Anti-diabetic Effect of Black Mulberry Leaves\textit{(Morus nigra, L.)} in Streptozotocin-Induced Diabetic Rats

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Abstract

The effect of different levels of black mulberry leaves (\textit{Morus nigra, L.}) 2.5, 5\% as powder and 250 & 500 mg/kg as extract on diabetic rats were evaluated using 36 rats which divided for 6 groups. Also, phenolics compounds were determined. Results showed that the values of total phenols, total flavonoid and anthocyanin contents of mulberry leaves were 13.80±0.93, 61.40±0.35 and 3.70±0.61 mg/g, respectively. The highest phenolic compounds of mulberry leaves recorded for pyrogallol and syringic acid. The mean values were 223.3 and 80.12 mg/100g, respectively. While, the lowest values recorded for gallic acid and vanillic acid. The mean values were 9.97 and 7.15 mg/100g, respectively. Group rats administrated with 500 mg/kg extract of black mulberry leaves showed highest values with significant differences of BWG, FI and FER compared with other groups. The highest reduction with significant difference in GOT, GPT and ALP levels recorded with group administrated with 500 mg/kg black mulberry leaves extract compared with other groups. The maximum reduction with significant difference in serum triglycerides and total cholesterol recorded with group administrated on 500 mg/kg black mulberry leaves extract. The highest reduction with significant difference in VLDL-c value recorded for group administrated with 5\% black mulberry powder, while, the maximum increment with significant difference in HDL-c value recorded with group administrated with 500 mg/kg black mulberry leaves extract. The maximum reduction with significant difference in LDL-c value recorded with group fed on 500 mg/kg black mulberry leaves extract. The highest reduction with significant difference in serum urea, uric
acid and creatinine value recorded with group administrated with 500 mg/kg black mulberry leaves extract.

**Key words:** Black mulberry leaves, Diabetic rats, Biochemical analysis and Active compounds.

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**Introduction**

Diabetes mellitus is a chronic disease that has affected humankind throughout the world. Diabetes is considered as a major health problem at all ages in developing countries. It is a powerful and independent risk factor for cardiovascular disease, which remains the major cause of death in diabetic person. Diabetes mellitus is caused due to deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a global problem and number of those affected is increasing day by day.

Diabetes is the world’s largest endocrine disease associated with increased morbidity and mortality rate. Diabetes mellitus is also associated with long term complications including retinopathy, nephropathy, neuropathy, angiopathy and several others (Sharma et al., 2010).

The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes (Mukherjee et al., 2006).

Since, ancient times, plants have played an important role in the treatment of many diseases. Different parts of medicinal plants such as leaf, root, flower and seed are used as extracts and chemical compounds to produce drugs (Ozgen et al., 2009).

According to world Health Organization (WHO, 1988), 80% of the World’s population is dependent on the traditional medicine (Maiyo et al., 2010).

*Morus nigra* L., belonging to the Moraceae family, is a deciduous tree widely cultivated in Europe and West Asia. It has a long history of medicinal use in Chinese medicine, as a remedy for many kinds of diseases (Pawlowska et al., 2008).

Moreover, in recent years, numerous research studies have associated the consumption of foods rich in polyphenols with the prevention of cardiovascular diseases, certain type of cancer and other diseases related to aging; thanks to their antioxidant properties (Borbalan et al., 2003).
With the aim of finding new sources of natural antioxidants, plants, fruits, vegetables and other plant materials that are known to possess antioxidant activity have been investigated. The mulberry plant has significant biological importance for its antioxidant and antimicrobial properties. The phenolic compounds of black mulberry showed moderate anti-oxidant and anti-bacterial properties. The results add to the use of phenolic compounds present in mulberries and to partially explain their reported pharmacological activities which include use as refreshing substances and as antibacterial (Ofentse et al., 2011).

Anti-diabetic use of mulberry leaves had also been popular; moreover, this indication became part of the local traditional medicine wherever the tree has been naturalized. Recent studies have reported that 1-deoxynojirimycin in white mulberry has anti-diabetic effects and it reduces postprandial blood glucose levels through inhibition of α-glucosidase and decrease of serum triglyceride levels (Kwon et al., 2011).

The leaves of mulberry species are consumed in Korea and Japan as anti-hyperglycemic nutraceutical food for patients with diabetes mellitus because the leaves are rich in alkaloid components, including 1-deoxynojirimycin, which is known to be one of the most potent α-glycosidase inhibitors that decreases blood sugar levels (Kim et al., 2003).

The fruits and leaves of mulberry plants contained many bioactive components, such as alkaloids, anthocyanins, and flavonoids. Mulberry leaves are rich in alkaloid components including 1-deoxynojirimycin (DNJ), which is known as one of the most potent glycosidase inhibitors that decreases blood sugar levels. Some methods have been established to detect DNJ in mulberry leaves (Kimura et al., 2007).

