BIOCHEMICAL STUDIES ON MULBERRY FRUITS AND THEIR METHANOLIC EXTRACT .

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ABSTRACT

In this study, total polyphenol ,flavonoid , anthocyanin and ascorbic acid contents were determined in black (Morus nigra L.) and white (Morus alba L) mulberry fruits grown in Egypt . Antioxidant activity , reducing power and antidiabetic effect were determined in fruits methanolic extract . The total polyphenols ranged from 114.88 to 417.37 mg GAE/g dry weight in black and white mulberry fruits , respectively . While total flavonoids ranged between 12.43 and 24.27 mg QE/g d.w., respectively . Black fruits contain a higher value of anthocyanins 19.33 than white fruits 1.36 mg as cyanidin–3–glucoside, respectively. Both Black and white fruits contain nearly the same values of ascorbic acid 35.61 and 36.74 mg/100g .d.w. Black fruits methanolic extract have higher values of antioxidant activity inhibition % and reducing power than that obtained from white fruits . Average value of 76.908 % , 0.172 at 100 mg / ml ; 69.084 % and 0.085 at 100 mg / ml for black and white fruits , respectively . The effect of black and white fruits methanolic extracts were investigated on STZ diabetic rats through the determination of serum glucose , total cholesterol , triglycerides and liver functions (ALT and AST) .The best results were observed for black mulberry fruits methanolic extract .

Keywords : total polyphenols , flavonoids , anthocyanin , antioxidant , antidiabetic .

INTRODUCTION

Mulberry trees belong to genus Morus of the family Moraceae . Mulberry fruits are a good source of sugars , organic acids and anthocyanin which are important constituents of juices , jam , beverages , extraction of colorants which sometimes are used in medicine because they are contain gama-aminobutyric acid and alanine used against high blood pressure (Machii et al ., 2000) . Mulberry fruits contain phenolic compounds which have a wide spectrum of biochemical activities such as antioxidant , antimutagenic and anticarcinogenic properties , as well as the ability to modify gene expression (Nakamura *et al* ., 2003) . Deep-colored fruits are a good sources of phenolics , including flavonoids and anthocyanins which play an important role in the maintenance of human health (Lin and Tang , 2007). Flavonoids are potent antioxidants , free radical scavengers and metal chelators . Also inhibit lipid peroxidation and exhibit various physiological activities (Middleton and Kandaswami ,1994) .

More attention has been notice to the role of natural antioxidants phenolic compounds which may have more antioxidant activity than vitamin C , E and β -carotene (Vinson *et al*., 1995) . Epidemiological studies showed that the consumption of vegetables and fruits can protect human against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames *et al*., 1993). Recently , mulberry fruits had been

reported to have several biological action such as anti-diabetic (Asano *et al* ., 2001)

The aim of the present study evaluate black and white mulberry fruits for their polyphenolic content and their , antioxidant activity , reducing power and their anti-diabetic action.

MATERIALS AND METHODS

Source and preparation of samples

The present study was carried out using the fruits of mulberry.White and black fruits were purchased from local market at Mansoura city in May 2007. Both white and black fruits were washed using tap water , cut into small pieces and dried in an electric oven at 50°C until reaching the suitable powder for analysis , then kept in polyethylene bags until uses .

Total phenolic contents:

Folin–Ciocalteu method as described by Lin and Tang . (2007)_was used for determination of total phenolic contents .

Total flavonoids :

Total flavonoids content were determined according to the method described by Chang *et al.*, (2002).

Total anthocyanins :

Total anthocyanins were determined in oven dried mulberry fruits (white and black) using spectrophotometric differential pH method, according to Lo Scalzo *et al* .,(2007).

Ascorbic acid :

Ascorbic acid was determined in oven dried mulberry fruits according to the methods of vitamin assay .,(1966).

Extraction of mulberry fruits :

One Kg of the powdered oven dried fruits of mulberry were exhaustively extracted with methanol (10 L) at room temperature. The methanolic extract was concentrated to syrupy (300 ml) under reduced pressure at 45°C and the crude methanol extract was kept at 4 °C.

Antioxidant activity :

The antioxidant activity of the crude methanolic extract of mulberry fruits was determined according to the method of Lee *et al*., (2007)

Reducing power:

The method of Chang *et al.*, (2002). was used to determine reducing power for the crude methanolic extract of mulberry fruits .

