European Journal of Chemistry

Hypoglycemic effect of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits in diabetic rat

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ARTICLE INFORMATION

ABSTRACT

The aim of the present study was to investigate the hypoglycemic effect of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits either used individually or in a combination on alloxan diabetic rats. These fruits are reported to be rich in antioxidants, flavonoids and phenolics that can potentially fight against diabetes mellitus. Male albino rats were divided into 5 groups: normal control, alloxan-diabetic control, diabetic rats treated with white mulberry fruit powder at 5% in the diet, diabetic rats treated with black mulberry fruit powder at 5% in the diet and diabetic rats treated with mixture of white and black mulberry fruits powder at 5% in the diet. After 4 weeks of treatment, blood glucose level, liver and kidney enzymes activity, lipid profile, lipid peroxidation and histopathological studies on liver, kidney and pancreas were evaluated. The mixture of white and black mulberry fruits showed the most significant ($p < 0.05$) improvement in feed efficiency ratio with increasing body weight gain, as well as decrease in blood glucose level and liver-kidney dysfunction when compared with diabetic control rats. Significant decrease in serum cholesterol, triglycerides and low density lipoprotein cholesterol (LDLc) as well as significant increase in high density lipoprotein cholesterol (HDLc) in diabetic rats was observed with all treatments. Moreover, mulberry fruits administration caused significant inhibition in lipid peroxidation and α-amylase activity. In addition, the beneficial effect of all treatments was further confirmed with histopathological examination of liver, kidney and pancreas. This study reveals hypoglycemic and hypolipidemic effects of white and black mulberry fruits either used individually or in combination as a dietary supplement in alloxan diabetic rats.

1. Introduction

Diabetes mellitus (DM) is a worldwide disease affecting more than 347 million people [1]. In Egypt, it is estimated that 9 million people will become diabetic by the year 2025 [2]. DM refers to a disorder of metabolism of fat, carbohydrate and protein [3]. DM occurs due to lack of insulin secretion or its action on cells or both that leads to high blood glucose level [4]. Alloxan is widely used to induce diabetes mellitus in experimental animal models suggesting a close relation between hyperglycemia and diabetic complications and allowing investigation of hypoglycemic agents for the treatment of diabetes [5,6]. Moreover, DM is associated with increase production of reactive oxygen species and reduction of antioxidant defense system leading to oxidative stress, which plays an important role in tissue damage [5,7].

Nutrition is the major determinant for controlling DM, avoiding complications, successful aging and improving the quality of life [8]. Fruits and vegetables are the simplest form of healthful or functional foods [9]. Their physiologically active constituents are attenuate adverse effects associated with chronic diseases such as DM, cancer and cardiovascular Disease (CVD) [10,11]. Searching for new antidiabetic medicaments

from natural sources is still attractive as most of these plant materials contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids and other bioactive chemical components that are frequently implicated as having antidiabetic effect [12,13].

White (*Morus alba* L.) and black (*Morus nigra* L.) mulberry belong to the family Moraceae. Mulberry fruits are widely known as a nutritious food, which can be eaten fresh, used in wine, juice and jam production or can be canned [14,15]. These berries are not only used as fruits, but also have been used effectively as natural medicines to cure sore throat, fever, hypertension and anemia [16,17]. Moreover, white mulberry fruits widely distributed in Egyptian agriculture are used to protect from liver and kidney damage, strengthen the joints, improve eyesight, and have anti-aging and radio protective effects [15,18,19]. In addition, 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 [20,21]. The black mulberry fruit (*M. nigra)* is reported to possess antioxidant effect due to presence of phenolic compounds and anthocyanins [22]. Furthermore the anti-oxidant activity of three different extracts of *M. nigra* fruits on haemoglobin glycosylation, peroxidative damage to human

erythrocytes, liver hepatocytes of rats and human low-density lipoprotein (LDL) showed that black mulberry fruits inhibit haemoglobin glycosylation induced by glucose to differing degrees [23]. Hansawasdi and Kawabata [24] reported that flavonoid extracted from *M. indica* has an antidiabetic activity in experimental animals. However, few reports have evaluated the antidiabetic and hypoglycemic effects of whole mulberry fruits as a dietary supplement in animal models. Thus, the present study aims to evaluate the effects of dietary intake of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits either used individually or in combination on some biochemical and histopathological parameters in alloxan-induced diabetic rats.

