and sacrificed. Blood samples were collected from the aorta in each rat. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum cholesterol **(Allain*et al.*, 1974)**, triglycerides **(Foster and Dumns,1973)**, high density lipoprotein HDL-c **(Lopes-Virella*et al.*,1977)**, low density lipoprotein LDL-c and VLDL-c **(Friedwald*et al.*, 1972)**, glucose **(Trinder, 1969)**, Aspartate Amine Transaminase (AST) and Alanine Amine Transaminase (ALT) **(Reitman and Frankel 1957),** Alkaline Phosphatase (ALP) **(Belfield and Goldberg, 1971),** uric acid **(Fossati*et al.*, 1980)**, urea nitrogen **(Patton and Crouch, 1977)** and creatinine**(Henry, 1974).**

Liver and kidney were separated from each rat and weighted to calculate the liver and kidney to body weight %.

Statistical analysis

Results of biological evaluation of each group were statistically analyzed (mean ± standard deviation and one-way ANOVA test) using SAS package and compared with each other using the suitable test (least significant differences at P< 0.05 **(SAS, 1996).**

Results and Discussion

Chemical Composition of Berries Fruit (g / 100g dry weight)

Results of chemical compositions, including water, protein, total lipid, carbohydrate, fiber and ash for white mulberry, black mulberry and blueberry, in addition to anthocyanins and phenolic components, are displayed in Table (1). Results in this Table were characterized with a high amount of total lipid, fibers and ash in blueberry, as compared to white and black mulberry (7.505, 7.400 and 6.132 g/100 g dry weight) vs. (7.203, 4.655 and 4.131 g/100 g dry weight) and (6.00, 5.913 and 5.104 g/100 g dry weight), respectively. The mean value of total carbohydrate of black mulberry increased than that of white mulberry and blueberry (73.631 vs. 72.204 and 69.545 g/100 g dry weight), respectively. While water and protein in the white mulberry increased than black mulberry and blueberry (5.107 and 6.700 g/100 g dry weight) vs. (4.132 and 5.22 g/100 g dry weight) and (4.612 and 4.806 g/100 g dry weight), respectively.

Table (1) Chemical composition of some types of berries fruit (g / 100g dry weight)

Types of Fruits / Nutrients	White Mulberry	Black Mulberry	Blueberry
Water	5.107	4.132	4.612
Protein	6.700	5.220	4.806
Total lipid	7.203	6.00	7.505
Carbohydrate	72.204	73.631	69.545
Fiber	4.655	5.913	7.400
Ash	4.131	5.104	6.132

These values are the average of two determinations.

Table (2) Identified the main phenolic compounds found in of some types of berries fruit (mg/100 g fresh weight)

Types of Fruits / Nutrients	White Mulberry	Black Mulberry	Blueberry
Anthocyanins	138.532	205.441	230.554
Phenolic components	322.308	465.500	580.708

These values are the average of two determinations.

The amounts of anthocyanins and phenolic components in blueberry increased than that of black and white mulberry (230.554 > 205.441 >138.532 mg/100 g fresh weight) and (580.708> 465.500>322.308 mg/100 g fresh weight), respectively.

In this respect, composition of freeze-dried blueberries (unit/50 g) reported by **Basu*et al.* (2010)**, total protein (g), carbohydrates (g), phenolic compounds (mg) and anthocyanins (mg) were 1.7g, 42.3g, 1624 mg and 742 mg, respectively. While **Koca*et al.* (2008)** reported that total anthocyanins and total phenolic ranged between (58.8–630 mg/kg) and (800.58–2056.31 mg/kg) in mulberry fruits, respectively.

Effect of a low-fat diet with or without some types of berries fruit on some nutritional parameters and organs weight/body weight% of rats suffering from hypercholesterolemia and diabetes

The impact of low fat hypercholesterolemic diet with or without some types of berries fruit on feed intake (g/day/each rat), bodyweight gain% and (liver and kidney) weight/body weight% of rats with hypercholesterolemia and diabetes is displayed in Table (3).