Experimental animals:

Animals :

Albino rats (100 – 120 g) were brought from the animal house of Nil company for medical industries , Cairo , Egypt . The rats were kept for adaptation under normal laboratory conditions for 7 days before beginning of the experiment . They were given balanced diet and allowed free acess of water .

Diabetes mellitus experiments :

The experimental rats were divided randomly into tow classes . The first class (about fourty rats) were fasted for 24 hours prior to experimental treatments but were allowed free access to water . Diabetes was induced in this class by the intraperitoneal injection of streptozotocin (STZ) freshly dissolved in citrate buffer (PH 4.5) at a dose of 4.5 mg /100g body weight according to Ghasemi *et al* .,(2007). After 72 hour from STZ injection , rats were fasted 48 hours , then the serum glucose was measured (Bionim-GM300) and rats with serum glucose levels over 250 mg/dl were considered as STZ-diabetic rats and ready for using in experiments .

This class was divided into seven groups (five rats in each group) as follow : **Group (1):** diabetic Control rats which treated with STZ only .

Groups (2,3and 4) : were treated with black fruits methanolic extract at 10.20 and 40 mg/100g b.w., respectively.

Groups (5,6 and 7) :were treated with white fruits methanolic extract at 10,20 and 40 mg/100g b.w ,respectively .

The methanolic extract of each sample was dissolved in saline solution (sodium chloride 0.9 %) before orally administration and continuously given by stomach tube , daily for 30 days .

The second class (about five rats) was untreated with STZ (non-diabetic rats) .Then initial serum glucose level and other parameters were determined

Blood samples were collected from the eye canthus every 10 days after the beginning of the experiment for three times. The blood samples were centrifuged about 10 min to obtain clear serum and the fasting serum glucose level were determined immediately. Blood samples was stored at refrigerator under freezing conditions till determination of triglycerides, total cholesterol and liver functions.

Chemical analysis of blood :

Serum glucose :

Glucose was determined in blood serum using a colorimetric enzymatic method as described in commercial kits by SPINREACT,S.A. (SPAIN) Serum total cholesterol :

Cholesterol was determined by enzymatic colorimetric method described in commercial kits by HUMAN GmbH (GERMANY).

Determination of triglycerides :

Triglyceride was determined by colorimetric enzymatic methods described in commercial kits by HUMAN GmbH (GERMANY).

ALT activity :

Alanine Aminotrnsferase (ALT) was determined according to the method of Reitman and Frankel (1957).

AST activity :

Aspartate Aminotransferase (AST) was determined according to method of Reitman and Frankel (1957)

Statistical analyses

Statistical analysis of all experimental data were done using the statistical software package CoStat (2005). All comparisons were first subjected to one way ANOVA and significant differences between

treatment means were determined using Duncan's multiple rang test at p<0.05 as the level of the significance (Duncan, 1955).

RESULTS AND DISCUSSION

Total polyphenolics , total flavonoids , total anthocyanins and ascorbic acid were determined in oven dried mulberry fruits . Antioxidant activity , reducing power and antidiabetic effect were determined in the mulberry fruits methanolic extract .

Total polyphenols , total flavonoids , anthocyanin and ascorbic acid content :

Data in Table (1) showed that both black and white mulberry fruits contain average values of 417.37 and 114.88 mg GAE/g dry weight for total polyphenols , respectively . It was clear that black fruits have the highest value , while white fruits contain the lowest one . The present results agreed with those mentioned by Ercishi and Orhan (2007) . They were reported that black mulberry fruits contained higher total polyphenols (1422 mg GAE / 100 g fresh matter) than the white fruits (181 mg / 100g fresh matter) . On the other hand polyphenols in black fruits (table 1) were higher than that found by Shaker and EI – Hadidy (2006) , they gave an average value of 90.5 mg / g D.W.

Total flavonoids as showed in Table (1) ranged between 12.43 and 24.27 mg QE / g D.W . It can be observed that black fruits contain the highest amount , however the white fruits have the lowest one . The obtained results were lower than that obtained by Shaker and El–Hadidy (2006) , who reported that total flavonoids in black mulberry fruits was 36.72 mg / g D.W .