This activity of mulberry leaves has been verified by a number of studies including several animal experiments and a few human trials as well, according to our knowledge, the active constituents and their role in the activity still remain to be fully described. Nevertheless, a complex cocktail of various bioactive constituents is thought to be responsible for this activity, among which the role of amino -sugars and
certain phenolics mainly chlorogenic acid and rutin might be the most significant (Hunyadi et al., 2012).
Sanchez-Salcedo et al., (2015) evaluated the phenolic content of white (Morus alba) and black mulberry (Morus nigra) fruits,. Black mulberry clones showed higher antioxidant activity and amounts of phenolic compounds than white mulberry clones. These results are keys for the design of future dietary intervention studies examining the role of mulberry fruits in disease risk reduction. They can also be used for the development of mulberry derived-products rich in phenolic compounds.

This work was conducted to study the effect of different concentrations of black mulberry (Morus nigra) leaves powder and its organic extract on biological and biochemical changes on diabetic rats.

Material & Methods:
Black mulberries samples:
Black mulberry leaves (Morus nigra) were obtained from local market, Menoufia Governorate, Egypt.

Chemicals:
Folin-Ciocalteu reagent and standard substances were purchased from Sigma Chemical Co., St. Louis, USA,. All reagents and standards were prepared using Milli-Q deionized water (Millipore, Bedford, USA). All other chemicals and reagents were of analytical reagent grade.
Casein, cellulose, choline chloride, and DL Methionine:
Casein, cellulose, choline chloride powder, and DL methionine powder, were obtained from Morgan Co. Cairo, Egypt.

Experimental animals:
A total of 36 adult normal male albino rats, Sprague Dawley strain weighing 140±10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

The chemical kits:
Chemical kits used for determination the (TC, TG, HDL-c, ALT, AST, ALP, bilirubin, urea, creatinine, albumin) were obtained from Al-Gomhoria Company, Cairo, Egypt.
Methods:

Preparations of mulberry leaves:
To prepare the dried mulberry leaves powder, leaves obtained from local market. Plant leaves were washed thoroughly under running tap water, shade dried, and ground to a fine powder using an air mill.

Extraction of mulberry leaves:
Extraction of antioxidants was determined according to the method of Arabshahi- Delouee and Urooj (2006). The dried leaves of mulberry (15 g) were extracted with 100 mL of various concentrations of ethanol (50, 60, 70 and 95 %v/v) in a mechanical shaker (Julabo Shake Temp., SW23) at 37°C for 18 hrs. Each extract was filtered with Whatman No. 1 filter paper. The extract was kept at -20°C until further analysis.

Analytical methods:

Determination of total phenolic contents:
Total phenolic contents (TPC) were determined spectrophotometrically by using Folin-Ciocalteu reagent (Kahkonen et al., 1999). The absorbance was measured at 765 nm and results were expressed as mg of chlorogenic acid equivalents (CAE) per mg/gram of dry extracts.

Determination of total flavonoids contents:
Total flavonoids content (TFC) was performed using a modified colorimetric method (Jia et al., 1999). The absorbance was measured at 510nm and results were expressed as mg of rutin equivalents (RE) per gram of dry extract. Triplicate tests were conducted for each sample. Spectrophotometric measurements were performed using a Vis spectrophotometer (Janwey6300, Germany).

HPLC Analysis of phenolic compounds:
The identification of phenolics compounds was according to Mazza et al., 1999, with some modifications as described by Radovanović and Radovanović, (2010). HPLC system Perkin Elmer PE200 was composed of a binary pump, a column thermostat and an auto sampler. The mass spectrometer used was a 3200QTRAP MS/MS with ESI ionization (Applied Biosystems / MDSSciex, Foster City, USA). The experimental conditions where: mobile phase A: 50% acetonitrile, 50% acetic acid (0.5%); mobile phase B: 2% acetic acid; gradient elution: 0 min 30% A,70%B; 10 min 30% A,70% B; 30 min
100% A, 0% B; 35 min 100% A, 0% B; 40 min 30% A, 70% B for reconditioning of the system; flow rate: 0.7 ml/min; injection volume: 20 µl; ionisation: ESI negative; dwell time 50 ms; multi plereaction monitoring (MRM) transitions. Stock solutions of standards were diluted in the mobile phase to obtain working standard solutions. Concentrations of the compounds were calculated from chromatogram peak areas on the basis of calibration curves. The method linearity was assessed by means of linear regression of the mass of compounds injected vs. its peak area. All solvents were of HPLC grade and were filtered and degassed before use.