In comparison to the negative control group, feed intake (g/day/each rat) the mean value of group which suffers from hypercholesterolemia and hyperglycemia (positive control group)[1] showed a significant decrease (p≤ 0.5). In contrast, feeding rats that suffer from hypercholesterolemia and hyperglycemia on a low-fat hypercholesterolemic diet (LFHD) (positive control group)[2] exhibited non-significant variations in feed intake in comparison to control positive group[1] which fed on a high-fat diet (HFD). All groups which were treated with LFHD containing two levels (2.5% and 5%) of berries fruit recorded non-significant changes with respect to feed intake, except rats' groups which were treated with 5% white and black mulberry in comparison to positive control group.

Table (3) Effect of a low-fat diet with or without some types of berries fruit on some nutritional parameters and organs weight/body weight% of rats suffering from hypercholesterolemia and diabetes

Groups \ Parameters		Feed intake (g/day/ rat)	BWG%	Organs weight/body weight%	
				Liver	Kidney
Negative control group (-ve), fed on basal diet		18.488 [a] ± 0.589	28.085 [a] ± 1.243	3.109 [g] ± 0.109	0.540 [f] ± 0.017
Positive control group (+ve)[1],fed on hypercholesterolemic diet		17.400 [c] ± 0.438	24.471 [b c] ± 1.445	5.418 [a] ± 0.161	0.848 [a] ± 0.030
Rats fed on low fat hypercholesterolemic diet (LFHD)	Only Positive control group (+ve)[2]	17.666 [b c] ± 0.421	25.486 [b] ± 1.030	4.492 [b] ± 0.168	0.775 [b] ± 0.024
	Containing 2.5% white mulberry	17.870 [b c] ± 0.643	23.972 [c] ± 0.580	4.121 [c] ± 0.128	0.703 [c] ± 0.026
	Containing 5% white mulberry	18.220 [a b] ± 0.496	23.627 [c d] ± 0.547	3.943 [d] ± 0.109	0.639 [d] ± 0.026
	Containing 2.5% black mulberry	17.955 [a b c] ± 0.428	22.626 [d] ± 0.679	3.922 [d] ± 0.098	0.643 [d] ± 0.051
	Containing 5% black mulberry	18.210 [a b] ± 0.347	20.709 [e] ± 0.647	3.778 [e] ± 0.107	0.596 [e] ± 0.014
	Containing 2.5% blueberries	17.573 [c] ± 0.388	20.448 [e] ± 0.790	3.721 [e] ± 0.109	0.594 [e] ± 0.036
	Containing 5% blueberries	17.955 [a b c] ± 0.539	18.928 [f] ± 1.100	3.554 [f] ± 0.069	0.582 [e] ± 0.018

Each value represents mean of three replicates± standard deviation . Means in the same column with different superscript letters are significantly different at P≤0.05.

In comparison to negative control group,the results provided by Table 2 showed that body weight gain% (BWG%) for (positive control group)[1] was found to decrease significantly (P≤ 0.05). Feeding rats' group with hypercholesterolemia and hyperglycemia on LFHD (positive control group)[2] recorded non-significant differences in BWG% in comparisonto (positive control group)[1] based on feeding of HFD. Feeding rats' group based on LFHD containing (2.5% and 5%) white mulberry, black mulberry and blueberries decreased. The mean value of BWG% significantly,in comparisonto (positive control group)[2] fed on LFHD only. The highest decrease inBWG% was found to be for group treated by means of 5% blueberries, followed by the groups treated with 5% black mulberry and 2.5% blueberries, respectively.

The mean values regarding liver as well as kidney weights/body weight% of positive control group[1] fed on HFD were found to increase significantly (P≤ 0.05) compared to negative control group with feeding based on basal diet. While feeding rats that suffer from hypercholesterolemia and hyperglycemia on LFHD (positive control group[2]) decreased significantly (P≤ 0.05)in liver and kidney weight/body weight% in comparison to rats' group which fed on HFD (positive control group[1]). All treated groups with LFHD containing (2.5 and 5%)berries fruits decreased the weights of these organs (liver and kidneys) significantly (P≤ 0.05) regarding in comparison to positive control groups. The highest decrease in liver, as well as kidney weights/ body weight%, was recorded for hypercholesterolemic and diabetic group, which was treated with 5% blueberries.