As shown in the same table , the results indicated that anthocyanins on black and white fruits gave average values of 19.33 and 1.36 mg/g D.W , respectively . The present results nearly agreed with that obtained by Bea and Suh , (2007) ,who found that total anthocyanins varied from 1.229 to 2.057 as mg cyanidin–3–glucoside / g D.W in white fruits cultivated in Korea . On the other hand this result was higher than lest that found by Shaker and El–Hadidy (2006) , they gave average value of 0.9895 mg cyanidin–3–glucoside / g D.W for the total anthocyanin in black mulberry fruits .

The variation of phenolic compounds in the fruits depends on many factors, such as degree of maturity at harvest, genetic differences and environment, etc.. (Zadernowski et al, 2005).

Data in Table (1) revealed that the two samples have nearly the same value of ascorbic acid. The average values are 35.61 and 36.74 mg / 100g D.W in mulberry black and white fruits, respectively. the obtained result for black fruits (35.61) is higher than that mentioned by Shaker and El – Hadidy (2006), who gave average value 18.0 mg/100g D.W. On the other hand these obtained results nearly agreed with that found by Ercishi and Orhan ,(2007), who noticed that white and black mulberry fruits approximately contain the same amount of ascorbic acid (22.4 and 21.8 mg/100ml, respectively).

components samples	Total polyphenols(1)	Total flavonoids(2)	Total anthocyanin (3)	Ascorbic acid (mg/100gD.W)
Black mulberry fruits	417.37	24.27	19.33	35.61
White mulberry fruits	114.88	12.43	1.36	36.74

Table (1) Total polyphenols, total flavonoids, anthocyanin and ascorbic acid of investigated samples:

(1)Calculated as mg gallic acid equivalent (GAE) /g dry weight .

(2)Calculated as mg Quercetin Equivalent (QE) /g dry weight .

(3)Calculated as mg cyanidin-3-glucoside

Reducing power:

In this investigation, the ability of extracts to reduce iron (III) to iron (II) was determined. Data from Table (2) showed the reducing power of the methanolic extracts of investigate mulberry samples. The data measured as absorbance at 700 nm of producing color against five concentrations (20, 40, 60, 80 and 100 mg/ml) for each sample. Black Mulberry fruits gave the highest absorbance ranged from 0.033 (at 20 mg) to 0.172 (at 100 mg/ml) while white fruits come in the second order with value started with 0.020 (at 20 mg) and ended with 0.085 (at 100 mg).

The high value of reducing power indicated the presence of some compounds with both electron donors could react with free radicals to convert them into more stable products. The present result agreed with that obtained by Bea and Such (2007), who reported that reducing power of white mulberry fruits increased by increasing of concentration. Shaker and El – Hadidy (2006) gave a higher reducing power value of 0.768 for black mulberry fruits (0.01 mg/ml).

Table (2) Reducing power of mulberry samples methanolic extract .*

Concentration (mg/ml)	Black mulberry fruits	White mulberry fruits
20	0.033	0.020
40	0.050	0.028
60	0.114	0.031
80	0.156	0.048
100	0.172	0.085

*Reducing power expressed as absorbance at 700 nm .

Antioxidant activity :

The ability of different mulberry methanolic extracts to scavenge the 2,2-Azino-bis (3-ethyl benzthiazoline-6-sulgonic acid)(ABTS*) radical was determined, compared with that of ascorbic acid and expressed as inhibition %. From Table (3), it could be observed that two extracts showed some degree of inhibition capacity, but their values were inferior that of ascorbic acid. Data shows clearly that methanolic extract of black mulberry fruits had the lowest absorbance value (0.121), accompanied with the highest inhibition percentage value of 76.908. On contrary white mulberry fruits extract showed the highest absorbance (0.162) and the least inhibition value (69.084 %). Comparing the obtained data with that of ascorbic acid (91.41 %) which is known as a strong reducing agent, it was observed that black mulberry

fruits extract gave a moderate inhibition value of 76.908%. Bea and Such , (2007) reported that the ethanolic extract of white mulberry fruits showed moderate inhibitory ability on lipid oxidation (23.7 - 47.6%) at 76 µg and high inhibitory activity (52.7 - 73.3%) at 225 µg of extract.

Different studies indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with antioxidant activity (Siddhuraju *et al* .,2002). From table (2 and 3) it was observed that methanolic extract of black mulberry fruits have the highest reducing power and antioxidant activity because it contained highest amount of polyphenols comparing with the white fruits .

