

The Biological Benefits of Blackmulberry (*Morus nigra*) Intake on Diabetic and non Diabetic Subjects

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Abstract: The intake of fresh blackmulberry on some blood categories and blood pressure in diabetic and non-diabetic subjects was studied. From Public Hospital District in Cairo, 12 Type II Diabetes Mellitus (DM) and 26 non-diabetic subjects (29-74 yrs) both sexes were choosing on purpose. Eating 100 g fresh black mulberry were given daily to each subject for one month. Blood pressure was measured then fasting blood samples were taken twice before and after the month of fruit intake (pre & post) for analysis. Personal data, dietary habits, body weight (WT) and height (HT) were recorded. Nutrients or hormone supplements were not permitted. Nutritive values of the fruits were assessed using Food Composition Tables and compared with Recommended Dietary Allowances (RDA) and Dietary References Intake (DRI). T-test and percent alteration were done for differences between the two groups and (pre & post). Some risky factors were found in the lifestyle of the two groups which lead them in poor nutritional status. Results obtained showed that blackmulberry has the highest minerals and energy compared with all fresh fruits and other berry types. One hundred grams of this fruit provides with 18.6% of RDA, DRI for iron, 22.22% of Vitamin C for adolescence, 13% of Zn for children (1-10) yrs. Blood glucose, total cholesterol, creatinine, uric acid and blood pressure were reduced significantly while hemoglobin was increased significantly by daily eating of this fruit. Significant differences were found between DM and non-DM groups as well as pre & post. Therefore, blackmulberry has a therapeutic and improvement effects in DM & non-DM subjects. It could be considered as supplement food for anemic, diabetic, hypertensive and other nutrition risk groups. Besides, it is the cheapest and available nutritious spring fruit in Egypt which need more attention for its cultivation, marketing, storage and industry.

Key words: Fresh blackmulberry, Therapeutic effects, Diabetes Mellitus, Recommended Dietary Allowances, Dietary References Intake

INTRODUCTION

Diabetes mellitus (DM) is a worldwide disease and about 189 millions are diabetics, expected to be doubled by year 2025^[1]. In Egypt, it is estimated to be 9 millions by 2025^[2]. Uncontrolling DM leads to devastating complications. In type II DM, 50% are neurologically affected, having stroke, amputation, blindness and kidney failure, myocardial infarction are increased by 2-4 times^[1].

Healthful nutrition is the major determinant for controlling DM, avoid complications, successful aging and improving the quality of life^[3]. Fruits and vegetables are the simplest form of healthful or functional foods^[4]. Their physiologically active components associated with reducing chronic diseases as DM, cancer, Cardiovascular Disease (CVD) and longer life expectancy^[5,6]. High fruit consumption reduces health costs in aging^[7].

Berries, citrus and melons make up 48% of fruit served, are rich in phytochemicals^[4]. Berries are grown either cultivated or wild in sandy areas in Northern Hemisphere. About 200 species belong to one genus

vaccinium myrtillus (family *Ericacea*). Common names are blueberry, bilberry, whortleberry^[8]. Berries are tiny small fruits have special flavour, range in color from bright blue, purple to shiny black. Its season is spring (April-June). Most of crop is frozen, pasteurized, processed for juice concentrates, canned or dehydrated or extracted for traditional foods and medicinal purposes. Little is stored for fresh eating^[8,9].

The genus *Morus* included 7 species in (1917) were increased to 30 species and 10 varieties in (1930) for complete Taxonomical Index^[10]. White mulberry (*Morus alba*) is originally Egyptian for its uses for sericulture production in Islamic time^[11]. The freshly leaves powder are mixed with wheat flour for "Paratha" a common food item in India^[12].

Blackmulberry (*Morus nigra*) was found in Hawarah Toombs in Fayoum Governorate, Egypt, but it was rare^[11]. It has a sweet, sour, musky, woody fresh aroma and unique (tangy) flavor due to Ethyl-Linolenate (the most active compound)^[13]. Blackmulberry has the highest antioxidant capacity inhibition for O₂, H₂O₂, OH radical among 41 fruits and vegetables^[14].