2. Experimental

2.1. *Materials*

White and black mulberry fruits were purchased from the Egyptian local market. The fresh berries were dried at 70 °C for 4 days and were grounded to measure their potential value and biological effects [25]. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Determination of total phenolics, total flavonoid content and total antioxidant activity in mulberry fruits

A 60 g portion of white and black mulberry fruits powder was extracted with 100 mL of methanol at room temperature for 5 h. After filtration, the filtrate was washed with 50 ml of methanol and the residue was extracted again. The extract was collected, and combined to determine the total antioxidant activity, total phenolics and total flavonoid content.

2.2.1.1. Determination of total phenolics content

The concentration of total phenolics content in mulberry fruits was measured by Follin-Ciocalteu colorimetric method [26]. Briefly, 0.5 mL of the methanolic extract solution (5.0 mg/mL) of mulberry fruits was mixed with 2.5 mL 10% Folin-Cioalteu's reagent (*v:v*) and 2.0 mL of 7.5% Na_2CO_3 was subsequently added. The reaction mixture was incubated at 45 °C for 40 min, and the absorbance was measured at 765 nm against blank using spectrophotometer. Gallic acid was chosen as a standard, and the results were expressed as milligram of Gallic Acid Equivalents (GAE) per 100 g fresh matter of fruit (mg GAE/100 g fruit).

2.2.1.2. Determination of total favonoid content

The total flavonoid content of mulberry fruits was determined using the aluminum chloride colorimetric method described by Chang *et al.* [27]. Briefly, 0.5 mL of the methanolic extract solution (1.0 mg/mL) of mulberry fruits was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against blank. Quercetin was chosen as a standard. Using a seven point standard curve (0-50 mg/L), the levels of total flavonoid contents in mulberry fruits were determined. The data were expressed as milligram of qurcitin equivalents (QE) per 100 g fresh fruit (mg QE/100 g fruit).

2.2.1.3. Determination of total antioxidant activity

Total antioxidant activity in white and black mulberry fruits was determined following the method described by Jakobek *et al.* [28]. Briefly, 0.1 mL of methanolic solution containing 0.04 to 0.20 mg of extract was mixed with 2 mL of distilled water and then 0.25 mL of 1 mM methanolic solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was added. The mixture was vortexed thoroughly for 1 min. Finally, the absorbance of the mixture was measured at 517 nm after standing at ambient temperature for 30 min.

2.2.2. Animals and Experimental design

Thirty male Sprague-Dawley rats, weighing 160-200 g were purchased from Agricultural Research Center, Giza, Egypt. Upon arrival, the animals were given two weeks acclimation period, during which they were fed *ad libitum* a standard rat chow diet, with alternated 12 h dark/light cycle, and the ambient temperature was held between 21-25 °C. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by the University Ethical Committee.

In the current study diabetes was induced experimentally in fasted rats by intra-peritoneal injection of a single dose of 150 mg/kg BW alloxan monohydrate dissolved in distilled water [29]. Animals were given 10% glucose solution to drink instead of tap water for three days until sustained hyperglycemia was established. The diabetic state was assessed by measuring the fasting plasma glucose concentration 72 h after alloxan treatment. The rats with a fasting plasma glucose level above 200 mg/dl were selected for the experiment and considered as diabetics [30]. Treatment was started after 3 days of induction of alloxan and continued for 4 weeks.

Animals were divided into the following groups (6 rats each):

Group I: Normal control group was fed standard rat chow diet (NC).

Group II: Diabetic control group injected with alloxan and fed standard rat chow diet (DC).

Group III: Diabetic rats received daily powdered white mulberry fruit at 5% level [31] mixed with the chow diet (D+WMF).

Group IV: Diabetic rats received daily powdered black mulberry fruit at 5% level mixed with the chow diet (D+BMF).

Group V: Diabetic rats received daily powdered of equal amounts of both white and black mulberry fruits at 5% level mixed with the chow diet (D+W and BMF).

2.2.3. Blood Sampling

Four weeks after mulberry fruits administration, food was withdrawn for 12 hours. The fasting animals were sacrificed and blood samples were collected into clean centrifuge tubes. The blood samples were allowed to coagulate and were centrifuged at 3000 rpm for 20 minutes to separate the blood serum. Separated serum was stored at -20 °C for subsequent biochemical analyses. Food consumption was monitored daily and body weight was determined once a week.