Samples	Absorbance	% inhibition*
Black mulberry fruits	0.121	76.908
White mulberry fruits	0.162	69.084
Ascorbic acid (positive control)	0.045	91.412
Negative control	0.524	0

Table (0) Antioxidant additity of anticient maiserry methanono excludes	Table (3	3) Antioxidant	activity of	different m	ulberry met	hanolic extracts
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percentage inhibition % = A_{734nm} (negative control) - A_{734nm} (sample) × 100 A_{734nm} (negative control)

Effect of mulberry fruits extracts on STZ induced diabetic rats:

The methanolic extract of mulberry fruits(black and white) were examined for their effect on the STZ diabetic rats through the determination of blood glucose, total cholesterol, triglyceride and liver functions (ALT and AST).

Effect on blood glucose level :

Data in table (4) and Fig (1) revealed that the injection of STZ at dose of 4.5 mg/100g b.w caused highly significant (P< 0.05) increase in blood glucose level from 124.67 to 388.33 mg / dl for non diabetic and diabetic rats at zero time , respectively . Gradual increase was observed during the experimental periods (10, 20 and 30 days) until reached to the maximum level of 461.33 mg/dl at the end of experiment . This increase may be due to the destructive effect of STZ on β -cells of islets of Langerhans which lead to insulin deficiency.

The present results agreed with several authors, for instance Terada *et al*.,(1998) who showed that the injection of STZ at a dose of 50 mg/kg b.w raise the blood glucose to 350 mg/dl after 9 weeks. Andallu and vardacharyulu, (2001) observed a significant elevation of 74 % in blood glucose in STZ diabetic rats when compared with normal rats at the end of 60 days. Also Hessien (2003) found that when alloxan was injected in rats at 8 mg / 100 g b.w, it caused a highly raise in serum glucose level after 30 days which reached to 468 mg/dl in comparison with 108.3 mg/dl before injection.

In addition, Hsu *et al.*,(2000) found that injection of STZ at 60 mg / Kg b.w raised serum glucose to more than 400 mg / dl. While Soltani *et al* (2007) reported that the injection of STZ at a dose of 40 mg / Kg b.w raised blood glucose to 250 mg / dl.

Grou	ps	Days	Zero time	10 days	20 days	β0 days
	~	Non –diabetic control	124.67 1	123.67 1	123.33 1	124.67 1
	y fruit	Diabetic control	388.33 bcde	432.67 ab	425.67 abc	461.33 a
	ulberr	10 mg / 100g b.w.	335.00 efg	326.00 gh	236.33 hi	177.67 <u>jk</u>
Treatments	llack m	20 mg / 100g b.w.	404.67 def	302.00 fgh	210.00 <u>ij</u>	162.67 <u>jkl</u>
	I	40 mg / 100g b.w.	301.67 abcd	292.00 cdef	172.00 <u>jk</u>	140.50 kl
	ts	Non –diabetic control	124.67 h	123.67 h	123.33 h	124.67 h
	ry frui	Diabetic control	388.33 bc	432.67 <u>ab</u>	425.67 ab	461.33 a
	nulber	10 mg / 100g <u>b.w</u>	423.67 <u>ab</u>	398.67 <u>bc</u>	265.67 e	190.00 g
	/hite r	20 mg / 100g b.w	467.67 a	437.67 <u>ab</u>	239.00 ef	165.67 gh
	м	40 mg / 100g <u>b.w</u>	370.00 <u>cd</u>	337.67 d	204.67 fg	138.00 h

Table (4) Effect of mulberry methanolic extracts on blood glucose level (mg/dl) in STZ diabetic rats :



Fig (1)Effect of mulberry methanolic extracts on blood glucose level in STZ diabetic rats

Data in Table (4) showed that the optimal hypoglycemic activity was demonstrated at a dose of 40 mg /100 g b.w for the two fruit extracts. The initial antidiabetic activity was observed after ten days and continued to increase in all groups during the experimental period until the 30 days. Data in Table (4) and Fig (1) showed that both black and white fruits

methanolic extract approximately caused a decrease in blood glucose for the

same values of 140.5 and 138 mg/dl after 30 days by using a dose of 40 mg/100 g b.w.