Our study results are consistent with **Wu *et al.* (2013)[a]** who stated that purified mulberry anthocyanin consumption could considerably inhibit body weight gain, cause insulin resistance reduction, lower adipocytes size, decrease accumulation of lipid and reduce secretion of leptin. On the other hand, the same authors alsodecided feeding mice on a high-fat diet (HFD), leading to a body weight increase, resistance towards insulin, serum and hepatic lipids. When it comes to blueberry and mulberry juice, they also impeded weight gain, reduced serum cholesterol, decreased insulin resistance, decreased accumulation of lipid and reduced secretion of leptin.

Effect of a low-fat diet with or without some types of berries fruit on lipid profile of rats suffering from hypercholesterolemia and diabetes

The effect of low fat hypercholesterolemic diet with or without some types of berries fruit on lipid profile including cholesterol, triglycerides, high density lipoprotein-cholesterol

(HDL-c), low and very low density lipoprotein-cholesterol (LDL-c and VLDL-c) of rats suffering from hypercholesterolemia and diabetes are presents in Table (4).The data in this Table revealed that feeding rats that suffer from hypercholesterolemia and hyperglycemia on a hypercholesterolemic diet increased serum cholesterol, triglycerides, LDL-c and VLDL-c, whereas HDL-c decreased significantly,compared to negative control group ($P \leq 0.05$). Feeding hypercholesterolemic diabetic rats on LFHD only (positive control group (+ve)[2] led to significant decrease (($P \leq 0.05$) in the mean value of serum cholesterol, triglycerides, LDL-c and VLDL-c. In contrast, HDL-c was found to significantly increase ($P \leq 0.05$) compared to hypercholesterolemic diabetic rats with feeding based on a hypercholesterolemic diet (positive control group +ve[2]). In contrast, feeding rats which suffer from hypercholesterolemia and hyperglycemia on LDHD containing (2.5% and 5%) white mulberry, black mulberry, and blueberries led to significant decrease in terms of all parameters, except for HDL-c, which recorded a significant increase ($P \leq 0.05$) compared to positive control groups [1&2]. The lipid profile's essential findings were recorded for the group treated with 5% blueberries, followed by the groups treated with 5% black mulberry and 5% blueberries, respectively.

Basu*et al.* (2010) indicated that blueberries enhanced metabolic syndrome in addition to cardiovascular risk factors, particularly in the case of obese men as well as women having metabolic syndrome, at achievable dietary doses.

Blueberries (BB) are an abundant supplier of flavonoids, particularly anthocyanins and flavanols**(Wu *et al.*, 2006)**. Blueberries (BB) cause oxidative stress inhibition, inflammation inhibition and promotion of beneficial vascular effects **(Norton *et al.*, 2005)** and **(Giacalone*et al.*, 2011)**. Based on these properties, **Erlund*et al.* (2008)** stated that berries consumption for two months for elderly individuals who suffer from cardiovascular diseases (CVD) led to convenient variations in platelet role, HDL-cholesterol in addition to blood pressure. **Rodriguez-Mateos*et al.* (2012)** also reported that consuming blueberries' dietary quantities can cause blood pressure lowering and enhance endothelial dysfunction motivated by a high-fat diet with high cholesterol.

Yang *et al.* (2010) demonstrated that feeding rats on a diet with both high fat and supplementation of (5 and 10%) mulberry fruit powder caused cholesterol, serum and liver triglyceride, in addition to LDL-c to decrease significantly, while HDL-c increased. Authors in this study proposed the existence of a hypolipidemic effect in mulberry fruits as it contains dietary fiber with a high amount in addition to linoleic acid. Besides, **Venkatesan*et al.* (2003)** suggested that fruits of mulberry contain dietary fiber, leading to hepatic lipogenesis inhibition and LDL-receptor activity increase.The positive roles of fiber in health and disease particularly in digestive tract health, energy balance, cancer, heart and diabetes justify the demand of increasing dietary fiber content in the daily diet. Dietary fiber is a collective term for a group of substances with varied chemical composition, structure, physical properties and physiological effects**(Kritchevsky, 1986).**

Table (4) Effect of a low-fat diet with or without some types of berries fruit on lipid profile of rats suffering from hypercholesterolemia and diabetes