2.2.4. Biochemical Analysis

Fasting blood glucose was measured enzymatically and colorimetrically in serum immediately [32]. Triglycerides TG, total Cholesterol TC and High density lipoprotein cholesterol HDLc were colorimetrically determined in rat serum using enzymatic methods [33-35]. Low density lipoprotein cholesterol LDLc was calculated by using formula given by Friedewald *et al.* [36] (mg/dl) as follows;

$$LDLc = TC - HDLc - (TG/5) \tag{1}$$

Liver function was determined by measuring the activities as aspartate aminotransferase (AST) and alanine amino-transferase (ALT) in serum using colorimetric method [37]. Blood urea nitrogen (BUN) was determined according to the methods of Fawcett [38].

Table 1. Total phenolic, total flavonoid content and total antioxidant activity of white and black mulberry fruits.

Species	Total phenolics (mg GAE/ 100 g fresh mass)	Total flavonoids (mg QE/100 g fresh mass)	Total antioxidant activity (µg/mL)
Morus alba	983	129	237
Morus nigra	1366	256	178

Table 2. Effects of white and black mulberry fruits on body weight and food efficiency ratio (FER) [a].

Group	Initial weight (g)	Final weight (g)	FER (%)
Group I: NC	180.2±3.42	208.4±6.07	4.96±0.22
Group II: DC	174.0±3.19	148.2±4.41 [b]	-4.56±0.43 [b]
Group III: D+WMF	175.6±3.39	182.2±5.27 [b,c]	2.18±0.18 [b,c]
Group IV: D+BMF	173.4±4.09	186.8 ±4.09 [b,c]	2.33±0.29 [b,c]
Group V: D+W and BMF	181.4±2.90	198.6±5.38 [c]	2.78±0.15 [b,c]

[a] The values are mean±SD of 6 rats in each group.
[b] Significantly different from normal control group at $p < 0.05$.
[c] Significantly different from diabetic control group at $p < 0.05$.

The determination of serum creatinine was performed according to Larsen [39]. Plasma lipid peroxide (Malondialdehyde) was determined according to Ohkawa *et al.* [40]. The activity of α-amylase was determined according to Rinderknecht *et al.* [41]. All the kits used in biochemical analysis were purchased from Biodiagnostic, Egypt.

2.2.5. Histopathological examination

Autopsy samples from liver, kidney and pancreas were taken from the rats in different groups. Then, samples were fixed in 10% formol saline solution for twenty four hours. Washing was done in tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope [42].

2.2.6. Statistical analysis

Means of results were calculated among 6 replicates, with their Standard Deviations (SD) for each group. Analysis of variance was used to make statistical comparisons (ANOVA) with Dunnett's post hoc test. SPSS computer program [43] was used to calculate the significance between groups at the same experiment at 5% probabilities.

3. Results and discussion

3.1. Total phenolics, total flavonoid content and total antioxidant activity of mulberry fruits

Control hyperglycaemia is highly valuable, since botanicals are spending time and efforts to discover effective edible polyphenols and flavonoids. The current research is a trial in many to make benefit for diabetic patients. The amounts of total phenolic compounds, total flavonoids and total antioxidant activity of white and black mulberry fruits were shown in Table 1. The results have demonstrated that mulberry fruits are a rich source of phenolics, especially for *M. nigra* (1366 mg GAE/100 g), which showed higher value than *M. alba* (983 mg GAE/100 g). In the same trend, *M. nigra* fruit has high total flavonoid values (256 mg QE/100 g fresh mass) than *M. alba* fruit (129 mg QE/100 g fresh mass). Unexpected data proved that *M. alba* fruit has high levels of total antioxidant activity (237 µg/mL) than *M. nigra* fruit (178 µg/mL). Our results were in agreement with Kaewkaen *et al.* [25] and Butkhup *et al.* [44]. Ercisli and Orhan [45] found that the highest total phenolic and flavonoid contents were observed in black mulberry fruit when compared with the fruit of the white

mulberry. The variation of phenolic compounds, total flavonoids and antioxidant capacity in mulberry fruits depends on many factors, such as degree of maturity at harvest, genetic differences, and environmental conditions during fruit development [46]. Many reports revealed that the physiological function of natural food could be related to the anti-oxidative capacity of its phenolic components.