**Experimental design:**

Thirty six adult male white albino rats, Sprague Dawley Strain, 10 weeks age, weighing (140±10g) were used in this experiment. All rats were fed on basal diet (casein diet) prepared according to American Institute of Nutrition (AIN) (1993) for 7 consecutive days. After this adaptation period, rats are divided into 6 groups, each group which consists of six rats as follows: group (I): rats fed on basal diet as negative control. Group (2): injected by streptozotocin a dose of 40 mg per kg of rat’s body weight and used as a positive control group at faculty of Home Economics, Menoufia Univ. Group (3): a group diabetic rats fed on mulberry leaves as powder by 2.5% (of diet) of the weight of the rat. Group (4): a group diabetic rats fed on mulberry leaves as powder by 5% (of diet) of the weight of the rat. Group (5): a group diabetic rats fed on the leaves of mulberry leaves extract administrated by 250 mg/kg of the weight of the rat. Group (6): a group diabetic rats fed on the leaves of mulberry leaves extract administrated by 500 mg/kg of the weight of the rat. During the experimental period, the body weight and feed intake were estimated, and the general behavior of rats was observed. The experiment continued for 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered and blood samples collected. Blood samples were centrifuged at 4000 rpm for ten minute to separate blood serum, and then kept in deep freezer till using.
Blood sampling:
After fasting for 12 hours, blood samples were obtained from hepatic portal vein at the end of each experiment. Two kinds of blood samples were taken. The blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 30 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen in deep freezer till analysis.

Body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER):
During the experimental period (28 days) the net feed intake was daily recorded, while body weight was weekly recorded. The net feed intake and gained body weight were used for the calculation of feed efficiency ratios (FER) according to Chapman et al., (1959) using the following formulas:

\[
\text{BWG (g/28 days)} = \text{Final weight} - \text{initial weight}
\]

\[
\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Feed intake (g)}}
\]

Relative organs weight:
The different organs of rats (spleen, liver and kidney,) were carefully removed, washed in saline solution blotted, dried between 2 filter papers and immediately weighted. The relative organ weight was calculated as following:

\[
\text{Relative organs weight \%} = \frac{\text{Organ weight (g)}}{\text{Total body weight (g)}} \times 100
\]

Biochemical Analysis:
Lipids profile:
Serum total cholesterol was determined according to the colorimetric method described by Thomas (1992).

Determination of serum triglycerides:
Serum triglycerides was determined by enzymatic method using kits according to the Young, (1975) and Fossati, (1982).

Determination of high density lipoprotein (HDL-c):
HDL-c was determined according to the method described by Fredewaid (1972) and Grodon and Amer (1977).
Calculation of very low density lipoprotein cholesterol (VLDL-c):

VLDL-c was calculated in mg/dl according to Lee and Nieman (1996) using the following formula:

\[ \text{VLDL-c (mg/dl)} = \frac{\text{Triglycerides}}{5} \]

Calculation of low density lipoprotein cholesterol (LDLc):

LDL-c was calculated in mg/dl according to Lee and Nieman (1996) as follows:

\[ \text{LDL-c (mg/dl)} = \text{Total cholesterol} – \text{HDL-c} – \text{VLDL-c} \]

Liver functions:

Determination of serum alanine aminotransferase (ALT), serum asparatate aminotransferase (AST), serum alkaline phosphatase (ALP) were carried out according to the method of (Clinica Chimica Acta, 1980, Hafkenscheid 1979 and Moss 1982), respectively.

Kidney functions:

Determination of serum urea:

Urea was determined according to the enzymatic method of (Patton and Crouch, 1977).

Determination of creatinine:

Creatinine was determined according to kinetic method of (Henry, 1974).

Determination of uric acid:

In the used method the intensity of the red color formed during determination is proportional to the uric acid concentration in the sample (Schultz, 1984).

Determination of blood glucose:

Enzymatic determination of plasma glucose was carried out calorimetrically according to the method of Tinder (1969).

Statistical analysis:

The data were analyzed using a completely randomized factorial design (SAS, 1988) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments at (P≤0.05) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

Results And Discussion

Data given in table (1) show the total phenols, total flavonoid and anthocyanin contents of mulberry leaves expressed as (mg/g). It is
worth to mention that the values of total phenols, total flavonoid and anthocyanin contents of mulberry leaves were 13.80±0.93, 61.40±0.35 and 3.70±0.61 mg/g, respectively. These results are in agreements of Ozgen et al., (2009). They reported that mulberry leaves extract have higher content of phenolic compounds. Phenols possess a wide spectrum of biological activities, and the results show that mulberry extracts could be good sources of these natural constituents. Imran et al., (2010), found that the contents of total phenolics in mulberry leaves were 6.64 mg/100 g fresh mass (Morus nigra) and 7.55 mg/100 g fresh mass (Morus alba). Also, Danijela et al., (2013) mentioned that extracts of fresh mulberry from South East Serbia, contain high levels of total phenols, flavonoids and anthocyanins, > 100 mg/100 g of fruits. The highest content of phenols was found in aqueous extract, flavonoids in ethanol extract and anthocyanins in ethanol / water extract. Extracts of mulberry have a high content of polymeric anthocyanins.