Groups \ Parameters		Cholesterol	Triglyceride	HDL-c	LDL-c	VLDL-c
		Lipid Profile (mg/dl)				
Negative control group (-ve), fed on basal diet		82.135 ^{g} ± 3.631	41.294 ^{g} ± 2.884	42.022 ^{a} ± 2.432	31.853 ^{h} ± 1.928	8.258 ^{g} ± 0.576
Positive control group (+ve)[1],fed on hypercholesterolemic diet		192.211 ^{a} ± 5.389	81.380 ^{a} ± 3.728	22.975 ^{f} ± 1.928	152.959 ^{a} ± 3.353	16.275 ^{a} ± 0.745
Rats fed on low fat hypercholesterolemic	Only (control +ve)[2]	158.007 ^{b} ± 5.108	66.212 ^{b} ± 3.462	29.976 ^{e} ± 1.464	114.788 ^{b} ± 3.170	13.242 ^{b} ± 0.692
	Containing 2.5% white mulberry	147.965 ^{c} ± 5.790	59.688 ^{c} ± 3.401	33.413 ^{d} ± 1.812	102.614 ^{c} ± 3.820	11.937 ^{c} ± 0.680
	Containing 5% white mulberry	138.771 ^{d} ± 4.792	53.500 ^{de} ± 3.458	37.052 ^{bc} ± 1.515	91.019 ^{e} ± 3.273	10.699 ^{de} ± 0.691

	Containing 2.5% black mulberry	142.667 ^{d} ± 4.636	56.085 ^{cd} ± 3.412	35.986 ^{c} ± 1.772	95.463 ^{d} ± 2.983	11.216 ^{cd} ± 0.682
	Containing 5% black mulberry	133.264 ^{e} ± 2.612	49.833 ^{ef} ± 3.211	38.186 ^{b} ± 0.995	85.111 ^{f} ± 2.002	9.966 ^{ef} ± 0.642
	Containing 2.5% blueberries	137.363 ^{de} ± 4.655	50.952 ^{ef} ± 3.181	38.285 ^{b} ± 1.460	88.888 ^{e} ± 3.203	10.190 ^{ef} ± 0.636
	Containing 5% blueberries	124.571 ^{f} ± 2.062	47.495 ^{f} ± 1.840	40.582 ^{a} ± 1.535	74.490 ^{g} ± 1.911	9.498 ^{f} ± 0.367

Each value represents mean of three replicates± standard deviation . Means in the same column with different superscript letters are significantly different at $P \leq 0.05$.

For 2.5 months, feeding white rabbits on a hypercholesterolemic diet in addition to water extract of mulberry fruits (0.5% or 1.0%) led to a significant decrease in total cholesterol, LDL-c and triglycerides over those with feeding using only lard oil diet. On the other hand, a significant reduction of severe atherosclerosis occurred in the aorta of rabbits fed on a hypercholesterolemic diet and treated with (mulberry fruits' water extract, 0.5% or 1%) by 42–63% **(Chen *et al*., 2005)**.

The present data are in accordance with that obtained by**Sirikanchanarod*et al.*, (2016)** stated that total serum cholesterol and LDL-c decreased significantly in 58 hypercholesterolemic adults consuming 45 g freeze-dried mulberry fruit for six weeks. The decrease in these parameters may be due to the presence of high amounts of anthocyanins in mulberry fruit. This work proposed mulberry fruits use as a substitute therapy to treat hypercholesterolemic patients.

Effect of a low-fat diet with or without some types of berries fruit on serum glucose and liver enzymes of rats suffering from hypercholesterolemia and diabetes

Low-fat hypercholesterolemic diet impacts the presence or absence of some types of berries fruit on serum glucose and liver enzymes involving (AST, ALT and ALP) of rats suffering from hypercholesterolemia and diabetes is displayed in Table (5).

