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Study the Potential Effect of Mulberry Leaves and Fruits on Experimental Animals Infected with Hyperlipidemia

Khaled ALy Abd El-Rahman shaheen, Samar wageh hashem

Faculty of Home Economics, Dept. of Nutrition & Food Science, Minufiya University

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The main objective of the study is to know the effect of mulberry leaves and fruits at different concentrations to protect against high blood fat, where 45 varieties of albino were used, weighing 140 - 180g. The rats were divided into 9 groups (5 mice in each group), one of which was the negative control group. as for the rest of the eight groups, 2% of cholesterol was added for a period of 15 days, one of these groups as a positive control group, the other groups were fed on mulberry leaves 2.5 - 5-7.5%, and groups also fed berries with a ratio of 2.5 - 5 - 7.5%. of mulberry leaves and the last group, a blood lipid-lowering drug was added, and the trial lasted for two months, and was estimated B.W.G% & FER, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density, very-low-density cholesterol, glucose, kidney and liver functions.

The results showed a remarkable decrease in kidney and liver functions, low total and low density lipoprotein cholesterol, a rise in high-density lipoprotein cholesterol and a significant decrease in blood glucose levels, especially in groups that fed mulberry leaves and fruits at a concentration of 7.5%

Introduction

Cardiovascular disease (CVD) is one of the most common causes of deaths, with about 17 million people die of CVD every year worldwide (**Townsend et al., 2016 and Maet et al., 2016**). It is estimated that CVD will continue to be the largest contributor to global mortality in the future (**Luet et al., 2016**).

Hyperlipidemia is one of the most important risk factors for CVD (**Nussbaumerova and Rosolova, 2018**). Therefore, an increasing focus has been reported in research studies that determine the effectiveness of

natural alternative medicine in reducing blood lipid levels (**Yanget al.,2010**).

This is because majority of the hypolipidemic drugs can potentially cause side effects and they are expensive (**Yanget al.,2010**).

Hyperlipidemia is condition in which there is an elevated serum levels of one or more of total cholesterol ,low density lipoprotein cholesterol ,very low density lipoprotein cholesterol or both total cholesterol and triglycerides.

Hyperlipidemia is life style disorder which seriously disturbs the human health

It leads to various cardiovascular disorders like angina pectoris, hypertension, atherosclerosis myocardial infraction, congestive heart failure(**Grundy,1986**)

The main cause of hyperlipidemia includes changes in life style habits, poor diet,high cholesterol life style contributors include obesity,poor physical activity and smoking other factors include diabetes, kidney disease,pregnancy and underactive thyroid gland (**Chen and Li 2007**)

American heart associatiojn defined hyperlipidemia is a high level of fats in the blood.These fats called lipids include cholesterol and triglycerides,There are different of hyperlipidemia depending on which lipid levels are high in the blood (**Kishor Jain et al.,2007**) .

Natural products have always been rich source of biologically active compounds (**Maand Zhang 2017,Zhang and Ma 2018**).

Medicinal plants have always been considered as a healthy source of life for all people due to its rich therapeutic properties and being 100% natural.

Medicinal plants are widely used by the majority of populations to cure various diseases and illness and have ahigh impaction the world's economy (**Edeoga et al.,2005**) .

However, the risk of hyperlipidemia would be reduced by consumption of flavonois,antioxidants phytochemicals and polyphenols such as mulberry .mulberry are important dietary sources of fibre and essential vitamins and minerals. They also contain a vest number of other phytochemicals for which there are no known deficiency conditions but which may have marked bioactivities in mammalian cells of potential health benefit. Extracts of these fruits act effectively as free radical in hibitors. **Mia et al.,(2002)** found that fiber content of mulberry may play an important role in plasma lipids, particularly soluble fiber decrease serum total cholesterol and serum LDLc without significant alteration in serum HDLc and triacylgle.

Mulberry fruits possess several potential pharmacological properties including anti-cholesterol, anti-diabetic anti-oxidative and

anti-obesity effects (Ye *et al* 2002, Kang *et al* 2006, Zhang and Shi 2010).these pharmacological properties are due to the presence of polyphenol compounds including anthocyanins. However ,different colours of mulberry fruits even from the same species may have different amounts of anthocyanins (Gerasopoulos and stavroulakis1997).

