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Potential Effect of Mulberry Leaves on Obesity in Experimental Rats

التأثير المحتمل لأوراق التوت على السمنة في فئران التجارب

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Potential Effect of Mulberry Leaves on Obesity in Experimental Rats

Abstract.

Obesity is an increasingly primary health concern and one of the leading causes of declining quality of life . The study was conducted to evaluate the effect of the mulberry leaves (ML) on obesity in experimental rats. Sixty adult males albino rats, which were divided into (٦) groups each group (١٠) rats. Group (١): normal control, Group (٢): positive control group , Group (٣): a healthy group fed on the basal diet plus ٥% mulberry leaves powder , Group (٤): a healthy group fed on basal diet and ١٠ % mulberry leaves powder, Group (٥): rats induced obesity fed on basal diet and ٥% mulberry leaves powder , Group (٦): rats induced obesity fed on basal diet and ١٠ % mulberry leaves powder .The results showed that mulberry leaves contain moisture , ash ,protein ,fat ,and total carbohydrates ٤,٦٤%, ٢١,٧٦%, ١٣,٤٥%, ١٤,٥%, ١٤,٥٦% and ٣١,٠٩%; respectively. Moreover, mulberry leaves ١٢ phenolic compounds ; the results revealed a significant decreases in serum cholesterol, triglycerides, glucose and LDL-C and an increased in serum HDL-C in rats induced obesity and treated with ٥% and ١٠% mulberry leaves. In addition , a significant decreased serum ALT, AST, ALP, GGT and LDH, activities at ($p \leq ٠,٠٥$), also, there was a significant reduced serum creatinine, urea and in uric acid levels, when compared with group(٤), So this study recommended to use mulberry leaves in diets for it is many benefits.

Keywords:

Obesity, Mulberry leaves, Lipids profile ,Liver and Kidney Functions

Introduction

Globally, obesity is a serious health problem due to its strong association with increased dyslipidemia, cardiovascular disease (including hypertension, stroke, and myocardial infarction), insulin resistance, glucose metabolism disorders, osteoarthritis and some cancers. This trend will have a significant impact on world health and the economy. (Haslam and James, 2005; Després and Lemieux, 2006).

Obesity a part of metabolic syndrome is a major lifestyle disorder throughout the world. The main causes of obesity are the delicious and energetic diets rich in fat, consumption imbalances, low momentum, fat, sugar and salt (Brown *et al.*, 2015).

Claudia *et al.*, (2015) showed that the complex pathogenesis of obesity indicates the need of different intervention strategies to confront this problem. Herbal supplements and diet-based therapies for weight loss are among the most common complementary and alternative medicine modalities. As an alternative treatment for obesity and its complications, in the market are a variety of natural products that includes medicinal plants, either as pure compounds or as extracts.

Edeoga *et al.*, (2005) related that medicinal plants have always been considered as a healthy source of life for all people due to their rich therapeutic properties and being 100% natural. The majority of populations widely use medicinal plants to cure various diseases and illness and have a high impact on the world's economy.

Twani and Melody (2016) reported that among the plants circulated since passing ages is the mulberry (*Morus alba* L.), which belongs to the *Moraceae* family. The mulberry was found in the tombs of Hawara and was used by the Pharaohs as food and in therapeutic recipes. Currently it is grown all over the world because of its importance in breeding silkworms. Mulberry trees are grown to take advantage of their fruits and leaves. They are also planted in public gardens to beautify the city and provide shade in the streets where Tree wood is used in the manufacture of furniture and musical instruments, and the manufacture of boats and supports for wooden houses. In traditional Chinese herbal medicine, Mulberry fruit was used to treat diabetes, high blood pressure, arthritis, anemia, and others. Recently, it was found that mulberry leaves are anti-inflammatory, ulcerative, laxative, and anthelmintic. It was also found that it treats cancer and strengthens the immune system because it contains various antioxidants.

Correspondingly, mulberry (*Morus alba L.*) leaves are a nutritional supplement that has long been widely used for health purposes. Owing to its nutritional value in many Asian countries, it has been widely used as a functional food, including beverages, noodles, and herbal tea. Mulberry leaves contain numerous bioactive compounds, such as flavonoids and phenolic acids, which are responsible for their antioxidant activity (Wang *et al.*, ٢٠١٨ and Zhang *et al.*, ٢٠٢٢). Therefore, the present study aims to show the potential effect of mulberry leaves on obesity in experimental rats.