Data presented in table (2) show the identified phenolic compounds of mulberry leaves. It is worthy mentioning that the highest phenolic levels of mulberry leaves recorded for pyrogallol and syringic acid. The mean values were 223.3 and 80.12 mg/100g, respectively. While, the lowest values recorded for gallic acid and vanillic acid, the mean values were 9.97 and 7.15 mg/100g, respectively. On the other hand, sinapic acid did not detect. These results are in agreement with that published by Zadernowski et al., (2005). They reported that the gallic acid, pyrocatehehunic, vanillic acid, caffeic acid, o-coumaric acid, and p-coumaric acid, and ferulic acid acids in black mulberry leaves were as 27.3, 121.8, 6.5, 117.2, 212.7, 761.8, and 34.1 mg/100g, respectively. Also, these results are in agreements of Memon et al., (2010).

The effect of different concentration of mulberry leaves on BWG (%), FI (for 28 days) and FER of diabetic rats are shown in table (3). The obtained results indicated that body weight gain for control positive was lower than control negative. The values were 8.4 and 28, respectively. Concerning BWG for 500 mg/kg extract of black mulberry leaves group showed highest value with significant differences as compared with control positive being (36.4and 8.4) g. While, the lowest value recorded with group fed on 250 mg/kg black mulberry leaves extract. The value was 16.8g. In case of feed intake
(FI), data indicated that feed intake for control positive was lower than control negative. The mean values were 504.0 and 445 g/28 day, respectively. Group administrated with 500 mg/kg black mulberry leaves extract showed highest value with significant differences as compared with other groups (2.5% & 5% mulberry leaves powder and 250 mg/kg black mulberry leaves extract). The values were 509.6, 498.4, 495.6±0.20 and 492± 0.50 g/28 day, respectively). On the other hand, feed efficiency ratio of control positive recorded the lowest value being, 0.019%. While, the highest value of feed efficiency ratio with significant difference recorded for group fed on 500mg/kg black mulberry extract. The value was 0.071%. Finally, it could be concluded that group fed on 500mg/kg black mulberry leaves extract showed the highest values of body weight gain, feed intake and feed efficiency ratio compared with other groups. These results are in agreement with those of Mahmoud (2013) who investigated the effects of different levels (2.5, 5 and 10%) of black and white mulberry fruits (Morus nigra and Morus alba, L.) on body weight gain, feed intake and feed efficiency ratio. They reported that positive control group had significant decrease in feed intake, body weight gain and feed efficiency ratio when rats fed on basil diet. Moreover, all rats fed with different levels (2.5, 5 and 10%) of black and white mulberry had significant difference increase in BWG, FI and FER comparing with control positive group.

Data of table (4) show the atrophy of internal organs due to diabetes mellitus, while the relative weight increased by mulberry leaves, especially for diabetic rats fed on 500 mg/kg extract.

Data presented in table (5) show the effect of different concentration of mulberry leaves on glucose of diabetic rats. The highest reduction with significant difference in glucose levels recorded with group administrated with 500 mg/kg black mulberry leaves extract with value 109.1mg/dl. While other groups fed on (2.5 & 5% mulberry powder and 250 mg/kg black mulberry leaves extract) recorded a moderate reduction with significant difference in glucose levels. The values were 111.7, 115.2 and 124.5mg/dl, respectively. Finally, it could be concluded that group fed on 500 mg/kg black mulberry extract showed the highest reduction with significant difference in glucose levels compared with other tested groups. These results are in
agreement with those of Shukla et al., (2000), they found that mulberry leaves powder had improved fasting blood sugar of the diabetic Indian subjects.

Data given in table (6) show the effect of different concentration of black mulberry leaves on ALP, GOT (AST) and GPT (ALT) of diabetic rats. The highest reduction with significant difference in alkaline phosphate levels recorded with group administrated on 500 mg/kg black mulberry leaves extract compared with positive control group. The value was 90 7µ/L. While, other groups fed on (2.5 & 5% mulberry powder and 250 mg/kg black mulberry leaves extract) recorded a moderate reduction with significant difference in alkaline phosphate levels. The values were 135, 105 and 123U/L, respectively. Finally, it could be concluded that group fed on 500 mg/kg black mulberry leaves extract showed the highest reduction with significant difference in alkaline phosphate levels compared with other tested groups. These results are in agreement with those of Chaurasia et al., (2011).