The antioxidant activity of mulberry leaves has also been reported by(Doi and Fujimoto (2000) who reported that 1- butanol extract of mulberry leaves scavenged the DPPH radical and inhibited the oxidative modification of rabbit and human LDL. Five flavonol glycosides (rutin , isoquercitrin , quercetin 3-(6- acetylglucoside) have been reported in mulberry leaves mulberry plants contain a wide array of free radical scavenging molecules, such as flavonoids that have antioxidant and hypolipidemic activities(Chenet *al* .,2005 and Choudhary *et al* 2005).

Materials and methods

Materials

Mulberry leaves and fruits which is popular in some localities of Egypt,were purchased freshly from local Farm at Shibin El Kom Menofiya Governorate,also, cholesterol as pure chemical fine used for inducing hyperlipidemia in rats.was obtain from El Gomhoria company.

A total number of 45 albino rats were purchased from Bio-diagnostic Co,Gize,Egypt.

Methods

Preparation of fruits and leaves mulberry:

Collection leaves and fruits and washed and dried under vacuum to maintain active compounds and saved it in glass ware sealed until used .

Animals and Experimental Design:

The work was carried out at the faculty of Home Economics,Menofia University Egypt .forty five male albino rats were fed a standard diet according to Ain,1993 for 7days as an adaptation period as normal control group while other rats were subjected to intraperitoneal injection of cholesterol 1.5 %mg/kg .

The animals (45 rats) were distributed in to 9 groups (n=5) according to the following scheme normal control (5 rats) fed basal diet.

While hyperlipidemia rats (35) were classified in to hyperlipidemia control (2.5,5,7.5%) mulberry fruits and (2.5,5,7.5%) mulberry leaves ,and rats take drugs.

Feed intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by determination of feed intake, body weight gain and feed efficiency ratio (FER) according to Chapman *et al.*, (1959) using the following formulae:

$$\text{FER} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}}$$

$$\text{Body weight gain (BWG\%)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100.$$

Blood samples were collected after 12 hours fasting at the end of the experiment (4 weeks). Using the retro-orbital method by means of a micro capillary glass tubes, blood was collected into a dry clean centrifugal tube and left to clot in a water bath (37°C) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum apart of was subjected to glucose determination and the remainder was carefully aspirated and transferred into clear quit fit plastic tubes and kept frozen at (-20°C) until analysis.

Chemical Analysis:

Moisture, protein, fat, fiber and ash contents of mulberry fruits and leaves were determined according to methods described by the **A.O.A.C. (2010)**.

Biochemical Analysis:

Serum glucose and serum insulin were estimated according to **Asatoor and King,(1954)and Wilson and Miles, (1977)**.

Serum total cholesterol, triglyceride (TG) and high-density lipoprotein cholesterol (HDLc) were determined by using enzymatic colorimetric methods of (**Allain, 1974,; Fassati and Prencipe, 1982 Schmidt-sommerfeld, (1981)**), respectively. The determinations of low-density lipoprotein cholesterol (LDLc) and very low-density lipoprotein cholesterol (VLDLc) were carried out according to the method of **Lee and Nieman, (1996)** as follows:

$$\text{VLDLc} = \text{TG}/5 \text{ and } \text{LDLc} = \text{Total Cholesterol} - \text{HDLc} + \text{VLDLc}.$$

Serum aspartate and alanine amino transferases (AST, ALT) were determined by using enzymatic colorimetric method (**Bergmeyer and Harder, 1986 and Kachmar and Moss, 1976**).Determination of serum creatinine and urea **Bartleset al., 1972 and Barham and Trinder, 1972** respectively.

Statistical Analysis:

Statistical analysis was performed by using computer program, Statistical Package for Social Science and compared with each other using the suitable tests (**SPSS,1998**).

Results and Discussion

Data in table (1) showed the chemical composition activity of dried fruits and leaves of mulberry. Data revealed that mulberry fruits and mulberry leavescontains (9.0 and 8.48) carbohydrate, (1.28 and

3.16%) protein (49.0 and 33.0) fat , (86.63 and 81.14%) moisture, (1.03 and 4.25) Ash , (1.57 and 2.64) fiber respectively.