Table (5) Effect of a low-fat diet with or without some types of berries fruit on serum glucose and liver enzymes of rats suffering from hypercholesterolemia and diabetes

Groups \ Parameters		Glucose (mg/dl)	AST	ALT	ALP
			Liver enzymes (U/l)		
Negative control group (-ve), fed on basal diet		85.833 ^{g} ± 3.179	65.431 ^{g} ± 2.447	16.301 ^{h} ± 1.106	76.979 ^{g} ± 2.402
Positive control group (+ve)[1],fed on hypercholesterolemicdiet		176.640 ^{a} ± 3.818	110.264 ^{a} ± 2.474	79.713 ^{a} ± 3.927	119.232 ^{a} ± 3.894
Rats fed on low fat hypercholesterolemic diet (LFHD)	Only (control +ve)[2]	158.408 ^{b} ± 6.311	97.334 ^{b} ± 3.232	68.049 ^{b} ± 4.317	106.723 ^{b} ± 3.441
	Containing 2.5% white mulberry	150.702 ^{c} ± 1.465	88.083 ^{c} ± 2.137	59.548 ^{c} ± 2.771	99.409 ^{c} ± 3.227
	Containing 5% white mulberry	143.128 ^{d} ± 1.279	82.450 ^{d} ± 2.102	54.484 ^{d} ± 2.593	93.052 ^{d} ± 2.597
	Containing 2.5% black mulberry	142.867 ^{d} ± 2.052	82.297 ^{d} ± 2.264	52.049 ^{de} ± 2.037	91.255 ^{d} ± 3.073
	Containing 5% black mulberry	134.909 ^{e} ± 1.186	78.026 ^{e} ± 2.473	49.166 ^{ef} ± 2.761	90.068 ^{de} ± 2.302
	Containing 2.5% blueberries	133.926 ^{e} ± 2.129	76.716 ^{ef} ± 2.606	47.461 ^{fg} ± 2.668	85.327 ^{f} ± 2.953
	Containing 5% blueberries	127.905 ^{f} ± 1.687	74.465 ^{f} ± 2.262	45.086 ^{g} ± 2.436	87.110 ^{ef} ± 2.375

Each value represents mean of three replicates± standard deviation . Means in the same column with different superscript letters are significantly different at $P \le 0.05$.

Feeding rats that suffer from hypercholesterolemia and hyperglycemia Control (+ve)[1] on a hypercholesterolemic diet demonstrated mean values increase of serum glucose and liver enzymes in comparison to rats with a healthy state with feeding based on basal diet (negative control group). Meanwhile, treating ratswith LFHD control (+ve)[2] decreased these parameters in comparison to positive control group (+ve)[1].Treating groups with LFHD containing 2.5% and 5% "white mulberry, black mulberry and blue berries" caused significant decrease ($p \leq 0.05$) in serum glucose, AST, ALT and ALP, as compared to the positive control groups [1&2]. On the other hand, using the high levels (5%) from all types of berries recorded more effect in decreasing serum glucose and liver enzymes, as compared to low levels (2.5%).Highly improved serum glucose and liver enzymes were recorded for group treated with LFHD containing 5% blueberries, followed by groups fed on LFHD comprising 2.5% blueberries and 5% black mulberry, respectively.

Increasing insulin sensitivity is important in preventing the development of T2DM. The improved insulin sensitivity after blueberry supplementation could possibly be due to the observed body weight and adiposity reduction in rodents **(Stull, 2016)**. Added blueberries to the diet of obese rodent, led to decrease in body weight gain and/or lipid accumulation in tissues with increased insulin sensitivity **(Roopchand*et al.*, 2013 and Wu *et al.*, 2013)**[b].

These results agree with that reported by**Yan *et al.*, (2016)** who found that feeding rats for 8 weeks with anthocyanin extracted from mulberry fruit (5 and 125 mg/kgb.w.) caused cholesterol, leptin hormone, fasting blood glucose, serum insulin and triglyceride in addition to adiponectin level upsurge to decrease significantly.**Guo*et al.*, (2013)** also found that feeding diabetic rats for 14 days on a diet containing mulberry fruit polysaccharides caused fasting blood glucose to decrease significantly. In this respect, **Jiao*et al.* (2017)** manifested that feeding with mulberry fruits can provide a substantial function to treat diabetes owing to their anti-hyperglycemic in addition to anti-hyperlipidemic influences.