In case of glutamic oxaloacetate transaminase (GOT) it is obvious that a markedly reduction in (GOT) levels in diabetic rats fed on different levels of black mulberry leaves was observed. The highest reduction with significant difference in (GOT) levels recorded with group administrated on 500 mg/kg black mulberry leaves extract compared with positive control group. The values were 17.21 and 55.82, respectively. While other groups fed on (2.5 & 5% mulberry powder and 250 mg/kg black mulberry extract) showed a moderate reduction with significant difference in GOT levels. The values were 39.4, 31.0 and 27.15, respectively. These results are in agreement with those of Chaurasia et al., (2011). They reported that treatment with a mixture of white and black mulberry leaves produce a marked significant decrease of the elevated GOT activities.

On the other hand, a markedly reduction in (GPT) levels by different rates in diabetic rats fed on different levels of mulberry leaves was observed. The positive control group had higher than negative control with significant difference. The mean values were 20.7 and 6.5 U/L, respectively. While, the highest reduction with significant difference in GPT levels recorded with group administrated with 500 mg/kg black mulberry compared with other diabetic groups. The mean
values were 6.0, 9.2, 10.93 and 8.81 U/L, respectively. Finally, it could be concluded that group fed on 500 mg/kg black mulberry leaves extract showed the highest reduction with significant difference in ALP, GOT and GPT levels compared with other tested groups. These results are in agreement with those of (Mahmoud et al., 2013). They mentioned the non-significant \( (p < 0.05) \) decrease in GPT level comparing to diabetic control rats.

The effect of different levels of black mulberry leaves on serum triglyceride (T.G), total cholesterol (T.C), very low density lipoprotein cholesterol (VLDL-c), high density lipoprotein cholesterol (HLDL-c) and low density lipoprotein cholesterol (LDL-c) of diabetic rats is shown in table (7). It is worth to mention that the serum triglycerides level of control positive group was higher than control negative group. The values were 135.15 and 55.81 mg/dl, respectively. On the other hand, the maximum reduction with significant difference in serum triglycerides value recorded with group administrated with 500 mg/kg black mulberry leaves extract. The value was 57.63 mg/dl. While, other groups fed on 2.5 & 5% mulberry powder and 250 mg/kg black mulberry extract showed high reduction with significant difference in serum triglycerides when compared with control (+) group. The values were 60.42, 78.33 and 70.14 mg/dl, respectively. These results are in agreement with those of (Anderson et al., 1999). Concerning of total cholesterol, the obtained result indicated that the total cholesterol level of control positive group was higher than control negative group. The values were 140.0 and 94.0 mg/dl, respectively. On the other hand, the maximum reduction with significant difference in total cholesterol value recorded with group fed on 500 mg/kg black mulberry leaves extract and 2.5% black mulberry leaves powder with values 103.0 and 107.0 mg/dl. While, the lower reduction values recorded for groups fed on 250 mg/kg black mulberry leaves extract and 5% black mulberry leaves powder. The mean values were 130.0 and 128.0 g/dl, respectively. These results are in agreement with those of (Sonali et al., 2013). They found that mulberry leaves powder incorporated products had improved lipid profile of the diabetic subjects studied. They indicated that maximum improvement was observed in the value of experimental group, which shows the hypoglycemic and hypocholesteromic effects of mulberry leaves.
The obtained results in table (7) showed that the very low density lipoprotein cholesterol (VLDL-c) level of control positive group was higher than control negative group. The values were 27.03 and 11.16 mg/dl, respectively. On the other hand, the maximum reduction with significant difference in VLDL-c value recorded for group administrated with 5% black mulberry leaves. The value was 11.53 mg/dl, while, other groups fed on 2.5&5% black mulberry leaves powder and 250 mg/kg black mulberry leaves extract showed moderate reduction with significant difference in very low density lipoprotein cholesterol value. The values were 12.08, 15.67 and 14.03 g/dl, respectively. These results are in agreement with those of (Andallu et al., 2009). They found that a significant decrease in cholesterol, triglyceride, free fatty acid LDL-c, VLDL-c levels and a significant rise in HDL-c levels in mulberry leaves treated group. In case of high density lipoprotein cholesterol (HDL-c), the obtained result showed that the HDL-c level of control positive group was lower than control negative group. The values were 27.67 and 43.05 mg/dl, respectively. On the other hand, the maximum increment with significant difference in HDL-c value recorded with group administrated with 500 mg/kg black mulberry leaves extract. The value was 45.51 mg/dl. While, other groups fed on 2.5&5% black mulberry powder and 250 mg/kg black mulberry leaves extract showed lower increment with significant difference. The values were 40.46, 37.61 and 39.94 g/dl, respectively. These results are in agreement with those of (Rodríguez-Morán et al., 1998). On the other hand, the LDL-c level of control positive group was higher than control negative group. The values were 85.30 and 39.79 mg/dl, respectively. Also, the maximum reduction with significant difference in LDL-c value recorded with group fed on 500 mg/kg black mulberry extract. The value was 45.96 mg/dl. While, the lowest reduction recorded for group fed on 250 mg/kg black mulberry extract being 76.03 g/dl. Finally, it could be concluded that group administrated by 500 mg/kg black mulberry extract showed the highest reduction with significant difference in TG, TC, VLDL-c, and LDL-c values compared with other tested groups. These results are in agreement with those of Tsuduki, et al., (2009). They reported that a significantly lower serum total cholesterol and markedly dose
dependently lower serum LDL-cholesterol and triglycerides indicated the possible diabetic effects of mulberry leaf.