Table (1): Chemical composition of dried mulberry leaves and fruits

Chemical composition	Carbohydrates	Protein	Fat	Moisture	Ash	Fiber
Mulberry fruits	9±1.13	1.28±0.18	.49±0.23	86.63±3.16	1.03±0.38	1.57±0.45
Mulberry leaves	8.48±0.11	3.16±0.51	.33±0.23	81.14±2.18	4.25±0.65	2.64±0.12

Values when each 3 samples.

Ke Yi-Fu, (1997) stated that mulberry contain 85% water (moisture) , 0.36% protein , free acid 1,86% invert sugar 9.19% , crude fiber 0.91% , Ash 0.66%

The fruit also rich in carotene, vitamin B2 ,phenolics .

Butt et al.,(2008)stated that mulberry leaves contain on dry weight 5.31% protein, 2.09 % fat, 9.9% crude fiber ,27.6% dietary fiber, 11.3% Ash previous studies have shown that leaves and dark fruits are the richest in bioactive compounds (**Sanchez et al 2015 and Jiang and Nie 2015**).

1 Feed efficiency ratio

Data in table (2) show the mean value of feed intake, body weight gain % and FER for normal and hyperlipidemia rats. It is clear that feed intake value was a higher significantly increased ($p \leq 0.01$) in control (-) group compared with control (+) group . The consumption of mulberry leaves 7.5 % and 2.5, 5, 7.5 % mulberry fruits showed no- significant differences as compared with the control (+). in contrast the consumption mulberry leaves 2.5, 5 % and drug show significantly increase ($p \leq 0.05$) compared with control (+) group . Body weight gain (BWG) for control (-) was a significantly increase ($p \leq 0.001$) compared with control (+) group. The consumption of mulberry leaves 5% and 5 & 7.5 % mulberry fruits showed a significant decrease ($p \leq 0.05$) as compared with to control (+) group. On the other side rats take drug showed a significant ($p \leq 0.01$) as compared to control (+) . Feed efficiency ratio (FER) for control (-) showed a highly significant increase ($p \leq 0.01$) compared with control (+) groups. The consumption of mulberry leaves and fruits (5 & 7.5 %) and drug showed a significant increases ($p \leq 0.05$) when compared with control (+) group. On the other side consumption 2.5 mulberry leaves and fruits showed no significant as compared with control (+) group.

Table (2): Effect of mulberry leaves and fruits on feed intake, body weight gain (%) and fee efficiency ratio (FER) of hyperlipidemia rats

Parameters groups	Control -ve	Contro l+ ve	Mulberry leaves			Mulberry fruits			drag
			2.5%	5%	7.5%	2.5%	5%	7.5%	
Body weight gain(g)	16.09 [±] 1.14 ^{***}	5.23 [±] 0.33	7.71 [±] 0.26	9.14 [±] 0.75 [*]	8.91 [±] 0.15	7.82 [±] 0.25	10.95 [±] 1.28 [*]	10.17 [±] 1.14 [*]	11.48 [±] 1.72 ^{**}
Feed intake (g)	14.93 [±] 1.12 ^{**}	11.28 [±] 1.55	12.18 [±] 1.10 [*]	12.51 [±] 0.69 [*]	11.13 [±] 0.82	11.25 [±] 0.11	11.81 [±] 1.19	11.88 [±] 1.36	12.38 [±] 1.44 [*]
FER	0.075 [±] 0.001 ^{**}	0.026 [±] 0.002	0.032 [±] 0.001	0.053 [±] 0.002 [*]	0.049 [±] 0.002 [*]	0.044 [±] 0.002	0.066 [±] 0.001 [*]	0.055 [±] 0.001 [*]	0.066 [±] 0.001 [*]

* Differences are significant at P<0.05 **Differences are highly significant at P<0.01

***Differences are highly significant at P<0.001

The results are agreement with (Mohamed 2001) who found that food intake of negative control rats was significantly higher (p≤0.05) than negative group.

(Lim et al.,2013)reported that high fat diet –induced obese mice fed with acombination of mulberry leaf extract and mulberry fruits extract at low and high doses had a significant decrease in body weight gain. The high does of combination of mulberry leaf extract and mulberry fruit extract had significantly improved the glucose control.