This study include several important topics: 1- In Egyptian blackmulberry is the cheapest fruit in spring, (April – 2nd week of June), available, but many people don't eat it. Besides, many don't know its health benefits or nutrient contents, compared with many countries. In Japan, people are struggling, the harvesting time is rainy season and they processed it rather fresh consumption^[15] which causes a reduction in its phenolic content^[16]. 2- 50% of other populations considered berries and soy functional foods^[4]. 3-Foods are no longer be evaluated with its nutrient contents, but its role in reducing the risk of diseases, (in persons consuming it at regular basis in varied diets), to be considered a functional food^[4].

4- Food Drug Administration (FDA) approved more than 40 published clinical trials for soy, whereas only a few clinical trials on cranberry juice and urinary tract infections^[17]. 5- Most research work was made only on juice, extract or dry powder of the fruit or leaves but not on the fresh fruit.

The present study is a clinical trial aiming to study the effects of dietary intake of fresh blackmulberry (*Morus nigra*) on some blood categories in diabetic and non-diabetic subjects.

MATERIALS AND METHODS

Subjects: Volunteer persons (38) from El-Zytoun district in Cairo, were two groups. First group consists of 12 diabetic subjects type II (7F+5M), were attending the outpatient clinic of Public Hospital in same district in Cairo. They were diagnosed through clinical examinations and laboratory assessment maintained on hypoglycaemic agents. Second group consists of 26 non-diabetic persons (16F + 10M). The age of subjects in both groups is ranged between 29-74 years. Subjects were choosed carefully on purpose since they have to agree for: a) Eating the blackmulberry (100g) was given daily for one month. b) Introducing the fasting blood samples and assessment of blood pressure in The Public hospital [twice pre & post treatment]. c) Cooperating with other personal information concerning the research besides body WT& HT. d) Not taking any supplementation or hormone therapy during the research.

Fresh blackmulberry was bought daily in spring (April–2nd week of June) season 2004/2005 from the local district market. Some experiences were acquired during this study with such delicate fruit which include: a) Blackmulberry must be bought in the early morning only up to 10 o'clock because the sun heat would spoil the sensory qualities of the fruit quickly as appearance, texture, vitality, brightness and juice. b) Not buying more than 1.5 kg in one package, avoiding the pressure of the

fruit themselves which induce the juice out. c) It must be put on the first refrigerator-shelf without washing or coverage, excluding the strange things or damaged fruits (no more than 5 days, it'd be spoiled with moulds).

Study Design:

- Assessment the nutritive value of the fresh blackmulberry using Food Composition Tables of the NNI^[18] and comparing it with other Egyptian fresh fruits and berry types and US Tables of Nutritive Value of Foods^[19,20]. Nutritive value of 100 g fruit for the person consumed it would be calculated according to RDA^[21] and DRI^[22].
- Personal data, dietary habits, body WT, HT for Body Mass Index (BMI, K g /M²)^[23] were taken for each person in the study as shown in table 1.
- Weighed 100 g fresh blackmulberry and distributed without washing in transparence plastic bags for each person daily for one month.
- Subjects were instructed to have their daily foods drinks, medications, no supplements or hormone therapy and just wash the blackmulberry before eating.
- Fasting blood samples were taken twice from each subject (pre & post the dietary blackmulberry) for determination of glucose^[24], total cholesterol (T. Ch)^[25] haemoglobin (Hb)^[26] creatinine^[27] and uric acid^[28]. Seated resting blood pressure was also obtained using mercury sphygmomanometer according the Egyptian Hypertension guide lines^[29]. All blood analysis and measurement of blood pressure were made by laboratory staff and physicians of the hospital.
- Statistical analysis was performed according the results obtained which include: mean ± SD, SE, T-test, percent Alteration (P-Alt.) between pre & post and between the two groups (p value of 0.05 or less is considered significant)^[30, 31].