Materials and Methods

Materials

Plant materials

One kilogram of mulberry leaves was obtained from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt).

Methods

Preparation of Mulberry leaves

Collection of mulberry leaves, washed and dried under vacuum to maintain active compounds and saved in glassware sealed until used.

Chemical methods

Kits were used to determine total cholesterol (T.C), triglycerides (T.G), high-density lipoprotein cholesterol (HDL- C), low-density lipoprotein-cholesterol (LDL-C), alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transaminase (GGT), Glucose, urea, creatinine and uric acid were obtained from Sigma Aldrich (St. Louis, MO, USA)

Determination of the chemical composition of Mulberry leaves

Moisture, ash, protein, crude fat and crude fiber were determined according to the method outlined by (A.O.A.C., ٢٠١٠).

Carbohydrate content

The total carbohydrate content of the studied mulberry leaves sample was calculated by difference ١٠٠ - (other nutrients composition) according to the method described in (A.O.A.C., ٢٠١٠).

Caloric value

The caloric value was calculated according to the methods of Select, (٢٠١٠).

Determination of phenols content

HPLC analysis was carried out using an Agilent ١٢٦٠ series and was determined using the method of (Kujala *et al.*, ٢٠٠٠).

Experimental design

Sixty adult male Wistar albino rats (*Rattus norvegicus*) weighting (100 ± 10 g) were obtained from Animal Colony, National Research Centre, Cairo, Egypt. The animals were housed in suitable plastic cages for one week for acclimation before the experimental study. Excess tap water and standard rodent food pellets [٢٠,٣% protein (٢٠% casein and ٠,٣% DL-Methionine), ٥% fat (corn oil), ٥% fibers, ٣,٧% salt mixture and ١% vitamin mixture; obtained from Meladco company for animals and rodents food pellets, El-Obour City, Cairo, Egypt] were always available, they received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to ethical committee of Faculty of Science, Al-Azhar University, Assuit, Egypt; however, this study was approved by the same ethical committee (AZHAR ١٣\٢٠٢٣). After the rats were acclimatized to experimental room conditions, they were divided randomly into six groups (١٠ rats each). Depending on the duration of treatment, the rats were randomly divided into the following groups:

Group (١): Rats was fed on a standard diet as a negative control group.

Group (٢): Comprise of healthy rats that were subjected to feed on mulberry leaves ٥ % for six weeks.

Group (٣): Comprise of healthy rats was subjected to feed on mulberry leaves ١٠ %.

Group (٤): Comprise of high-fat diet as a positive control group.

Groups (٥): Comprise of a high-fat diet by oral administration and mulberry leaves ٥ %.

Groups (٦): Comprise of a high-fat diet by oral administration and mulberry leaves ١٠ %.

Blood sampling

At the end of the study period, rats fasted overnight, and following diethyl ether anesthesia, about ٠,٥ ml of blood sample was

collected into heparinized vacutainer tube immediately for the hematological investigations; while non-heparinized blood specimens (3-5 ml) from each rat) were drawn from the retro-orbital plexus using sterile glass capillary (single draw vacutainer needle) into open vacutainer collecting tubes. The non-heparinized blood specimens were left for 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using a cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -8°C until biochemical measurements could be carried out as soon as possible.

Biochemical determinations

Lipids profile

Serum, total cholesterol (T.C), triglycerides (T.G), high-density lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c) were determined according to (Cole *et al.*, 1997; Artiss and Zak, 1997; Lopes-Virella *et al.*, 1977 and Wieland and Seidel, 1983); respectively.

Serum glucose

Serum Glucose level was determined according to the CHOP-PAP method by the photometric system described by Young (2001)

Liver functions

Serum aspartate amino transferases (AST) , alanine amino transferases (ALT), alkaline phosphatase (ALP), gamma-glutamyl transaminase (GGT) and lactate dehydrogenase (LDH) were determined according to (Schumann and Klauke, 2003; Moss and Henderson, 1999, IFCC, 1983; Trinder, 1969 and Tietz *et al.*, 1983) ; respectively.

Kidney functions

Serum creatinine activity was determined according to the kinetic method described by Young (2001).

Serum urea activity was determined according to the enzymatic colorimetric method described by Young (2001).