3.2. Changes in body weight and food consumption

The final body weight showed significant increase from the initial body weight in all the groups except in the diabetic group, in which there was significant decrease in final body weight compared with the initial one (Table 2). The failure of diabetic rats to gain weight during the 4-week period corresponded with the hyperglycemia seen during this period. Rats of treated mulberry groups (III, IV and V) showed higher gain in weight as compared with those in the diabetic control group but less than those in the normal control group. In addition, significant increase revealed in food efficiency ratio (FER) for all groups (diabetic rats) fed on white and black mulberry as well as its mixture comparing to the diabetic control group. Our findings are, in fact, in a good agreement with that for Andallu and Varadacharyulu [47]. Another theory stated that alloxan decreases body weight due to depressed synthesis of DNA and RNA in the diabetic animals [48]. In the same while, increasing food consumption and decreasing body weight noticed in diabetic control rats are remarks for the polyphagic condition. Also, the loss of weight is probable due to the excessive break down of tissue proteins [49]. Mulberry fruit administration decreases food consumption and improves body weight in the same direction. Obviously, that is indicating control over the polyphagia and muscle wasting due to the hyperglycemic condition.

3.3. Changes in fasting blood glucose

Table 3 shows the changes in fasting blood glucose level over 4 weeks. Normal control rats did not show any significant variation in the blood glucose throughout the experimental period. Intra-peritoneal injection of alloxan led to over 3-fold elevation of blood glucose levels in all diabetic rats. Individual treatment of either *M. alba* or *M. nigra* fruits decreased the hyperglycemia significantly ($p < 0.05$) by 47 and 45%, respectively, as compared to the diabetic control group, although no one of the mulberries alone failed to restore the level of blood glucose to that of the normal control. On the other hand, the mixture of *M. alba* and *M. nigra* fruits decreased the diabetic blood glucose levels to almost catch the normal control level. The obtained data are in a parallel with that of Singab *et al.* [50] and Ma *et al.* [51]. Grover *et al.* [52] stated that mulberry exhibited a potent hypoglycemic activity in fasted and non-fasted streptozotocin diabetic mice.

Table 3. Effects of white and black mulberry fruits on fasting blood glucose level [a].

Group	Fasting blood glucose level (mg/dl)			
	At the time of grouping	Days of white and black mulberry or its mixture supplement		
		0	14	28
NC	87.6±2.15	90.8±2.77	87.4±3.06	89.4 ±2.11
DC	84.9±2.69	245.4±7.75 [b]	242.6 ±8.88 [b]	244.7 ±7.28 [b]
D+WMF	88.2±3.19	239.2±6.61 [b]	187.4±6.30 [b,c]	129.4±4.70 [b,c]
D+BMF	87.0±1.53	238.7±8.61 [b]	189.6±5.96 [b,c]	133.6±5.86 [b,c]
D+W and BMF	85.4±2.99	246.5±5.95 [b]	169.3 ±5.58 [b,c]	101.3±2.98 [c]

[a] The values are mean±SD of 6 rats in each group.
[b] Significantly different from normal control group at $p < 0.05$.
[c] Significantly different from diabetic control group at $p < 0.05$.

Table 4. Effects of white and black mulberry fruits on serum AST, ALT, BUN and creatinine level [a].

Group	Liver dysfunction indices		Kidney toxicity indices	
	AST (IU/dl)	ALT (IU/dl)	BUN (mg/dl)	Creatinine (mg/dl)
NC	19.60±0.84	9.50±0.27	36.04±1.06	1.08 ±0.06
DC	35.77±1.12 [b]	33.75±0.75 [b]	62.06±2.28 [b]	1.82±0.08 [b]
D+WMF	31.00±1.09 [b]	27.25±1.11 [b,c]	56.04±1.30 [b,c]	1.59±0.06 [b,c]
D+BMF	30.97±1.18 [b]	26.75±1.07 [b,c]	57.56±1.76 [b,c]	1.63±0.03 [b,c]
D+W and BMF	21.17±0.78 [c]	13.50±0.95 [c]	39.13±1.18 [c]	1.17 ±0.06 [c]

[a] The values are mean±SD of 6 rats in each group.
[b] Significantly different from normal control group at $p < 0.05$.
[c] Significantly different from diabetic control group at $p < 0.05$.

Table 5. Effects of white and black mulberry fruits on serum TG, TC, HDLc and LDLc level [a].

Group	TG (mg/dl)	TC (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)
NC	42.26±1.45	97.64±3.09	60.16±2.05	29.02±0.93
DC	66.42±2.03 [b]	131.57±4.27 [b]	32.13±1.29 [b]	86.49±2.18 [b]
D+WMF	49.94±1.18 [b,c]	110.91±4.05 [b,c]	41.98±1.34 [b,c]	59.60±1.88 [b,c]
D+BMF	51.30±1.23 [b,c]	112.78±4.89 [b,c]	49.13±1.52 [b,c]	52.02±1.93 [b,c]
D+W and BMF	45.26±1.45 [c]	103.90±3.01 [c]	53.89±1.68 [c]	41.09±1.03

[a] The values are mean±SD of 6 rats in each group.
[b] Significantly different from normal control group at $p < 0.05$.
[c] Significantly different from diabetic control group at $p < 0.05$.