RESULTS AND DISCUSSIONS

Personal data and risky factors: Data in Table 1 represents risk factors as: sedentary work type, abdomen obesity, not practicing exercise, family history with diabetes and the combination of diabetes + hypertension is considered a bad combination. Besides dietary factors which increase the risk on health status as shown in the same table, most of the two groups are adopting wrong dietary habits as omitting breakfast, or one of the main meals depending on 1-2 big meals/day. Or the lower time between last meal and go to bed less than 2-3 hrs, which increases the abdomen obesity and other digestive factors. Body Mass Index showed that some are in the leanness category < 18.5, some are obese having BMI > 30

Table 1: Personal Data and Some Risk Factors Among the Diabetic and Non-Diabetic

Categories	Groups							
	Non Diabetics				Diabetics			
	F (16) +		M (10) = 26		F (7) +		M (5) =12	
Sex	No.	%	No.	%	No.	%	No.	%
Age (year): 29-50	5	19.23	2	7.692	2	16.67	1	8.333
> 50	11	42.31	8	30.77	5	41.67	4	33.33
Ed. Level: Secondary	2	7.692	8	30.77	5	41.67	3	25
High Ed.	8	30.77	8	30.77	2	16.67	2	16.67
Working Type Sedentary	6	23.08	10	38.46	0	0	0	0
Active	4	15.38	4	15.38	3	25	1	8.333
Retired	0	0	2	7.692	4	33.33	4	33.33
Family History: DM	6	23.08	12	46.15	6	50	5	41.67
Abdomen Obesity	2	7.692	4	15.38	4	33.33	2	16.67
Duration DM (y) >20	0	0	0	0	5	41.67	4	33.33
<20	0	0	0	0	2	16.67	1	8.333
DM + Hypertension	0	0	0	0	4	33.33	2	16.67
Practicing Exercise: (≤ 0.5h/d 3 times /w)	0	0	8	30.77	0	0	0	0
Time/h from last meal to bed	2	7.692	4	15.38	4	33.33	2	16.67
Regular breakfast	6	23.08	8	30.77	7	58.33	5	41.67
Meals/d 3	1	3.846	0	0	6	50	2	16.67
1-2	9	34.62	16	61.54	1	8.333	3	25
Early morning water	4	15.38	7	26.92	4	33.33	1	8.333
Water cups/d as recommended	7	26.92	8	30.77	5	41.67	3	25
Vegetable exch. /d as recommended	4	15.38	4	15.38	5	41.67	2	16.67
Fruit exch. /d as recommended	6	23.08	8	30.77	4	33.33	4	33.33
Dairy exch. /d as recommended	5	16.67	19.23	6	23.08	4	33.33	2
BMI < 18.5	2	7.692	0	0	0	0	0	0
18.5 – 25	4	15.38	2	7.692	0	0	3	25
25 – 30	8	30.77	6	23.08	3	25	0	0
≥ 30	2	7.692	2	7.692	4	33.33	2	16.67

DM : Diabetes mellitus Ed : Education level

BMI : Body Mass Index

category and only 23-25% of non diabetic and 25% of diabetic groups are considered in normal BMI category. The present study showed that some of the subjects had only one type of fruit (blackmulberry) day due to the poor economy or the work hours. This

shows the importance of nutritional education for all age groups. But the great therapeutic effects of the intakes of blackmulberry in the two groups are shown in tables (3,4,5 and 6) could fulfill the nutrient gaps for those skipping meals.

Nutritive values of Blackmulberry (*Morus nigra*): Results in table 2 shows that blackmulberry has the highest levels of protein, fat, calcium, iron, zinc, sodium compared with other fresh fruits [18]. The tested fruit has highest levels of phosphorous (P) except guava, highest copper except spiked fig and lime. Besides Egyptian blackmulberry has the highest energy, protein, fat, sugar, calcium, zinc, sodium, potassium, phosphorous and copper compared with Egyptian Raspberry [18] and other berry types [19,20]. Egyptian type has the higher sugar content (16.5 g) than in Turkey type (11.3 – 16.2 g) [13]. The present study showed that one hundred grams of blackmulberry could provide with 28%, 18.66% of iron according to RDA and DRI [21,22] for males and females (11-50) yrs. respectively. Also it provides with 22.22% of vitamin C for (both sexes) of adolescents and 13%, 10.8% of zinc for children (both sexes, 1-10 y) and adults (females up to 50 yrs). It could increase plasma vitamin C hence the ingestion of 500 ml juice increased plasma vitamin C by 30% in healthy females [32].