Statistical analysis

The obtained data were statistically analyzed by SPSS computer software and expressed as mean \pm SD. Effects of different treatments

were analyzed in one way (ANOVA) followed by Duncan's multiple range tests . Differences were considered significant at $P < 0.05$ according to **Snedecor and Cochran** (1986).

Results and discussion

Gross chemical composition and caloric values of Mulberry leaves on a dry weight basis

The data in **Table (1)**: revealed that moisture, ash, protein, crude fat, crude fiber, and total carbohydrates in mulberry leaves were 4.64%, 21.77%, 13.45%, 14.56%, and 32.09% while, caloric value was 308.66 K.Cal./100g. These results agree with **Ewa et al.**, (2013) they reported that moisture, protein, and ash were 5.40±0.07, 12.70±1.73 and 22.70±0.13 respectively, While these results disagree with **Butt et al.**, (2008) & **Monika and Ewa**, (2016) they obtained that proximate analysis in mulberry leaves was 5.31% protein, 4.3%; ash 11.3%, 8.5% and fat 2.09% , 11.7% respectively. Variations in moisture contents, ash, protein, crude fiber, crude fat and carbohydrate due to several factors can these compositions such as climate, growing, postharvest management and processing conditions.

Table (1): Gross chemical composition and caloric values of Mulberry leaves on dry weight basis (mg / 100g)

Sample	Moisture %	Ash %	Protein %	Crude Fat %	Crude Fiber %	Total Carbohydrates %	Caloric Value (K.cal/ 100g)
Mulberry leaves	4.64±2.3	21.77±1.09	13.45±0.9	14.56±6.22	14.56±7.25	32.09±10.90	308.66 ±107.8

-Mean of three replicates

Phenolic constituents of Mulberry leaves

As shown in **Table (2)**: 12 phenolic compounds were identified in Mulberry leaves using High-Performance Liquid Chromatography (HPLC) analysis. The compounds phenolic identified mulberry leaves were found to include high contents of chlorogenic acid, gallic acid, and rutin. while, the lowest values were in coumaric acid , methyl gallate , cinnamic acid, and Coffeic acid . These results disagree with **Lingyu et al.**, (2022) who found that 9 identified compounds were in ML by HPLC, rutin, hyperoside, astragaline, luteolin, and glucoside. In this respect **Hao et al.**, (2021) and **Huang et al.**, (2022) found these

compounds have been identified in Mulberry leaves and the flavonoids were major components in ML.

Table (٢): Phenolic constituents of the Mulberry leaves (ML)

Parameters	($\mu\text{g/ml}$)
Gallic acid	٦٣٤,٨٦
Chlorogenic acid	٢٣٠٦,٣١
Catechin	N.D
Methyl gallate	٢,٦٠
Coffeic acid	٨,٤٣
Syringic acid	١٠٥,٢٧
Rutin	٢٧٤,٤٠
Ellagic acid	N.D
Coumaric acid	٠,٧٢
Vanillin	N.D
Ferulic acid	٢٣,٨٩
Naringenin	٣٠,٧٨
Rosmarinic acid	٩٦,٢٤
Daidzein	N.D
Querectin	١٠,٨٩
Cinnamic acid	٦,٠٣

- N.D. Not detected

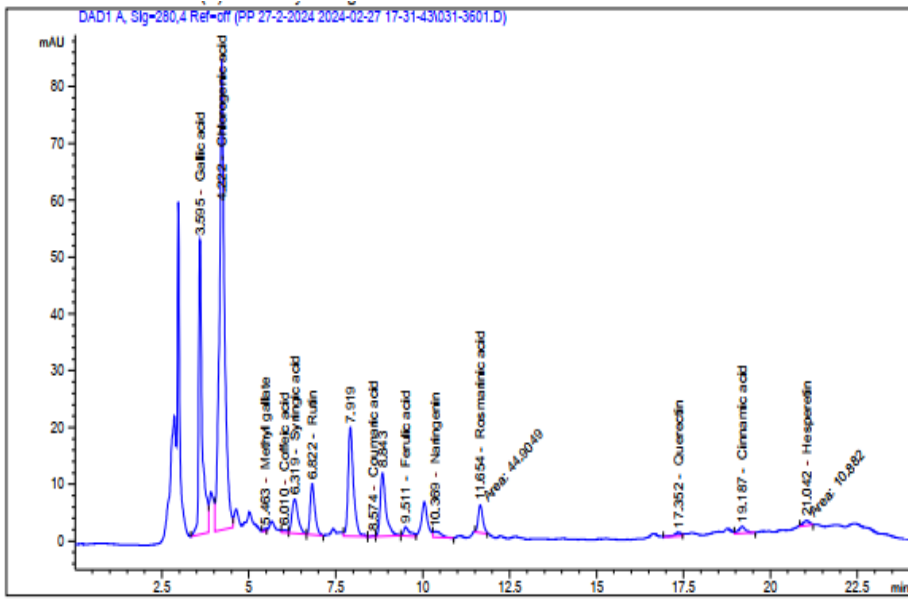


Fig. (1): HPLC analysis of phenolic constituents Mulberry leaves .