Liver function

From results recorded in Table (5 and 6) and drown in Fig (2 and 3) it was clear that average values of 36.67 and 25.33 U/L were recorded for AST and ALT in non-diabetic rats at zero time which reached to 70.67 and 58.0 U/L after injection of STZ, respectively. Such increase may be due to the damage in liver tissues.

The present results agreed with several authors for instance Andallu and vardacharyulu (2001) found that injection of STZ elevated AST and ALT activity from 53.8 to 94.6 and from 23.2 to 79.6 U /L, respectively. Hessien (2003) mentioned that induction of alloxon raised AST and ALT activity from 30.9 to 50.2 U / L and from 19 to 40 U / L at zero time, respectively. While Ju-Jung *et al.*,(2008) found that diabetic rats showed more activities of serum AST and ALT compared with normal rats by 3.9 and 2.6 times.

Data in Tables (5 and 6) and Fig. (2 and 3) showed that the most effect was observed by treatment with methanolic extract of black mulberry fruits, the AST activity decreased to 42.50 U/L after 30 days and at 40 mg /100g b.w comparing with 70.67 U/L at zero time for STZ induced diabetic rats. While ALT decreased to 27.00 U/L after the same period and using the same dose comparing with 58 U/L at zero time for diabetic rats. The methanolic extracts of white fruits decreased AST and ALT to 47.00 and 31.33 U/L after 30 days and using 40 mg /100 g b.w, respectively

JU-Jung *et al.*, (2008) found that ethanolic extract of Chinese juniper berries decreased AST and ALT activity from 288 and 161 U / L to 250 and 93 U/ L after 9 weeks, respectively.

Table (5) Effect of mulberry	methanolic	extracts	on AST	(U/L) in STZ
diabetic rats :				

Grou	ps	Days	Zero time	10 days	20 days	30 days
		Non –diabetic control	36.67 hi	35.00 į	36.00 hi	36.33 hi
	y fruit	Diabetic control	70.67 bc	74.33 ab	72.33 bc	79.33 a
	ulber	10 mg / 100g b.w.	65.67 <u>cd</u>	71.33 bc	53.67 de	53.33 <u>ef</u>
	lack m	20 mg / 100g b.w.	68.67 <u>cd</u>	71.00 bc	49.33 fg	48.67 g
nents	-	40 mg / 100g b.w.	55.00 bcd	59.00 bcd	39.67 hi	42.50 h
reati	2	Non –diabetic control	36.67 g	35.00 g	36.00 g	36.33 g
[ry frui	Diabetic control	70.67 bc	74.33 ab	72.33 bc	79.33 a
	nulber	10 mg / 100g b.w.	68.00 <u>bc</u>	73.33 <u>bc</u>	67.00 c	58.33 d
	White 1	20 mg / 100g b.w.	70.33 <u>bc</u>	70.67 <u>bc</u>	55.67 de	52.00 ef
	*	40 mg / 100g b.w	69.00 <u>bc</u>	70.33 <u>bc</u>	51.00 ef	47.00 f



Fig (2)Effect of mulberry methanolic extracts on AST level in STZ diabetic rats.

Grou	ps	Days	Zero time	10 days	20 days	30 days
		Non –diabetic control	25.33 gh	24.00 h	23.33 h	23.67 h
	y fruit	Diabetic control	58.00 bcd	62.33 b	59.00 bc	71.33 a
	ulber	10 mg / 100g b.w.	50.67 e	61.67 <u>bc</u>	36.67 f	33.33 f
nents	Black n	20 mg / 100g b.w.	55.33 de	58.00 <u>bc</u>	35.00 f	26.33 g
		40 mg / 100g b.w.	43.67 cde	47.67 bcd	29.67 g	27.00 gh
Ireat	ts	Non –diabetic control	25.33 g	24.00 g	23.33 g	23.67 g
.	ry frui	Diabetic control	58.00 bc	62.33 b	59.00 bc	71.33 a
	nulber	10 mg / 100g <u>b.w</u>	54.00 c	61.00 ъ	45.33 d	41.00 d
	Vhite 1	20 mg / 100g <u>b.w</u>	58.00 bc	59.33 bc	41.00 de	36.33 ef
	A	40 mg / 100g b.w	53.00 c	59.00 bc	33.00 f	31.33 f

Table (6) Effect of mulberry methanolic extracts on ALT (U/L) in STZ diabetic rats :

Serum total cholesterol :