Data in table (3) show the mean values of serum lipids patterns of normal and hyperlipidemia groups. The value of cholesterol of to control (-) group showed a highly/significantdecrease (p≤0.001) as compared to control (+) group .The consumption of mulberry leaves (7.5 %) and drug showed significantly decrease (p≤0.01) when compared control (+) group, on the other side consumption mulberry leaves 2.5 % and mulberry fruits 2.5 , 5 % showed no significantly as compared to control (+) group . The value of triglyceride of control (-) showed a highly significant decrease (p≤0.01) when compared to compared to control (+) group. The consumption of mulberry leaves and fruits 7.5 % and drug showed significantly decrease (p ≤0.01) when compared to control (+) group, on the other side consumption of mulberry leaves and fruits 5% showed significantly (p ≤0.05) as compared with control (+) group. The value of HDLc of control (-) showed a highly significant increase (p≤ 0.001) as compared with control (+) group. The consumption of mulberry fruits 7.5 % and drug showed significantly increase (p ≤0.01) when compared to control (+) group while consumption mulberry leaves 7.5 % and mulberry fruits 5% showed significantly (p≤0.05). The value of LDLc of control (-) group showed highly significant decrease (p≤0.001) as compared with control (+)

group. The consu a significant decrease ($p \leq 0.01$) when compared to control(+)while consumption mulberry leaves and fruits showed significant ($p \leq 0.05$) as compared with control (+) group. The value of VLDLc of control (-) showed decrease significant ($p \leq 0.01$) when compared with control (+) group. The consumption of drug showed a highly significant decrease ($p \leq 0.01$) as compared to control (+) group while consumption of mulberry leaves 5, 7.5 % and mulberry fruits 7.5 % showed significant ($p \leq 0.05$) control (+) group. The result agree with **Yang et al .,2010** reported that rat fed with high fat diet supplemented with 5% or 10% mulberry fruits powder had asignificant decrease in the concentration of serum and liver triglyceride,total cholesterol and serum LDL cholesterol.

Table (3): Effect of mulberry leaves and fruits on lipid profile for normal and hyperlipidemia rats.

parameters groups	control -ve	control +ve	Mulberry leaves			Mulberry fruits			drug
			2.5%	5%	7.5%	2.5%	5%	7.5%	
T.CH Mg/dl	95.26 [±] 1.16 ^{***}	168.48 [±] 3.45	132.74 [±] 2.19	130.37 [±] 1.77 [*]	123.66 [±] 1.11 ^{**}	142.41 ±5.23	137.53 [±] 3.26	132.21 [±] 2.15 [*]	114.72 [±] 5.31 ^{**}
T.G Mg/dl	64.28 ^{**} 2.84 [±]	98.51 [±] 3.11	84.79 [±] 2.62	80.21 [±] 1.52 [*]	75.65 [±] 1.37 ^{**}	82.4 ±1.24	81.92 [±] 2.33 [*]	79.82 [±] 3.68 ^{**}	71.86 [±] 1.12 ^{**}
HDL Mg/dl	52.56 [±] 1.15 ^{***}	33.23 [±] 2.77	36.63 [±] 1.42	40.83 [±] 1.19	43.22 [±] 1.42 [*]	40.37 [±] 1.64	43.31 [±] 2.35 [*]	46.31 [±] 1.49 ^{**}	48.11 [±] 1.75 ^{**}
VLDL Mg/dl	12.85 [±] 1.08 ^{**}	19.70 [±] 1.71	16.95 [±] 1.11	16.04 [±] 1.52 [*]	15.13 [±] 1.23 [*]	16.4 8 [±] 1.16	16.38 [±] 1.44	15.96 [±] 1.28 [*]	14.73 [±] 1.33 ^{**}
LDL Mg/dl	55.55 [±] 1.12 ^{***}	155.31 [±] 3.54	112.78 [±] 5.26	105.58 [±] 3.18 [*]	95.57 [±] 1.78 ^{**}	118.16 [±] 3.11	110.60 [±] 2.58 [*]	101.86 [±] 3.55 ^{**}	80.98 [±] 1.58 ^{**}

* Differences are significant at $P \leq 0.05$ **Differences are highly significant at $P \leq 0.01$

***Differences are highly significant at $P \leq 0.001$

An increase in the serum high – density lipoprotein (HDL)cholesterol was reported in rat fed with high fat diet supplemented with 5% or 10% mulberry fruit powder(**Yang et al., 2010**). It is suggested that the presence of dietary fiber in mulberry fruits in hibits the hepatic lipogenesis and increases LDL-receptor activity (**Venkales and Devaraj 2003**) .In addition , the authors suggested that mulberry fruits might have a hypolipidemic effect because mulberry fruits have high content of dietary fiber and linoleic acid(**Yang et al .,2010**).