Effect of Mulberry leaves on the lipids profile of rats

The obtained results in **Table (٣)** revealed that the obesity rats showed a significant at ($p \leq 0.05$) increased in cholesterol, triglycerides, glucose and LDL-C matched with significant decreased HDL-C in group (٤) when compared with normal control group (١); however the treatment of mulberry leaves (ML) for six weeks induced non-significant changes in serum cholesterol, triglycerides, HDL-C and LDL-C levels. Moreover, the obesity rats treated with ML showed at ($p \leq 0.05$) significant decreases in serum cholesterol, triglycerides, glucose and LDL-C matched with significant decreases in serum HDL-C and insulin in group (٥,٦) when compared to group (٤). These results agree with (**Khaled and Mohamed**, ٢٠١٩) they found that the obese rats treated with ML showed significant at ($p \leq 0.05$) decreases in serum cholesterol, triglycerides, and LDL-C matched with a significant increase in serum HDL-C when compared to obesity group. These results agree with **yinzhaio et al**., (٢٠٢٠) they founded that serum levels of glucose, TG, T.C and HDL were significantly increased in the high fatty diet (HFD) group compared to the control group at ($p < 0.05$). Flavonoids from Mulberry leaves (FML) administration decreased serum levels of TG at ($p < 0.05$) and HDL. **Alkudhayri et al.**, (٢٠٢١) stated that ML contains several bioactive compounds, such as rutin, chlorogenic acid, and gallic acid, which have been shown to have antiobesity, antihyperglycemic, and anti-inflammatory effects This can explain the reduction TC, TG, and LDL levels in HFD-fed rats treated with ML.

Table (٣): Effect of Mulberry leaves (ML) on the lipids profile and glucose in experimental rats.

The same column means that different superscript letters are significantly different at ($p \leq 0,05$)

Parameters Groups	T.C (mg/dl)	T.G (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	glucose (mg/dl)
Group (١) Control (-)	١٤١±٥,٧	١٥٨,٦±٦,١	٤٤±٠,٨١	٦٥,٥±٦,٣	٣١,٧±١,٢	٧٤,٤±٣,٤
Group (٢) ML (٥%)	١٣٩,٥±٢,٩	١٤٨,٢±٥,٣	٤٣,٥±٠,٥٢	٦٦,٣±٢,٩	٢٩,٦±١,٠٦	٧٢,٩±٥,٠
Group (٣) ML (١٠%)	١٣٩,٧±٢,٧	١٤٨±٦,١٤	٤٣,٧±٥±١,٠٧	٦٦,٤±٢,٣	٢٩,٦±١,٢	٧٤,٣±٤,٤
Group (٤) Obesity Control (+)	٢٥٤,٣±١٢,٢*	٢٦٦,٦±١٣,٠*	٣٥,٣±٠,٦٢*	١٦٥,٦±١٤,٢*	٥٣,٣±٢,٦*	١٢٥,٣±٤,٦*
Group (٥) Obesity + ML (٥%)	١٣٧,٦±١٠,٣#	١٢٩,٣±٨,٤#	٤٣±٠,٠٤#	٦٨,٨±١,٦#	٢٥,٨±٢,٣#	٨٠,٤±٠,٠١٧#
Group (٦) Obesity + ML (١٠%)	١٢٣,٣±٧,٢#	١٢٣,٣±٥,٩#	٤٤±٠,٤٠#	٤٥,٦±٨,٨#	٢٤,٦±١,١٨#	٧٨,٦±١١,٦#

Symbol (*) is significantly different from control group; symbol (#) is significantly different from obesity group at $p \leq 0,05$ level

- (T.C) Total cholesterol
- (T.G) Triglycerides
- (HDL-c) High-density lipoprotein-cholesterol
- (LDL-c) Low-density lipoprotein-cholesterol
- (VLDL- c) Very Low density lipoprotein-cholesterol