Fasting blood glucose concentrations at the initial and final stages confirm uncontrolled hyperglycemia in untreated diabetic rats, whereas white and black mulberry fruits treatment remarkably decreased blood glucose concentrations in diabetic rats. The effect of white and black mulberry fruits in controlling hyperglycemia could be explained as follow: (I) the N-containing pseudo-sugar isolated form mulberry fruits which inhibit the functions of α-glucosidase, α-mannosidase and β-galactosidase, (II) fagomine which strengthens the glucose induced insulin secretion similar to the action of glibenclamide, a sulfonylurea drug which stimulates the secretion of endogenous insulin, and (III) increase in tissue uptake of glucose by both white and black mulberry fruits [53].

3.4. Changes in serum liver-kidney dysfunction indices

The efficacy of white and black mulberry fruit as well as its mixture on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine is shown in Table 4. These parameters were significantly (p < 0.05) altered in alloxan induced diabetes comparing to normal control rats. In diabetic rats, administration of either white or black mulberry fruits individually led to non-significant (p < 0.05) decrease in AST, ALT, BUN and creatinine level comparing to diabetic control rats. On the other hand, administration a mixture of white and black mulberry fruits significantly (p < 0.05) reduced AST, ALT, BUN and creatinine level by 40, 60, 37 and 36%, respectively, compared with the diabetic control rats. In the current study, increased the liver function activities of AST and ALT were observed in the diabetic animals. These results are in agreement with the results published by Zhang *et al.* [54]. It has already been demonstrated that tissue antioxidant status is an important factor in the development of diabetic complications [55]. The increase in the level of these enzymes in diabetes may be as a result of its leakage from the tissues and migration into the bloodstream as a result of alloxan acute toxicity which leads to the liver damage [56]. Furthermore, treatment with a mixture of white and black mulberry fruits produce a marked significant decrease of the elevated AST and ALT activities. Chaurasia *et al.* [57] attributed this decrease to the good hepato-protective and antioxidant activity of mulberry fruits which due to the presence of a number of constituents, such as flavonoids, anthocyanins, carotenoids and tannins. Since antioxidants are known to reduce the development of chemically induced liver damage [58]. In the same trend, Jarald *et al.* [59] showed that diabetic rats had a significant increase in creatinine and BUN levels as compared to the normal animals. Kidney dysfunctions in the diabetic rats may be related to the generation of reactive oxygen species and lipid peroxidation which are associated with tissue injury. The diabetic rats had higher values of plasma BUN than control rats [60]. In addition, Shah *et al.* [61] reported that increased oxidative stress and reduce antioxidative ability in diabetes results in renal tubular injury, proteinuria and leads to gradual loss of renal function.

3.5. Changes in serum TG, TC, HDLc and LDLc level

Data in Table 5 revealed that diabetic control group showed a significant (p < 0.05) increase in the values of TG, TC and LDLc and decrease in HDLc when compared with normal control. Either white or black mulberry treated groups (III and IV) showed a significant decrease in TC, TG and LDLc and a significant increase in HDLc compared with diabetic control. The best reduction in lipids profile was recorded for the combination between white and black mulberry supplement; the levels of triglycerides, total cholesterol, and LDLc were decreased by 31.8, 22.9, and 52.3%, respectively, compared with diabetic control. On the other hand, administration of a mixture of white and black mulberry fruits caused a significant increase (p < 0.05) in the HDLc level by 40.3% when compared with diabetic control.

Table 6. Effects of white and black mulberry fruits on plasma α-amalyse activity and lipid peroxide (MDA) [a].

Group	Plasma α-amalyse activity (U/L)	MDA (nmol/mL)
NC	51.42±1.80	3.21±0.13
DC	206.22±8.30 [b]	6.46±0.22 [b]
D+WMF	97.78±3.19 [b,c]	4.58±0.18 [b,c]
D+BMF	90.44±3.09 [b,c]	4.70±0.15 [b,c]
D+W and BMF	63.22±1.90 [c]	3.93±0.19 [c]

[a] The values are mean±SD of 6 rats in each group.
[b] Significantly different from normal control group at $p < 0.05$.
[c] Significantly different from diabetic control group at $p < 0.05$.