Data in table (4) showed the value of AST& ALT& Urea and creatinine for normal and hyperlipidemia rats. Levels of a Sparta amino transferees(AST) in control (-) showed highly significant decrease ($p \leq 0.01$) as compared with control (+) group. The consumption of

mulberry leaves 7.5 % , mulberry fruits 5,7.5 % and drug showed a significant decrease ($p \leq 0.001$) as compared with control (+) group, while consumption of mulberry leaves 2.5 ,5 % and mulberry fruits 2.5 % showed a significant decrease ($p \leq 0.05$) as compared with of control (+)group.. The value of alannamino transferees (ALT) in control (-) group showed highly significant decrease ($p \leq 0.001$) as compared with control (+) .The consumption of mulberry fruits 7.5 % and drug showed highly significant decrease ($p \leq 0.001$) as compared with control (+) group while consumption of mulberry leaves (2.5, 5, 7.5) % and mulberry fruits 2.5% showed significant ($p \leq 0.05$) as compared with control (+) group. The value of urea in control (-) showed highly significant decrease ($p \leq 0.001$) as compared with control (+) group. The consumption of drug showed significant decrease ($p \leq 0.01$) compared with control (+) group while consumption of mulberry leaves and fruits 5,7.5 % showed significant ($p \leq 0.05$) as compared with control group. The value of creatinne urea in control (-) showed highly a significant decrease ($p \leq 0.001$) when compared with control (+) group. The consumption of drug showed highly significant decrease ($p \leq 0.001$) compared with control (+) group, while consumption of mulberry fruits 7.5 % showed a significant ($p \leq 0.01$) as compared with control (+) group

Table (4): Effect of mulberry leaves and fruits on AST,ALT,Urea and creatinnefor normal and hyperlipidemia rats.

parameters groups	Control -ve	Control +ve	Mulberry leaves			Mulberry fruits			drug
			2.5%	5%	7.5%	2.5%	5%	7.5%	
AST (□/l)	31.34± 1.16**	53.62± 3.77	42.08± 2.11*	41.25± 1.38*	36.52± 1.85*	41.03± 2.75*	38.99± 1.08**	34.3± 1.23**	35.1± 1.13**
ALT (□/l)	31.28± 1.42***	50.22± 1.31	41.71± 1.06*	38.99± 1.11*	38.14± 1.84*	40.01± 2.19*	36.69± 1.33**	33.73± 1.51***	32.61± 2.27***
Urea (mg/dl)	40.39± 2.16***	56.86± 3.56	53.01± 4.37	46.16± 2.15*	45.01± 1.11*	52.02± 3.33	49.92± 2.19*	48.62± 2.83*	43.3± 1.49**
Creatinne (mg/dl)	0.68± 0.01***	1.47± 0.08	1.18± 0.01	0.86± 0.02*	0.81± 0.01*	1.12± 0.06	0.82± 0.03*	0.78± 0.02**	0.75± 0.02***

* Differences are significant at $P \leq 0.05$ **Differences are highly significant at $P \leq 0.01$

***Differences are highly significant at $P \leq 0.001$

Li et al., 2016 reported that rats fed with mulberry fruit marc anthocyanins had a decrease in the levels of ALT, aspartate amino transferase ,collagen type III hyaluroidase acid and hydroxyproline.**Hussein et al., (2010)** studied the liver protective effect of mulberry and calendula officinalis extracts against hepatotoxicity induced by CCL4 induced toxicity in isolated rat hepatocytes mulberry

reduced the levels of alanine aminotransferase (ALT) , (AST) and LDH and maintained the integrity of isolated hepatocytes.