Liver Functions

The obtained results in **Table (٤)** revealed that the obesity rats showed a significant at ($p \leq 0,05$) increased in ALT, AST, ALP, GGT and LDH activities in group (٤) when compared with the normal control group (١) ; however, the treatment of ML for six weeks induced non-

significant changes in serum ALT, AST, ALP and GGT activities respectively. Moreover, the obesity rats treated with ML showed significant at ($p \leq 0,05$) and decreased in serum ALT, AST, ALP, GGT, and LDH activities in group (2,6) when compared to group (4). In general, this finding pointed to that, treatment of obesity rats with ML induced a marked amelioration serum ALT, AST, ALP, GGT and LDH activities. These results agree with **Khaled and samar**, (2020) they found that Levels of asparta amino transferees (AST) and alanine amino transferees (ALT) in control (-) showed a highly significant decrease ($p \leq 0,01$) as compared with control (+) group. The consumption of mulberry leaves 3,0 %, mulberry fruits 0,7,0 % and drug showed a significant decrease as ($p \leq 0,01$) compared with the control (+) group, while consumption of mulberry leaves 2,0, 0 % and mulberry fruits 2,0

% showed a significant decrease ($p \leq 0,05$) as compared with of control (+) group. **Hussein et al.**, (2010) studied the liver protective effect of mulberry and calendula officinal 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.

Ann et al., (2015) reported that mulberry leaves (ML) have beneficial effects on obesity-related fatty liver disease by regulating hepatic lipid metabolism, fibrosis, and antioxidant defense system. MLE supplementation might be a potential therapeutic approach for obesity-related diseases including non-alcoholic fatty liver disease.

Table (٤): Effect of Mulberry leaves of serum ALT, AST, ALP, GGT, and LDH (U/L) activities in experimental rats

Parameters Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Group (1) Control (-)	٥٧,٢±٠,٧٤	١٧٦±١٣,٦	٢٣٠,٥±٤,٠٨	٨,٠٥±٠,٤٩	٢١٣١±١٥٥
Group (٢) ML (٥%)	٥٢,٣±٠,٩١	١٦٩±٣,٤	٢٣٢,٢±١٠,٢	٧,٩±٠,٣٨	٢١٢٢,٤±١٢٣
Group (٣) ML (١٠%)	٥٢,٥±٥,٥	١٧٠,٨±١٤,٣	٢٣١,٥±٧,٧	٧,٤±٠,٤	٢٠٧٦,٨±١٠٦
Group (٤) Obesity Control (+)	٩٣±٠,٥١*	٢١٤,٩±١٢,٣*	٣٣٧±٢٢,٨*	١٦,٦±٢,٠٧*	٣٤٨١,٥±١٦٥*
Group (٥) Obesity + ML (٥%)	٣١,٤±٣,٨#	١٦٩,٤±٢,٦#	٢٤٩±٢٥,٣#	٨±٠,٦٢#	١٧٢٣,٥±٧٠#
Group (٦) Obesity + ML (١٠%)	٢٨,٤±٤,٣٩#	١٦٢,١±٥,٥#	٢٠٢±٢٩,٢#	٧±١,٠	١٦٦٤,٣±٧٩#

The same column, means with different superscript letters are significantly different at ($p \leq 0,05$)

Symbol (*) is significantly different from control group; symbol (#) is significantly different from obesity group at $p \leq 0,05$ level

- Alanine amino transferees (ALT)
- Aspartate amino transferees (AST)
- Alkaline phosphatase (ALP),
- Gamma glutamyl transaminase (GGT)
- Lactate dehydrogenase (LDH)

Kidney functions

The obtained results in **Table (٥)** revealed that group (٤) showed a significant difference increased at ($p \leq 0,05$) group (٤) in creatinine, urea and uric acid when in group (٤) compared with group (١) normal control; however group (١) the treatment of ML for six weeks induced non-significant changes in serum creatinine, urea, and uric acid; respectively. Moreover, the obese rats treated with ML showed significant at ($p \leq 0,05$) and decreased serum creatinine, blood urea and uric acid levels in group (٥,٦) when compared with group (٤). In general, this finding pointed to the treatment of obese rats with the ML induced a marked amelioration of serum creatinine, urea and uric acid levels. These results agree with **Olfat et al., (٢٠٢٢)** they found that diabetic groups treated with ML (٢% and ٤%) had decreased kidney functions (urea, uric acid, and creatinine) compared to diabetic groups.