Supporting to our findings, Andallu and Varadacharyulu [47] found 16% decrease in triglycerides in type 2 diabetic patients after treatment with mulberry powder-filled capsules. They also reported that cholesterol, LDL cholesterol, and vLDL cholesterol were reduced by 12, 23, and 17%, respectively, in type 2 diabetic patients after treatment with mulberry powder. Diabetes mellitus is also strictly related to other metabolic disorders, in which the most important one is lipid abnormalities, characterized mainly by high triglyceride, cholesterol, LDL-cholesterol and low HDL-cholesterol levels [62], which were also observed in our alloxan-induced diabetic control rats. Increased levels of triglycerides are a risk factor for atherosclerotic coronary disease. LDL and vLDL carry cholesterol to the peripheral tissues where it is deposited; hence, high levels of LDL and vLDL are atherogenic. HDL transports cholesterol from peripheral tissues to the liver and thus aids in its excretion (protective effect). Consumption of plant material like mulberry fruits, containing antioxidants, carotenoids, polyphenol and phytonutrients increases the antioxidant status in blood and tissues, as these compounds are capable of modulating LDL oxidation through several mechanisms [63]. At the same time, mulberry fruit might also be influencing lipoprotein associated cholesterol fractions and probably the phytocomponents exert action similar to the drugs lovastatin and simvastatin that are used for controlling the imbalance in plasma lipoprotein in diabetes.

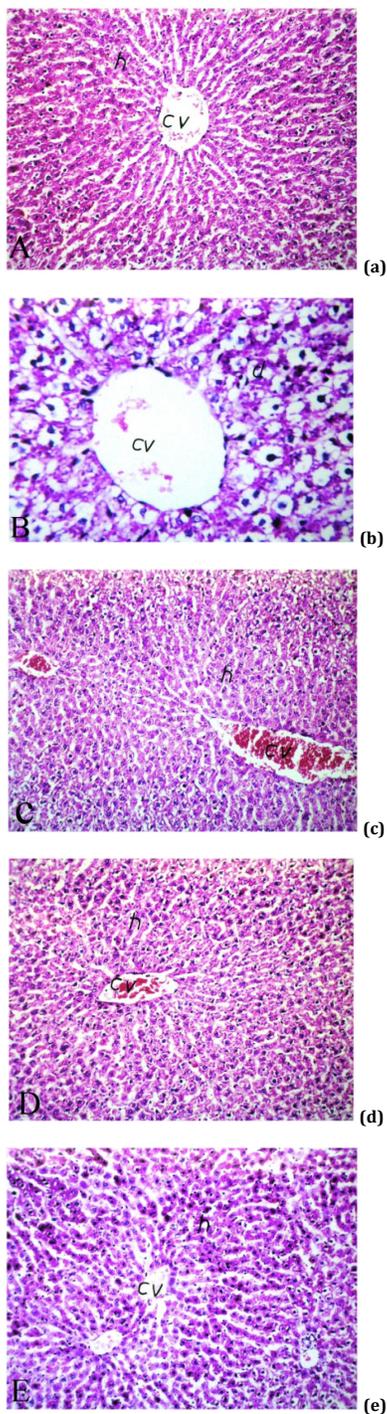### 3.6. Changes in α-amylase activity and lipid peroxidation (MDA)

Compared the normal control group with its counterpart the diabetic control group, a significant increase in terms of plasma α-amylase activity reached up to 301% (Table 6). The findings of the present study showed that the administration of white and black mulberry fruits or its combination to surviving diabetic rats significantly reduced α-amylase activity, which plays a key role in the digestion of carbohydrates. A significant increase in plasma lipid peroxide (MDA) levels (101%) was observed in the diabetic control group compared to the normal control. Diet supplemented with white and black mulberry fruits either individually or in combination induced a significant decrease of MDA levels in plasma compared to the diabetic control group (Table 6). α-Amylase is the main enzyme in humans responsible for the breakdown of starch into simple sugars (dextrin, maltotriose, maltose and glucose). α-amylase is well known enzyme in the management of hyperglycemia linked to type 2 diabetes. α-amylase inhibitors have been thought to improve glucose tolerance in diabetic patients [64]. In the current study, administration of white and black mulberry or its combination to diabetic rats significantly reduced α-amylase activity. This was indicative of lowered levels of absorbable glucose being formed from the digestion of carbohydrate and leading to reduced levels of blood glucose. The inhibition of α-amylase activity in the human digestive tract represents one of the therapeutic approaches commonly used for the control and prevention of postprandial hyperglycemia in non-insulin-dependent diabetic patients through reducing the uptake of glucose released by those enzymes from starch [65]. Oxidation remark in our experiment was parallel to the data of Ma *et al.* [48] which reported that *M.*