The effect of different levels of black mulberry leaves on serum urea and serum uric acid of diabetic rats is shown in table (8). It is clear to notice that the urea level of control positive group was higher than control negative group. The values were 73.65 and 42.20 mg/dl, respectively. On the other hand, the highest reduction with significant difference in serum urea value recorded with group administrated with 500 mg/kg black mulberry leaves extract. The value was 46.25 mg/dl. While, the lowest reduction with significant difference recorded for 250 mg/kg black mulberry leaves extract. The value was, 60.03 g/dl, respectively.

In case of serum uric acid, in table (8) the serum uric acid level of control positive group was higher than control negative group. The values were 3.97 and 2.11 mg/dl, respectively. On the other hand, the maximum reduction with non-significant difference in serum uric acid value recorded with group administrated by 500 mg/kg black mulberry leaves extract. The value was 1.95 mg/dl. While, other groups fed on 2.5% & 5% black mulberry powder and 250 mg/kg black mulberry leaves extract showed high reduction with no significant difference in uric acid value by different rates. The values were 2.27, 2.60 and 2.91 g/dl, respectively.

On the other hand, serum creatinine level of control positive group was higher than control negative group. The values were 0.63 and 0.35 mg/dl, respectively. The highest reduction in serum creatinine level recorded for 500 mg/kg black mulberry leaves extract being, 0.37 mg/dl while, the lowest reduction recorded for 2.5% black mulberry leaves powder being, 0.51 mg/dl. These results are in agreement with those of (Jarald et al., 2008). They showed that diabetic rats had a significant increase in creatinine and BUN levels as compared to the normal animals. Also, Wilson and Shahidul (2015) found a significantly decreases in serum urea, uric acid and creatinine with Morus alba diets.
Table (1): Total phenol, anthocyanin and flavonoid contents of black mulberry leaves expressed as (mg/g)

<table>
<thead>
<tr>
<th>Total phenols</th>
<th>Total flavonoids</th>
<th>Anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.80±0.93</td>
<td>61.40±0.35</td>
<td>3.70±0.61</td>
</tr>
</tbody>
</table>

Means under the same column bearing different superscript letters are different significantly (p < 0.05).

Table (2): Phenolic compounds fractions of black mulberry leaves

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>mg/100g of dry extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrogallol</td>
<td>223.30</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>9.97</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>49.31</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>19.66</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>7.15</td>
</tr>
<tr>
<td>Caffeine</td>
<td>13.13</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>15.89</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>80.12</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not Detected