Blackmulberry could fulfill the nutrient requirements for diabetic and non-diabetic subjects in special who are at nutritional risk, or skipping meals which leads to a drop in blood glucose or causes prompt fatigue, irritability, headache and inability to concentrate. It is considered a healthy snack which provides vitality to brain functioning and body strength due to all nutrients and antioxidants it contains [13, 14, 18].

Blood analysis and blood pressure: Data in tables 3 and 4) show that eating 100 g blackmulberry daily for 30 days produced significant and valuable improvements in blood categories either in diabetic or non-diabetic groups. Differences were significant between the two groups as well as pre and post intake of the fruit including blood glucose levels (p= 0.01), T. Ch (post intake) (p= 0.05) and blood creatinine at p= 0.01. The other blood values (hemoglobin and uric acid, systolic, diastolic and pulse pressure) showed no significant differences between the two groups.

Table 2: Nutritive Values of Berry Types (100 g).

Nutrients	Egyptian Berries		U.S. berries		
	Blackmulberry	Raspberry	Blackberry	Blueberry	Raspberry
Energy / Kcal	77	66	52.08	55.17	48.78
Protein /g	1.6	1.3	0.69	0.68	0.81
Fat /g	1.5	0.5	0.69	0.68	0.81
CHO/g	16.5	14	12.5	13.79	11.38
Water /g	79.6	83.5	59.72	58.62	70.73
Vit. C /mg	10	22	20.83	13.10	10.56
Vit.A / IU	0.0	110	166.66	103.45	130.08
Na / mg	12	2	Tr.	6.21	Tr.
Ca / mg	55	30	31.94	6.21	21.95
Fe / mg	2.8	1.20	0.55	0.14	0.57
K / mg	236	155	195.83	88.97	152.03
P/ mg	35	27	20.83	10.35	12.19
Zn /mg	1.3	0.35	0.27	* 0.11	* 0.46
CU /mg	0.22	0.11	-	-	-
Mg /mg	17	25	-	-	-
B1 /mg	0.05	0.04	0.02	0.05	0.03
B2 /mg	0.07	0.7	0.04	0.05	0.09
Folic*/ mg	-	-	* 39.02	* 6.20	* 26.82
Vit. E* /mg	-	-	* 0.49	* 0.30	* 0.30
Fiber /g	1.2	3.5	-	2.3	-

*Analysis according to ref. 18, 19, 20

Table 3: Comparison between the Two Groups in blood analysis (Pre and Post).

		Intakes of blackmulberry										
subjects	No. of Cases		Glucose (mg /dl)		T.Ch (mg /dl)		Hb (mg /dl)		Creatinine(mg /dl)		U r i c a c i d (mg /dl)	
			Pre	post	Pre	post	Pre	post	Pre	p o s t		
Diabetic	12	Mean	183.33	156.67	276.17	250.83	12.983	14.967	1.23	0.8	7.5	6.8
		± S.D.	42.395	37.941	78.769	50.817	1.0312	0.5416	0.144	0.1595	1.133	0.645
		S.E.	12.238	10.953	22.739	14.67	0.2977	0.1563	0.041	0.0461	0.327	0.186
Non-Diabetic	26	Mean	120.42	108.47	234.68	209.53	14.242	15.263	0.97	0.9195	5.15	4.78
		± S.D.	49.875	40.585	59.451	50.095	2.7684	2.246	0.243	0.2544	0.769	0.837
		S.E.	8.091	6.584	9.644	8.127	0.4491	0.3644	0.039	0.0413	0.125	0.136
Difference of mean Diabetic and Non diabetic			62.91	48.2	41.49	41.3	-1.259	-0.296	0.26	-0.12	2 . 3 5	
t			-3.937	-3.639	-1.946	-2.482	1.533	0.45	-3.492	1.528	-0.184	0.761
P			**	**	N.S.	*	N.S.	N.S.	**	N.S.	N.S.	N.S.