Blueberry has prevention and protection impacts on hepatic fibrosis mediated by carbon tetrachloride (CCl_4) through hepatocyte injury reduction in addition to peroxidation of lipid **(Ping Wang*et al.*, 2010)**. Besides,**Liu *et al.*, (2019)** demonstrated that blueberry extract's impact on mice having liver injury showed a positive correlation with blueberry extract concentration. This exhibited similarity to that of silymarin drug used towards liver injury, proposing that BE possessed a considerable impact for preventing liver injury.

Such data could be interpreted through the study of **Li *et al.*, (2016)** demonstrated that using mulberry fruit marc anthocyanins towards feeding Sprague Dawley rats, which suffer from fibrosis, showed levels reduction for ALT, AST, collagen type-III hyaluronidase acid and hydroxyproline**.** Therefore, **Chang *et al.*, (2013)** stated that the mulberry fruits could preclude non-alcoholic fatty liver disease.

Effect of a low-fat diet with or without some types of berries fruit on kidney functions of rats suffering from hypercholesterolemia and diabetes

The effect of low fat hypercholesterolemic diet with or without some types of berries fruit on kidney functions including (uric acid, urea nitrogen and creatinine) of rats suffering from hypercholesterolemia and diabetes are presents in Table (6).

The data revealed that serum uric acid, urea nitrogen, in addition to creatinine mean values, were found to increase significantly ($P \leq 0.05$)for positive control group[1] fed on a hypercholesterolemic diet in comparison to negative control group fed on a basal diet. While low-fat hypercholesterolemic diet (LFHD) (positive control group[1]) was found to decrease significantly ($P \leq 0.05$) for these parameters compared to (positive control group[1]).

In general,all the species of Morus is a rich source of phenolic compounds, including flavonoids and anthocyanins, of great biological, pharmacological, and structural interest because of their antioxidant properties (**Kumar and Chauhan, 2008)**. The two levels of white mulberry, black mulberry and blueberries with LFHD used in this study demonstrated that uric acid, urea nitrogen in addition to creatinine in rats which suffer from hypercholesterolemia and hyperglycemia was found decrease significantly ($P \leq 0.05$) in comparison to positive control groups [1&2]. Such findings in this Table revealed that blueberries and black mulberry have a stronger effect in improving kidney function than white mulberry. The best-recorded findings in kidney function were for group treated by means of 5% blueberries.

Traditionally, the species of *Morus* are used for the prevention of some diseases such as, liver and kidney, joint damage, and anti-aging, due to their antioxidant properties **(Mena *et al.*, 2016)**. In addition, it has been shown to be an ally in the treatment of type 2 diabetes mellitus (DM2), due to its hypoglycemic effects **(Sánchez-Salcedo*et al.*, 2017)**. Other study found that, fruits, roots, and leaves of *Morus alba* have a protective effect against atherosclerosis, liver and kidney disorders, and inflammation **(Jiao *et al.*, 2017)**.

Table (6) Effect of a low-fat diet with or without some types of berries fruit on kidney functions of rats suffering from hypercholesterolemia and diabetes

Groups / Parameters		Uric acid	Urea nitrogen	Creatinine
		Kidney functions (mg/dl)		
Negative control group (-ve), fed on basal diet		1.280 ^{g} ± 0.046	23.161 ^{f} ± 0.644	0.548 ^{f} ± 0.034
Positive control group (+ve)[1],fed on hypercholesterolemic diet		2.252 ^{a} ± 0.132	39.878 ^{a} ± 1.939	1.207 ^{a} ± 0.093
Rats fed on low fat hypercholesterolemic diet "LFHD"	Only (control +ve)[2]	2.054 ^{b} ± 0.112	35.072 ^{b} ± 1.952	0.993 ^{b} ± 0.054
	Containing 2.5% white mulberry	1.917 ^{c} ± 0.118	32.029 ^{c} ± 1.353	0.903 ^{c} ± 0.054
	Containing 5% white mulberry	1.766 ^{d} ± 0.044	29.475 ^{d} ± 1.162	0.769 ^{d} ± 0.044
	Containing 2.5% black mulberry	1.758 ^{d} ± 0.063	29.464 ^{d} ± 1.772	0.779 ^{d} ± 0.035
	Containing 5% black mulberry	1.450 $^{e f}$ ± 0.091	27.197 ^{e} ± 1.049	0.676 ^{e} ± 0.042
	Containing 2.5% blueberries	1.517 ^{e} ± 0.053	27.603 ^{e} ± 1.224	0.681 ^{e} ± 0.055
	Containing 5% blueberries	1.387 ^{f} ± 0.035	25.923 ^{e} ± 0.788	0.638 ^{e} ± 0.030

Each value represents mean of three replicates± standard deviation . Means in the same column with different superscript letters are significantly different at P≤0.05.