Wang et al.,(2011) reported that mulberroside A shows uricosuric and nephroprotective effects. In hyperuricemia mice it decreases the serum level of urea nitrogen, creatinine, urinary Nacetyl β-D-glucosaminidase action, albumin, β₂ βmicroglobulin and enhanced the creatinine clearance Further research is required in order to explore the nephroprotective constituents in *M. alba*.They also conducted an experimental study on rabbits to evaluate the nephroprotective effect of *M. alba* against isoniazid induced nephrotoxicity. Parameters used for the analysis of nephrotoxicity were blood urea nitrogen and creatinine along with histopathological studies. It wasreported that creatinine and urea clearance are the primary functions of glomerulus (**Garba et al., 2011**).

Data showed that Fasting serum glucose (mg/dl) for different groups of hyperlipidemia rats fed on leaves and fruits of mulberry in table (5).

Serum glucose in control (-) showed highly significantly decrease ($p \leq 0.001$) as compared with control (+) group. The consumption of drug showed a significant decrease ($p \leq 0.001$) as compared with control (+) group while consumption of mulberry leaves (2.5,5,7.5)% and 7.5% mulberry fruits showed significant decrease ($p \leq 0.05$) as compared with positive group. However no significant decreased in glucose in group 2.5, 5 % mulberry fruits. The result agree with **Wang et al., (2013)** reported that diabetic rats fed with ethyl acetate –soluble extract of mulberry fruits for 2 weeks had a significant decrease in the levels of fasting blood glucose and glycosylated serum protein. Similar findings were also reported by **Guo et al.,(2013)** who found that diabetic rats fed with mulberry fruit poly saccharides for 2 weeks had a decrease in fasting blood glucose.

Table (5): Effect of mulberry leaves and fruits on serum glucose for normal and hyperlipidemia rats.

parameters groups	Control - ve	Control + ve	Mulberry leaves			Mulberry fruits			drug
			2.5	5	7.5	2.5	5	7.5	
Glucose (mg/dl)	87.77 ±2.15***	135.75 ±5.85	110.64 ±3.19*	108.85± 6.32*	108.39 ±2.13*	124.38 ±3.54	117.59± 2.77	107.2± .38*	105.3± 1.33**

* Differences are significant at $P \leq 0.05$ **Differences are highly significant at $P \leq 0.01$

***Differences are highly significant at $P \leq 0.001$

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دراسة التأثير المحتمل لأوراق وثمار التوت على حيوانات التجارب المصابة بارتفاع دهون الدم

خالد علي عبد الرحمن شاهين ، سمر وجية هاشم
قسم التغذية وعلوم الأطعمة- كلية الاقتصاد المنزلي-جامعة المنوفية

الملخص العربي:

الهدف الرئيسى من الدراسة هو معرفة تأثير اوراق وثمار التوت بتركيزات مختلفة للحماية ضد ارتفاع دهون الدم 0 تم استخدام 45 فار من النوع الالينو يتراوح وزنها (140 – 180) جم تم تقسيم الفئران الي 9 مجموعات (5 فئران فى كل مجموعه) احدهما المجموعة الضابطة السالبة اما باقى المجموعات الثمانية تم اضافته 2 % من الكوليسترول لمدة 15 يوما احدى هذه المجموعات كمجموعه ضابطه موجبه والمجموعات الاخرى تغذت علي اوراق التوت بنسبه (2.5- 5- 7.5) % و مجموعات تغذت ايضا علي ثمار التوت بنسبه (2.5- 5- 7.5) % والمجموعة الاخيرة تم اضافته عقار خافض لدهون الدم واستمرت التجربة لمدة شهرين وتم تقدير كلا من % FER&B.W.G والكوليسترول الكلي وثلاثى الجلسريد والكوليسترول عالي الكثافة والكوليسترول منخفض الكثافة ومنخفض الكثافة جدا والجلوكوز ووظائف الكلي و الكبد)

قد اوضحت النتائج انخفاض ملحوظ في وظائف الكلي والكبد و انخفاض الكوليسترول الكلي ومنخفض الكثافة وارتفاع في الكوليسترول عالي الكثافة وانخفاض مستويات الجلوكوز في الدم بشكل ملحوظ وخاصة في المجموعات التي تغذت على اوراق وثمار التوت بتركيز 7.5 %.