* : Significant P (<0.05) t = t Test S.D. : Standard Deviation
 ** : Significant P (<0.01) P= Significant S.E. : Standard Error
 N.S. : Not Significant

Table 4: Comparison between the two groups in blood pressure (Pre and Post).

		intakes of blackmulberry										
subjects	No. of Cases		Systolic (mm Hg)		Diastolic (mm Hg)		pulse pressure (mm Hg)					
			Pre	post	Pre	post	Pre	post				
Diabetic	12	Mean	160	140.5	100	95	60	45.5				
		± S.D.	24.171	20.597	10	5	21.12	16.9				
		S.E.	6.978	5.946	2.887	1.443	6.097	4.879				
Non-Diabetic	26	Mean	130.47	85	120.63	80.32	9.84	4.68				
		± S.D.	23.082	20.492	10.301	8.108	16.791	14.27				
		S.E.	3.744	3.324	1.671	1.315	2.724	2.315				
Difference of mean Diabetic and Non diabetic			29.53	55.5	-20.63	14.68	5 0 . 1 6					
T			-0.758	-0.594	-0.233	-0.878	-0.664	-0.364				
P			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.				

* : Significant P (<0.05) t = t Test S.D. :Standard Deviation
 ** : Significant P (<0.01) P= Significant S.E. :Standard Error
 N.S. : Not Significant

Therapeutic effects in diabetic group and improvement in non-diabetic group resulted from eating blackmulberry (*Morus nigra*) as shown in (Tables 5 and 6) produced a significant reduction in blood glucose level by 14.54, 9.90% (at p= 0.01) in diabetic and non diabetic subjects respectively. These results are consistent with another study which used blackmulberry and other antidiabetic plant extracts in diabetic induced (NOD) mice for 7 days. The extracts caused a significant reduction in blood glucose level by 10, 20% respectively [33]. This clears that consumption of the

whole fresh fruit resulted better and greater therapeutic effects than fruit extracts. Besides processing operation reduces the phenolic contents [16,34].

Blackmulberry fruit deserves the recommendations made by herbalists, naturopaths and practioners in Morocco and Quebec, Canada that this fruit and blueberry (all berry types) are considered the Top Ten Antidiabetic Plants [35]. *Morus nigra* was known as fruit that has nutritional qualities, flavour, antioxidant capacity and therapeutic effects in controlling DM by mixing it with water [36].

Table 5: Percent alteration (pre & Post) intakes of blackmulberry on blood analysis in the two groups.

		Blood Analysis									
Categories		Glucose (mg /dl)		T.Ch (mg /dl)		H.B (mg /dl)		Creatinine (mg /dl)		Uricacid (mg /dl)	
Group		Pre	post	pre	Post	pre	post	pre	post	pre	post
Diabetic	Mean	183.33	156.67	276.17	250.83	12.983	14.967	1.23	0.8	7.5	6.8
	± S.D.	42.395	37.941	78.769	50.817	1.0312	0.5416	0.144	0.16	1.133	0.645
	% Alt	-14.542		-9.176		15.282		-34.959		9.333	
	t	12.649		2.708		-5.928		6.994		3.45	
	P	0		0.02		0		0		0.005	
		**		*		**		**		**	
Non-Diabetic	Mean	120.42	108.47	234.68	209.53	14.242	15.263	0.97	0.9195	5.15	4.78
	± S.D.	49.875	40.585	59.451	50.095	2.7684	2.246	0.243	0.2544	0.769	0.837
	% Alt	-9.924		-10.717		7.169		-5.206		-7.184	
	t	5.879		6.618		-5.362		0.857		4.197	
	P	0		0		0		0.397		0	
		**		= **		**		N.S.		**	

* : Significant P (<0.05) S.D. : Standard Deviation t = t Test
 ** : Significant P (<0.01) P= Significant
 N.S. : Not Significant % Alt = Difference between Pre & Post X 100 Pre

Table 6: Percent alteration (Pre & Post) intakes of blackmulberry on blood pressure in the two groups.