In conclusion, the present study has demonstrated the blueberries and black mulberry have a stronger effect in improving BWG%, organs weight/body weight%, lipid profile, liver enzymes, glucose and kidney function than white mulberryfor rats suffering from hypercholesterolemia and diabetes, especially the high levels (5%). All of these effects could be attributed to the high antioxidant activities as the result of high levels of phenolic compounds, including flavonoids and anthocyanins.

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تأثير النظام الغذائي منخفض الدهن المحتوي على ثلاثة أنواع من فاكهة التوت أو بدونهم على الفئران المصابة بارتفاع مستوى الكوليسترول و تعاني من مرض السكر

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الملخص العربي

تهدف الدراسة إلى دراسة تأثير النظام الغذائي منخفض الدهن المحتوي على التوت الأبيض و الأسود و الأزرق أو بدونهم على الفئران المصابة بارتفاع مستوى الكوليسترول و تعاني من مرض السكر ،بالإضافة لتقدير التركيب الكيميائي و المركبات الفينولية. 54 فأر ذكر ألبينو (الاسبراجو داولي)، تم تقسيمهم الي مجموعتين رئيسيتين. المجموعة الرئيسية الأولي (6 فئران) تم تغذيتهم علي غذاء اساسي، واستخدمت كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية (48 فأر) تم تغذيتهم لمدة 6 أسابيع علي نظام غذائي محتوي على الكوليسترول لإحداث ارتفاع في مستوى الكوليسترول، ويتبع ذلك قياس مستوى الكوليسترول و الدهون الثلاثية للتأكد من إصابتهم بارتفاع مستوى الكوليسترول. و تم حقن فئران المجموعة الثانية بمادة الألوكسان (150 ملجم / كجم من الوزن) لإحداث الاصابة بمرض السكر . بعد 4 أيام تم تقدير مستوى الجلوكوز في المجموعتين الرئيسيتين الأولى و الثانية للتأكد من الإصابة بمرض السكر. تم تقسيم المجموعة الرئيسية الثانية الي 8 مجموعات فرعية كالتالي. المجموعة الفرعية (1): تم تغذيتهم علي نظام غذائي مرتفع الكوليسترول (كمجموعة ضابطة موجبة أ). المجموعة الفرعية (2): تم تغذيتهم علي نظام غذائي مرتفع الكوليسترول يحتوى علي (12,5%) من زيت فول الصويا و يستخدم كنظام غذائي منخفض الدهن مرتفع الكوليسترول (كمجموعة ضابطة موجبة ب). المجموعات الفرعية (3 ، 4 ، 5 ، 6 ، 7 ، 8): تم تغذيتهم علي نظام غذائي

منخفض الدهن يحتوى علي 2,5% ، 5% من التوت الأبيض و الأسود و الأزرق، علي التوالي. أظهرت النتائج أن التوت الأبيض و الأسود و الأزرق قد أدى إلى تحسن في كل التقديرات الغذائية و الكيميائية للفئران المصابة بارتفاع في مستوى الكوليسترول وتعاني من مرض السكر. ومن جهة أخرى فإن التوت الأزرق و الأسود قد أدى للتأثير الأقوى لتحسن النسبة المئوية للزيادة في الوزن ، وزن الأعضاء منسوبا كنسبة مئوية لوزن الجسم، صورة الدهون، إنزيمات الكبد ، الجلوكوز ، وظائف الكلى مقارنة بالتوت الأبيض للفئران المصابة بارتفاع في مستوى الكوليسترول وتعاني من مرض السكر، خاصة في أعلى مستوى (5%).

الكلمات المفتاحية: التوت – صورة الدهن – الجلوكوز – إنزيمات الكبد – وظائف الكلى.