Table (7) and Fig (4) showed that STZ injection caused a significant increase in serum total cholesterol from 194.67 mg / dl in non-diabetic rats to 382.67 mg / dl in diabetic rats at zero time, respectively. An increase in total cholesterol values by (5 and 6 %) using 10 mg / 100 mg b.w of methanolic extract after 10 days were observed for mulberry fruits (black and white), respectively, While after 20 days, a sudden decrease (23.25 and 44.6 %) was obtained with the previous two methanolic extracts using 10 mg / 100 g

b.w. The Same observation was shown by using 20 mg / 100 g b.w. On the other hand using 40 mg / 100 g b.w caused a decrease after 30 days for all extracts. The methanolic extract of mulberry black fruits was the most effective in lowering serum cholesterol level which reached to 209.67 mg / dl (45.21%) after 30 days , while white fruits methanolic extract decreased the level of total cholesterol to 224.67 mg / dl (41.29 %), respectively .



Fig (3) Effect of mulberry methanolic extracts on ALT level in STZ diabetic rats

Hurtado and Dummi (1998) found that total cholesterol increased from 90 to 130 mg / dl in non-diabetic and diabetic rats, respectively. Hessein (2003) mentioned that alloxan injection caused a markedly increase in serum total cholesterol which reached about above five times (10mg / L) of those obtained in case of non-diabetic rats (2.1 mg / L) at zero time . Also , twenty mg crude extract of mulberry leaves / 100 g b.w caused a decrease in total cholesterol after 30 days of experiment . Also JU-Jung *et al* (2008) reported that alloxan-injection increased serum total cholesterol from 62 in normal to 76 mg / dl diabetic rats. They found also that ethanol and water extracts of Chinese juniper barriers decreased total cholesterol from 76 to 74 and 43 mg / dl , respectively .

Serum Triglyceride

Data recorded in Table(8) and Fig (5) revealed that serum triglyceride increased from 174.67 to 288 mg /dl by injection of STZ. After 30 days and using 10 mg black fruits extract /100g b.w. triglyceride level decreased to reach 226.67mg/dl. Treatment with 20 mg / 100 g b.w of mulberry black fruits methanolic extract caused a decrease in serum triglyceride to 196.67 mg / dl (35.42%), while the decrease reached to 178.67 mg / 100g b.w after 30 days by using 40 mg / 100g b.w of the same methanolic extract. The same trend nearly was observed using white fruits methanolic extract , a decrease in serum triglyceride reached to about 209mg/dl after 30 days using 40 mg /

100g b.w was observed comparing with 248.67 and 209.33 mg/dl for 10 and 20 mg / 100g after 30 days, respectively.



Table (7) Effect of mulberry methanolic extracts on Serum total cholesterol (mg/dl) in STZ induced diabetic rats :



From these findings, it could be concluded that methanolic extract at the level of 40 mg / 100g b.w for both black and white fruits after 30 days gave the most effective decrease in serum triglyceride.

Group	ps	Days	Zero time	10 days	20 days	30 days
		Non –diabetic control	174.67 į	171.00 į	181.67 į	172.67 į
	y fruit	Diabetic control	288.00 bcd	300.67 <u>ab</u>	307.00 a	295.00 abc
	ulberr	10 mg / 100g b.w.	287.00	295.67	265.33	226.67
10	lack n	20 mg / 100g b.w.	288.00	289.00	241.33	196.67
Treatments	В	40 mg / 100g b.w.	252.33	246.67	220.50	178.67
	hite mulberry fruits	Non –diabetic control	174.67 e	171.00 e	172.67 e	181.67 e
		Diabetic control	288.00 b	300.67 <u>ab</u>	295.00 <u>ab</u>	307.00 a
		10 mg / 100g b.w.	288.00	296.33	287.00	248.67
		20 mg / 100g b.w.	288.67	294.00	219.00	209.33
	A	40 mg / 100g b.w.	287.67	288.33	209.67	209.00
3!	50					
30	00					

Table (8) Effect of mulberry methanolic extracts on Serum triglycerides (mg/dl) in STZ induced diabetic rats :





Hurtado and Dummi (1998) reported that triglyceride increased from 80 to 390 mg / dl in non-diabetic and diabetic rats , respectively. In this respect , Hessien (2003) found that alloxan injection caused a significant increase in triglyceride level which reached to 280.5 mg / dl comparing with 166.5 mg / dl for non-diabetic rats at zero time . She added also that the treatment with 20 mg methanolic crude extract of mulberry leaves / 100g b.w decreased the level of serum triglyceride to 153 mg / dl after 30 days. While JU-Jung *et al*, (2008) revealed that serum triglyceride increased from 83 to 262 mg / dl in non-diabetic rats, respectively. They found that the ethanol and water extract of Chinese juniper berries decreased triglyceride to 195 and 96 mg / dl for those extracts, respectively.