		Blood pressure					
Categories		Systolic (mm Hg)		Diastolic (mm Hg)		Pulse pressure (mm Hg)	
Group		pre	post	pre	post	pre	post
Diabetic	Mean	160	140.5	100	95	60	45.5
	± S.D.	24.171	20.597	10	5	21.12	16.9
	% Alt	-12.188		-5.000		-24.167	
	T	3.112		1.318		2.104	
	P	0.01		0.214		0.059	
		**		N.S.		N.S.	
Non-Diabetic	Mean	123.47	112.63	84.21	80.32	38.74	32.37
	± S.D.	23.082	20.492	10.301	8.108	16.791	14.272
	% Alt	-8.779		-4.619		-16.443	
	T	6.643		3.797		3.418	
	P	0		0.001		0.002	
		**		**		**	

* : Significant P (<0.05) S.D. : Standard Deviation
 ** : Significant P (<0.01)P= Significant
 N.S. : Not Significant % Alt = Difference between Pre & Post X 100
 t = t Test Pre

Concerning total cholesterol the levels were 276.17, 234.68 mg/dl in diabetic and non diabetic groups respectively before treatment. These were classified as high and borderline - High cholesterol [25].

The two levels are considered at risk for heart diseases but blackmulberry intervention resulted a significant reduction for these levels (Tables 3 and 5). This supports that this fruit is best source for antioxidants

which have the capacity for some chronic diseases resulted from lipid oxidation and other cellular components as (CVD) [6,7]. Also the present results are matched with other study showed that berries have proactive effects in reducing serum cholesterol, triglycerides in primary dislipidemia. Where proanthocyanins and anthocyanins in blackmulberry work as proactive for cancer and improve the ischemic stroke outcomes [37]. In other clinical study applied on males which consumed high fat diet, the data showed that: supplementation with freeze dried wild blueberry powder significantly increased serum antioxidants ($p=0.02$). That powder was containing 25 anthocyanins and 19 out of 25 were found in plasma [38]. It could be correlated with reducing many chronic degenerative diseases and delaying or reversing aging symptoms as documented in rats [33,38,39].

Creatinine and uric acid were reduced significantly (at $p = 0.01$) in diabetics, while uric acid only reduced in non-diabetics sign (Table 5). This clearly reflexes the improvements in kidney function in the two tested groups. These data are consistent with that found in treatment gout when given blueberry (like other cherries) and suggested that flavonoid components are able to reduce both uric acid levels and tissue deterioration [8]. Besides the tradition information was mentioned that cranberry either juice or dried as other berries are recognized by 74% of parents as the first therapeutic home aid for urinary tract infection prevention treatment. It is flushing the kidneys when things don't seem right and the juice used for not less than one month [40]. European countries and USA use blueberry for treating blood vessels through anthocyanin usefulness in urinary tract infection and digestive disorders [41].

The present study also showed that blood pressure was lowered significantly by fruit intake in the two tested groups (Tables 4 and 6). This agreed with the previous studies recorded that consuming fruits and vegetables has been associated with lower risk of Coronary Heart Disease (CHD), stroke and favorable levels of (CVD) and blood pressure, either systolic, diastolic or pulse pressure [42].

Our data showed that three subjects in the non-diabetic group were having HB below normal level were improved after the intake of blackmulberry. A great benefit was also resulted by this fruit hence, an increase in blood hemoglobin level was noticed by (15.282 and 7.169%) in diabetic and non-diabetic groups respectively, (Tables 3 and 5). This could be related to that fruit blackmulberry has the highest content of iron compared with all fresh fruits and other berry types [18,19,20] as shown in (Table 2). It could be considered a cheap nutritional source for treating anemia which is widely spread in infants, children and adolescence. Information clears that iron deficiency

either alone or in combination with folate or B12 accounted for approximately one third of anemia [43].

Conclusion and Recommendations: The present clinical study is scientifically proved that fresh blackmulberry intake (in its season) produced great therapeutic effects in diabetic subjects and improvements in non-diabetic group in blood glucose, T.Ch, creatinine, uric acid and blood pressure. Significant increases in blood HB levels in subjects of both groups were noticed. Besides, the nutrition assessment of the fruit cleared that the Egyptian blackmulberry (*Morus nigra*) has high nutrient contents of sugar, P, F, Ca, Fe, Zn, Na, P, K and Cu compared with other fresh fruits or other berry types inside or outside Egypt. It could be considered a cheapest nutritious food supplement for sensitive groups who are at risk nutritionally and for (anemic, diabetic and hypertensive subjects). Raising the nutrition awareness with such fruit type for its qualities and biological effects, it deserves and paying attention for its cultivation, marketing, storage and technological food industries.

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