alba flavonoids reduced the hepatic MDA level in model group compared with streptozotocin-diabetic rats. Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species [66,67]. It was reported that diabetics are highly sensitive to oxidative stress [68]. In alloxan-diabetic animals, the alloxan generates nitric oxide, which is a powerful free radical oxidant results in an increase in serum level of lipid peroxides due to oxidation of cells [48]. In our experiment, administration of *M. alba* or *M. nigra* suppresses the elevation of lipid peroxides in alloxan-diabetic rats compared with diabetic control rats. The data included in this work suggested that mulberry fruits prevents cellular damage induced by alloxan via inhibition of lipid peroxidation possibly through antioxidant mechanisms due to its high flavonoids content, which is in accordance with the reported data on this kind of compounds [69], as well as it preserves the capability of insulin secretion. Also, this finding is in agreement with those of Coskun *et al.* [70], who reported that a natural antioxidant quercetin has a protective effect in diabetes by decreasing the oxidative stress and preservation of pancreatic cell integrity.

3.7. Histopathological investigations

The normal liver tissue showed no histopathological finding with normal histological structure of the central vein and surrounding hepatocytes, where the liver tissue of diabetic rat showed ballooning degeneration in hepatocytes associated with dilation in the central vein (Figure 1A and B). The white mulberry fruit administration brought back the cellular arrangement around the central vein and only mild congestion was noticed in the central veins (Figure 1C). Treating diabetic rats with black mulberry or with the mixture of white and black mulberry did not show any significant change of liver histology (Figure 1D and E). At the same time, kidney sections of alloxan-diabetic rat showed congestion in the glomerular tuft associated with degeneration in the tubular lining epithelial cells of the cortex (Figure 2B) comparing with normal histological structure of the glomeruli and tubules at the cortex in the kidney of normal control rat (Figure 2A). Kidney of white mulberry fruit treated diabetic rat showed swelling degeneration in the lining epithelial cells of the cortical tubules (Figure 2C). Microscopic examination of the kidney of the treated diabetic rats with black mulberry fruit showed degeneration in the lining tubular epithelial cells in the cortical portion (Figure 2D), while an improvement in kidney structure of diabetic rat administrated with mixture of white and black mulberry fruits occurred and only mild congestion was noticed in the cortical blood vessels (Figure 2E). In general, berries are able to facilitate a very strong drug for diabetic rats as has been shown from the physiological histogram sections in different organs. Liver section results run parallel to the study of Ma *et al.* [51] which indicated that rats treated with 100 or 200 mg/kg mulberry flavonoid showed considerably lower hepatic lipid accumulation and more liver injury recovery than those of the untreated diabetic rats.
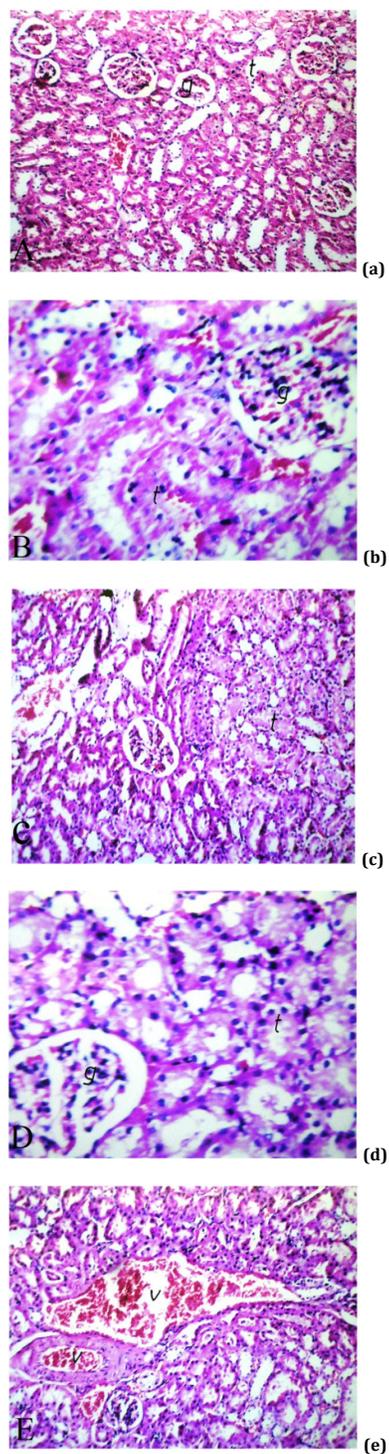
The light microscropic examination by specific staining of pancreas in normal tissues section shows normal histological structure of the island of Langerhans cells as endocrine and the acini as excocrine (Figure 3A).

antioxidant activity with improvements for histopathological organ sections. At the same time, this study correlate with the reports of Mohammadi and Naik [71], who stated that the histopathologic studies undertaken on the islets demonstrated the recovery of damaged islets and an improvement in the number of B cells after treatment with *M. alba* extract.