REFERENCES

- Ames , B . M ; shigena , M . K . and Hagen , T . M . (1993). Oxidants , antioxidants and the degenerative diseases of aging . Proc . Natl . Acad . Sci . USA , 90:7915 .
- Andallu , B and Vardacharyulu , N (2001). Effect of mulberry leaves on diabetes . INT. J . DJAB . DEV . countries , vol . 21 .
- Asano, N; Yamashita, T; Yasuda, K; Ikeda, K;Kizu, H; Kameda, Y ;Kato, A;Nash, J.R.;Lee, H.S and Ryu, K.s.(2001).Polyhydroxylated alkaloids isolated from mulberry trees (Mours alba L) and skilworms (Bombyx mori L.) J.Agri. Food chem. 49(9):4208–213.
- Bea , S . H and Suh , H . J .,(2007) . Antioxidant activities of five different mulberry cultivars in Korea . Availble on line at <u>www.Science-direct.com</u> LWT 40 (955 962) .
- Chang, C. C; Yang, M. H; Wen, H. M; and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis,10:178-182.
- CoStat program, Version 6.311(2005). CoHort Software, 798 Lighthouse Ave. PMB 320, Monterey, CA, 3940, USA. http://www.cohort.com
- Duncan, (1955) Multiple rang and multiple F test. Biometrics 11: 1-42.
- Ercishi, S and Orhan , E (2007). Chemical composition of white (Morus alba), red (Morus rubra) and black (Morus nigra) mulberry fruits. Food Chemistry 103 :1380–1384.
- Ghasemi,M; Sadeghipour,H; Asadi,S and Ahmed ,R.D.(2007). Timedependent alteration in cromakalim-induced relaxation of corpus cavernosum from streptozocin-induced diabetic rats. Life Sciences 81: 960–969.
- Hessien , Rania .A.A(2003). Bio chemical studies on mulberry (Morus alba) and prickly – pear (Opuntia Sp) . M.Sc. Thesis , Agri . Bio chem. Dept ., Fac of Ain Shams Univ. Cairo .
- Hurtado , G.E and Dummi (1998). Lipid dismetabolism in Leydig and Sertoli cells isolated from streptozotocin-diabetic rats . The Int . J . of Biochemistry and cell Biology 30 , 1001 1010 .

Hsu , F . L ; Chen , Y.C . and Cheng , J.T. (2000) Caffeic acid as active principle from the fruit of Xanthium strumarium to lower plasma glucose

in diabetic rats . Planta Medica 66,228-230 .