Table (3): Effect of different concentration of mulberry leaves on BWG, FI and FER of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Body Weight Gain (g)</th>
<th>Feed Intake (g/day)</th>
<th>Feed Efficiency Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G/28 day</td>
<td>G/28 day</td>
<td>Ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 C (-)</td>
<td></td>
<td>28³±0.40</td>
<td>504.0³±0.70</td>
<td>0.056³±0.007</td>
</tr>
<tr>
<td>G2 C (+)</td>
<td></td>
<td>8.4³±0.20</td>
<td>445.0³±0.60</td>
<td>0.019³±0.002</td>
</tr>
<tr>
<td>G3 (2.5% Mulberry leaves powder)</td>
<td></td>
<td>21³±0.80</td>
<td>498.4³±1.00</td>
<td>0.042³±0.005</td>
</tr>
<tr>
<td>G4 (5% Mulberry leaves powder)</td>
<td></td>
<td>25.2³±0.60</td>
<td>495.6³±0.20</td>
<td>0.051³±0.003</td>
</tr>
<tr>
<td>G5 (250mg/kg Mulberry leaves extract)</td>
<td></td>
<td>16.8³±0.90</td>
<td>492.0³±0.50</td>
<td>0.034³±0.004</td>
</tr>
<tr>
<td>G6 (500mg/kg Mulberry leaves extract)</td>
<td></td>
<td>36.4³±0.50</td>
<td>509.6³±0.70</td>
<td>0.071³±0.008</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>1.092</td>
<td>1.18</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Each value is presented as mean ± standard deviation (n = 6).
Means under the same column bearing different superscript letters are different significantly (p < 0.05).
Table (4): Effect of different concentration of mulberry leaves on internal organs relative weight of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Spleen (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ C (-)</td>
<td>7.50 ± 0.90</td>
<td>1.3 ± 0.70</td>
<td>1.10 ± 0.78</td>
</tr>
<tr>
<td>G₂ C (+)</td>
<td>6.10 ± 0.20</td>
<td>0.70 ± 0.15</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>G₃ (2.5% Mulberry leaves powder)</td>
<td>6.80 ± 0.50</td>
<td>0.90 ± 0.32</td>
<td>0.86 ± 0.32</td>
</tr>
<tr>
<td>G₄ (5% Mulberry leaves powder)</td>
<td>6.60 ± 0.70</td>
<td>1.15 ± 0.60</td>
<td>0.80 ± 0.44</td>
</tr>
<tr>
<td>G₅ (250mg/kg Mulberry leaves extract)</td>
<td>6.90 ± 0.40</td>
<td>1.0 ± 0.50</td>
<td>0.70 ± 0.27</td>
</tr>
<tr>
<td>G₆ (500mg/kg Mulberry leaves extract)</td>
<td>7.0 ± 0.80</td>
<td>1.5 ± 0.44</td>
<td>0.90 ± 0.58</td>
</tr>
</tbody>
</table>

LSD 1.02 0.86 0.83

Each value is presented as mean ± standard deviation (n = 6). Means under the same column bearing different superscript letters are different significantly (p < 0.05).

Table (5): Effect of different concentration of mulberry leaves on serum glucose of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ C (-)</td>
<td>108 ± 0.70</td>
</tr>
<tr>
<td>G₂ C (+)</td>
<td>230 ± 1.10</td>
</tr>
<tr>
<td>G₃ (2.5% Mulberry leaves powder)</td>
<td>111.7 ± 0.50</td>
</tr>
<tr>
<td>G₄ (5% Mulberry leaves powder)</td>
<td>115.2 ± 0.80</td>
</tr>
<tr>
<td>G₅ (250mg/kg Mulberry leaves extract)</td>
<td>124.5 ± 0.90</td>
</tr>
<tr>
<td>G₆ (500mg/kg Mulberry leaves extract)</td>
<td>109.1 ± 0.40</td>
</tr>
</tbody>
</table>

LSD 1.37

Each value is presented as mean ± standard deviation (n = 6). Means under the same column bearing different superscript letters are different significantly (p < 0.05).
Table (6): Effect of different concentration of mulberry leaves on (ALP), (GOT) and (GPT) of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>(ALP) U/L</th>
<th>(GOT) U/L</th>
<th>(GPT) U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 C (-)</td>
<td>95± 1.70</td>
<td>9.22± 1.10</td>
<td>6.50± 0.80</td>
</tr>
<tr>
<td>G2 C (+)</td>
<td>197± 0.90</td>
<td>55.82± 1.35</td>
<td>20.70± 0.40</td>
</tr>
<tr>
<td>G3 (2.5% Mulberry leaves powder)</td>
<td>135b± 2.10</td>
<td>39.4b± 2.05</td>
<td>9.20± 1.20</td>
</tr>
<tr>
<td>G4 (5% Mulberry leaves powder )</td>
<td>105d± 1.10</td>
<td>31.0± 0.60</td>
<td>10.93± 0.90</td>
</tr>
<tr>
<td>G5 (250mg/kg Mulberry leaves extract)</td>
<td>123d± 0.50</td>
<td>27.15d± 1.25</td>
<td>8.81± 0.50</td>
</tr>
<tr>
<td>G6 (500mg/kg Mulberry leaves extract)</td>
<td>90f± 0.80</td>
<td>17.21f± 0.90</td>
<td>6.0f± 0.60</td>
</tr>
<tr>
<td>LSD</td>
<td>2.32</td>
<td>2.29</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Means under the same column bearing different superscript letters are different significantly (p < 0.05).