Figure 1. Liver histological structure of rats in normal control group (A), untreated diabetic control group (B), diabetic rats treated with white mulberry fruits (C), black mulberry fruits (D) and mixture of white and black mulberry fruits (E) (НХ+ЕЧ164). [CV; central vein - h; surrounding hepatocytes – d; degenerated hepatocytes].

The alloxan diabetic rats showed atrophy in the island of Langerhans cells, associated with focal haemorrhage in between the acini and lobules (Figure 3B). However, treatment of alloxan-diabetic rats with either white and black mulberry or its mixture made recovery in the pancreas structure and only atrophy was noticed in the island of langerhans cells (Figure 3C, D and E). In the histogram sections for berries, there was a good correlation between potent valuable compounds and

Figure 2. Histological examinations of kidney tissues of rats in normal control group (A), untreated diabetic control group (B), diabetic rats treated with white mulberry fruits group (C), black mulberry fruits group (D) and mixture of white and black mulberry fruits group (E) (НХ+ЕЧ160). [g; glomeruli - t; surrounding cortex tubules - v; cortical blood vessels congestion].
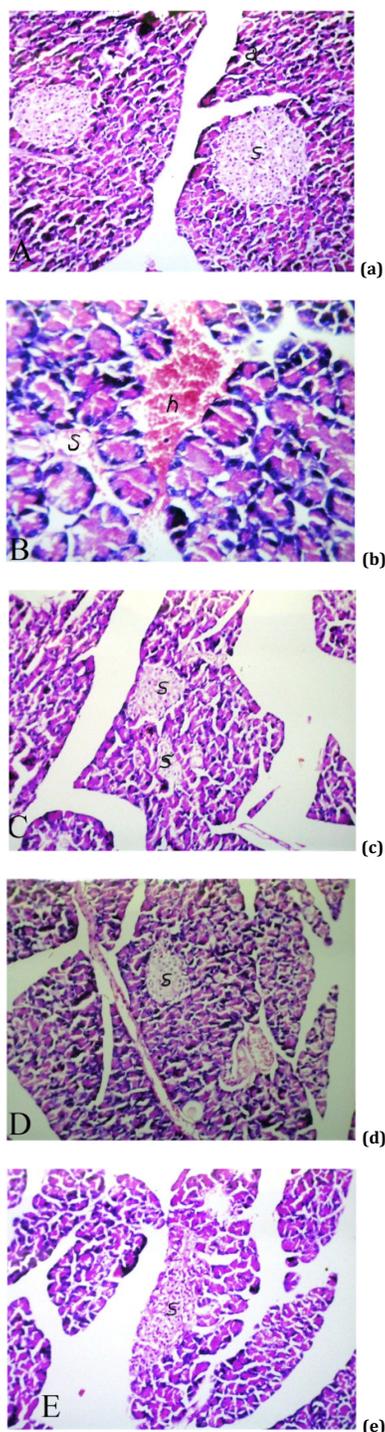
Figure 3. A photomicrograph of the pancreas of rats in normal control group (A), untreated diabetic control group (B), diabetic rats treated with white mulberry fruits group (C), black mulberry fruits group (D) and mixture of white and black mulberry fruits group (E) (HX+EЧ160). [s; endocrine Langerhans cells - a; exocrine acini - h; focal haemorrhage].

The regenerative effect of the pancreatic cells by white and black mulberry fruits or its mixture via exocrine cells of pancreas may enlighten the positive effects of these fruits on the production of insulin.

4. Conclusion

In conclusion, our study revealed that white and black mulberry fruits either used individually or in combination have pronounced antidiabetic and hypoglycemic effects in rats. The efficacy of these fruits was expressed by lowered blood glucose level, improvement of lipid profile and inhibition of lipid peroxidation and α-amylase activity. In addition, our results were further confirmed with histopathological examination of liver, kidney and pancreas showed normal histoarchitecture. In general a combination of the white and black mulberry fruits is more effective than individual supplement in the treatment of diabetic rats for synergistic and ameliorating effects attributed to the high content of phenolics and flavonoids that possess antioxidant activity.

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