Table (٥): Effect of Mulberry leaves on serum urea , Creatinine, and uric acid

Parameters Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group (١) Control (-)	٤٨,٦±٣,٢	٠,٧٤±٠,٠٥	٢,١٧±٠,١٨
Group (٢) ML (٥%)	٤٧,٦±١,٧	٠,٧٧±٠,٠٤	٢,١٧±٠,٥٧
Group (٣) ML (١٠%)	٣٩,١±٠,٩٦	٠,٧١±٠,٠٣	٢,١١±٠,٠٩
Group (٤) Obesity Control (+)	٧٣,٨±٤,٧*	١,٢±٠,٠٧٦*	٦,٤٠±٠,٠٨*
Group (٥) Obesity + ML (٥%)	٣٧,٦±٣,٠٢#	٠,٥٧±٠,٠٠٤#	٣,٣±٠,١٤#
Group (٦) Obesity + ML (١٠%)	٣١,٠٥±١,٩#	٠,٥٠±٠,٠٣#	٢,٣١±٠,٣٩#

The same column, means with different superscript letters are significantly different at ($p \leq 0,05$)

Symbol (*) is significantly different from control group; symbol (#) is significantly different from obesity group at $p \leq 0,05$ level

Conclusion

In conclusion, we found that Mulberry leaves induced a marked amelioration in serum ALAT, ASAT, ALP, GGT, and LDH activities, induced a marked amelioration in serum cholesterol, triglycerides, HDL-C, LDL-C and glucose levels and induced a marked amelioration serum creatinine, urea and uric acid levels . So this study recommended to use mulberry leaves in diets for it is many benefits.

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التأثير المحتمل لاوراق التوت على السمنة في فئران التجارب

المستخلص:

السمنة هي مشكلة صحية أولية متزايدة الأهمية وأحد الأسباب الرئيسية لتدهور نوعية الحياة، أجريت الدراسة لتقييم تأثير أوراق التوت على السمنة في فئران التجارب. ستون من ذكور الفئران الألبينو البالغة ، والتي تم تقسيمها إلى (٦) مجموعات كل مجموعة (١٠) فئران. المجموعة (١) المجموعة الضابطة السالبة، المجموعة (٢) المجموعة الضابطة الموجبة، المجموعة (٣) مجموعة صحية تتغذى على النظام الغذائي الأساسي ومسحوق أوراق التوت بنسبة ٥٪، المجموعة (٤) مجموعة صحية تتغذى على النظام الغذائي الأساسي ومسحوق أوراق التوت بنسبة ١٠٪، المجموعة (٥) فئران مصابة بالسمنة و تتغذى على النظام الغذائي الأساسي ومسحوق أوراق التوت بنسبة ٥٪، المجموعة (٦) فئران مصابة بالسمنة و تتغذى على النظام الغذائي الأساسي ومسحوق أوراق التوت بنسبة ١٠٪. وأظهرت النتائج أن أوراق التوت تحتوي على رطوبة ورماد وبروتين ودهون وكربوهيدرات إجمالية ٤,٦٤% و ٢١,٧٦% و ١٣,٤٥% و ١٤,٥٠% و ١٤,٥٦% و ٣١,٠٩% على التوالي. علاوة على ذلك، يحتوي مستخلص أوراق التوت على ١٢ مركب فينولي؛ أظهرت النتائج انخفاضاً معنوياً في مستويات الكولسترول والدهون الثلاثية والجلوكوز والكولسترول الضار في مصل الدم وزيادة في مستويات الكولسترول الجيد في مصل الدم في الفئران المصابة بالسمنة والتي عولجت بأوراق التوت بنسبة ٥% و ١٠%، بالإضافة إلى انخفاض نشاط إنزيمي ALT و AST و ALP و GGT و LDH في مصل الدم عند $(p \leq 0,05)$ ، كما انخفض مستوى الكريتيانين واليوريا وحمض البوليك في مصل الدم عند مقارنتها بالمجموعة (٤) وهي المجموعة الضابطة الموجبة ؛ لذا أوصت هذه الدراسة باستخدام أوراق التوت في الوجبات الغذائية لفوائدها العديدة.

الكلمات المفتاحية:

السمنة، أوراق التوت، دهون الدم ووظائف الكلى والكبد .