Table (7): Effect of different concentration of mulberry leaves on Serum T.G, TC, VLDL-C, HDL-C and LDL-C of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>(TG) mg/dl</th>
<th>(TC) mg/dl</th>
<th>(VLDL-C) (mg/dl)</th>
<th>(HDL-C) (mg/dl)</th>
<th>(LDL-C) (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 C (-)</td>
<td>55.81± 0.52</td>
<td>94.00± 0.70</td>
<td>11.16± 0.69</td>
<td>43.05± 2.80</td>
<td>39.79± 0.93</td>
</tr>
<tr>
<td>G2 C (+)</td>
<td>135.15± 3.81</td>
<td>140.00± 1.10</td>
<td>27.03± 1.20</td>
<td>27.67± 1.71</td>
<td>85.30± 1.58</td>
</tr>
<tr>
<td>G3 (2.5% Mulberry leaves powder)</td>
<td>60.42± 0.70</td>
<td>107.00± 1.10</td>
<td>15.67± 1.22</td>
<td>40.46± 1.38</td>
<td>54.46± 1.91</td>
</tr>
<tr>
<td>G4 (5% Mulberry leaves powder )</td>
<td>78.33± 1.10</td>
<td>128.00± 0.50</td>
<td>12.08± 0.60</td>
<td>40.46± 1.38</td>
<td>74.72± 0.93</td>
</tr>
<tr>
<td>G5 (250mg/kg Mulberry leaves extract)</td>
<td>70.14± 2.15</td>
<td>130.00± 0.30</td>
<td>14.03± 0.90</td>
<td>39.94± 0.90</td>
<td>76.03± 2.41</td>
</tr>
<tr>
<td>G6 (500mg/kg Mulberry leaves extract)</td>
<td>57.63± 2.66</td>
<td>103.00± 0.80</td>
<td>11.53± 2.20</td>
<td>45.51± 1.9</td>
<td>45.96± 2.15</td>
</tr>
<tr>
<td>LSD</td>
<td>3.67</td>
<td>1.27</td>
<td>2.63</td>
<td>3.02</td>
<td>3.10</td>
</tr>
</tbody>
</table>

TG= triglyceride  TC= total cholesterol  VLDL-C = very low density lipoprotein  HDL = High density lipoprotein  LDL = Low density lipoprotein  
Each value is presented as mean ± standard deviation (n = 6).  
Means under the same column bearing different superscript letters are different significantly (p < 0.05).
Table (8): Effect of different concentration of mulberry leaves on serum urea, serum uric acid and creatinine of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 C (-)</td>
<td>42.20± 2.10</td>
<td>2.11b± 0.20</td>
<td>0.35d± 0.10</td>
</tr>
<tr>
<td>G2 C (+)</td>
<td>73.65a±3.20</td>
<td>3.97a± 0.90</td>
<td>0.63a± 0.40</td>
</tr>
<tr>
<td>G3 (2.5% Mulberry leaves powder)</td>
<td>50.96a±1.60</td>
<td>2.27b± 0.60</td>
<td>0.51b± 0.20</td>
</tr>
<tr>
<td>G4 (5% Mulberry leaves powder)</td>
<td>58.27b± 0.90</td>
<td>2.60b± 0.30</td>
<td>0.43c± 0.05</td>
</tr>
<tr>
<td>G5 (250mg/kg Mulberry leaves extract)</td>
<td>60.03b± 1.30</td>
<td>2.91ab± 0.70</td>
<td>0.40c± 0.30</td>
</tr>
<tr>
<td>G6 (500mg/kg Mulberry leaves extract)</td>
<td>46.25d± 0.50</td>
<td>1.95b± 1.10</td>
<td>0.37d± 0.10</td>
</tr>
<tr>
<td>LSD</td>
<td>3.24</td>
<td>1.26</td>
<td>2.13</td>
</tr>
</tbody>
</table>

Each value is presented as mean ± standard deviation (n = 6). Means under the same column bearing different superscript letters are different significantly (p < 0.05).

REFERENCES:


التأثير المضاد للسكر لأوراق التوابل الأسيد في الفئران المصببة بسكر بتأثير الأستيربتيزول

سمى عزيز خضر

قسم التغذية وعلوم الأطعمة كلية الاقتصاد المنزلي - جامعة المنوفية

الملخص العربي

تم تقييم تأثير مستويات مختلفة من أوراق التوت الأسود (2.5 % تي) على شكل مسحوق و 500 ملجم / كجم في صورة مستخلص. كما تم تقييم المستخلص على الفئران المصاببة بداءة السكري باستخدام 36 فار، والتي قسمت إلى 3 مجموعات. أيضا، تم تقييم المراعيات الفيئنولية. وظهرت النتائج أن فئون الفئران المصاببة بالكلية والألوان ذات الأوراق الأسود كانت 13.81 ± 3.30% و 12.94 ± 3.16% للفئران التي سُلبت الأوراق والثائرة. مع ذلك، كان هناك تأثير ملحوظ على الوزن الإجمالي للجسم. ومع ذلك، فإن النتائج تشير إلى أن مستخلص أوراق التوت الأسود يمكن استخدامه كمصدر طبيعي لعلاج السكري البديل والسكري النوع الثاني.

الكلمات الدالة: أوراق التوت الأسود - الفئران المصاببة بالسمنة - التحاليل الكيميائية الحيوية - المركبات الفعالة