- Ju Tung , B ; Kima Ji Su ; Chang ,W.C ; Hae K. L ;Tae-Kyun Oha and Sei Chang Kim . (2008). Comparison between ethanolic and aqueous extracts from Chinese juniper berries for hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats. Journal of Ethnopharmacology 115 : 110–115.
- Lee .C.Y ;Sim .S.M and cheng , H.M (2007). Systemic absorption of antioxidants from mulberry (Morus alba L)leaf extracts using an in situ rat intestinal preparation .Nutrition Ressearch 27:492-497
- Lin , J . Y and Tang , C . Y (2007). Determination of total phenolics and flavonoids contents in selected fruits and vegetables , as well as their stimulatory effects on mouse splenocyte proliferation . Food chemistry 101 : 140 147.
- Lo Scalzo ,R ; Genna , A ; Branca , F ; Chedin , M ; and Chassaigne , H.(2007) . Anthocyanin composition of cauliflower (Brassica oleracea L. var. botrytis) and cabbage (B. oleracea L. var. capitata) and its stability in relation to thermal treatments. Food Chemistry 107 : 136– 144.
- Machii , H ; Koyama , A and Yamanouchi , H.(2000) .Charactrization and safety evaluation of traditional Greek fruits distillate " Mouro " by flavor compounds and minerals analysis . Food chemistry 86 :625-636
- Methods of vitamin assay , the association of vitamin chemists , interscience puplishers , New York , 3rd ed ., 287,(1966).
- Middleton, E. Jr and Kandaswami, C (1994) The impact of plant flavonoids on mammalian biology : implications for immunity, inflammation and cancer. In the flavonoids . advances in research since 1986 ed. Harborne, J.B.pp.619 – 65.London.
- Nakamura, Y., Watanabe, S., Miyake, N., Kohno, H., & Osawa, T. (2003). Dihydrochalcones: evaluation as novel radical scavenging antioxidants. Journal of Agricultural and Food Chemistry, 51, 3309– 3312.
- Reitman, S and Frankel, S . Amer . j . Clin . Path ., (1957) ; 28 56 .
- Shaker , E and El-Hadidy , E (2006).Could Mulberry and Roselle Anthocyanins do the Expected Role as Natural Valuable Colorants?Ist International conference & Exhibition on food & Tourism 1-3 March , 2006. Cairo , Egypt .
- Siddhuraju, P; Mohan, P.S and Becker, K. (2002). Studies on the antioxidant activity of Indian laburnum (Casia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruits pulp. Food chemistry 79, 61 – 67.
- Soltani,N; Keshavarz,M and Dehpour,A.(2007).Effect of oral magnesium sulfate administration on blood pressure and lipid profile in streptozocin diabetic rat. European Journal of Pharmacology, 560: 201–205.

- Terada, M ; Yasuda, H and Kikkawa, R (1998). Delayed wallerian degenration and increased neurofilament phosphorylation in sciatic nerves of rats with streptozotocin-induced diabetes. Journal of Neurological Science 155, 23-30.
- Vinson, J. A; Dabbag, Y. A; Serry, M. M. and Jang, J. (1995). Plant flavonoids, especially tea flavonols are powerful antioxidants using in vitro oxidation model for heart disease. J. Agri. Food Chem; 43:2800.
- Zadernowski, R.; Naczk, M and Nesterpwicz, J.(2005). Phenolic acid profile in some small berries. Journal of Agriculture and food chemistry , 53, 2118 – 2124.

دراسات بيوكيميائية على تمار التوت و المستخلص الميثانولى لها . صفاء محمد على ، سامي طلعت أبو طالب ، أحمد محمد يوسف و سامح عبد الرؤف قش . قسم الكيمياء الزراعية ، كلية الزراعة ، جامعة المنصورة .

في هذا البحث تم تقدير محتوى ثمار التوت السوداء و البيضاء التي تنمو في مصر من الفينولات الكليه و الفلافونيدات الكليه و الانثوسيانين وحامض الاسكوربيك حيث أوضحت النتائج أن الفينولات الكليه تراوحت قيمتها بين(A17.37 mg GAE/g dry weight) في الثمار السوداء و البيضاء عل التوالي .

و من ناحية أخرى وجد أن الانثوسيانين يوجد بنسبة اكبر في الثمار السوداء عنها في الثمار البيضاء وحيث تراوحت النسبة بين (–3–19.33 and 1.36 mg cyanidin)على التوالي وكانت نسبة حامض الاسكوربيك في الثمار السوداء 35.61 mg/100g على الثمار البيضاء d.w. 36.74 mg/100g .d.w وقد تم تقدير القدرة الاختزالية و النشاط كمضاد للأكسدة في المستخلص الميثانولى لكل من

وقد تم تقدير القدرة الاختزالية و النشاط كمضاد للأكسدة في المستخلص الميثانولى لكل من الثمار السوداء و البيضاء وأوضحت النتائج أن قدرة المستخلص للعمل كمضاد أكسدة و كذلك القدرة الاختزالية للثمار السوداء كانت and 0.172 at 100 mg / ml % 80.908 بينما كانت في الثمار البيضاء كانت 69.084 % 80.085 at 100 mg / ml .

بالإضافة إلى ذلك تم دراسة تأثير المستخلص الميثانولى لثمار التوت عل الفئران المصابة بمرض السكر من خلال تقدير نسبة سكر الدم و الكولسترول و الجليسردات الثلاثية وظائف الكبد من خلال تقدير إنزيمات الكبد (ALT , AST).

وأُثبتت الدراسة أن أفضل النتائج كانت لثمار التوت السوداء و كذلك المستخلص الميثانولي للثمار السوداء .