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ANTIOXIDANT ACTIVITY AND CHOLESTEROL LOWERING EFFECT OF THE EGYPTIAN DATE PALM (*Phoenix dactylifera* L.) TIP SHOOT FLAVONOIDS IN HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

Date palm (*Phoenix dactylifear* L.) tip shoot was extracted with ethanol 80% (Et) and ethylacetate before hydrolysis with HCl (EA-1) and after hydrolysis (EA-2) to obtain flavonoids. The yield and flavonoids content of every extract were determined.

Polyphenols and flavonoids were fractionated from ethanolic extract by HPLC, eighteen compounds were identified and the major constituents were myricetin 14.48%, coumarin 13.66 and pyrogallic acid 8.21%.

Free radical and superoxide radical scavenging activity of different extracts (Et, EA-1, EA-2) were determined using 2,2-diphenyl-1-picrylhydryzyl (DPPH˙) and nitro blue tetrazolium (NBT) reduction method. The obtained results revealed that ethanolic extract Et has high effect in free radical scavenging (92.12%) compared with rutin 94.75%) and superoxide radical scavenging (91.15% compared with rutin 96.54%).

The hypocholesterolemic effect of ethanolic extract was investigated on hypercholesterolemic rats by using three concentrations from ethanolic extract (50, 75 and 100mg/kg body weight for 30 days).

The results indicated that high flavonoids content in the extract caused significant increase in body weight gain and feed efficiency. Also, significant decrease in serum total cholesterol, total lipids, triglycerides and LDL-cholesterol and significant increase in HDL-cholesterol compared with hypercholesterolemic rats. On the other hand, high flavonoids content improvement from liver and kidney functions.

Keywords: Date Palm, (*Phoenix dactylifera* L.), tip shoot, flavonoids, antioxidant activity, hypercholesterolemia, lipid pattern, rats lipoproteins, liver functions, kidney functions.

INTRODUCTION

Date palm (*Phoenix dactylifera* L. c.v. Seewy and Zagleol ... etc) is one of the most important fruit crop in Egypt. The total number of trees in Egypt is around 6.7 million and the production is around 631400 ton / year FAO (2001). Date palm is employed to provide food, shelter, fuel, fiber, clothing, furniture and other misellaneous implements products Al-Bakr (1972).

Plants produce a broad variety of secondary metabolites. In general, one may assume that these compounds play a role in the interaction of plant with its environment, e.g. phytoalexin, antifeedant or attractant of pollinators Castelluccio *et al.* (1995) and Ahmed *et al.* (2000).

Plant phenols are mostly products of the phenyl propanoid pathway and comprise a large variety of compounds, cinnamic acids, benzoic acids, flavonoids and coumarins. They are strong antioxidant and might prevent oxidative damage of biomolecules such as DNA, lipids and proteins that play a role in chronic diseases such as cancer and cardiovascular disease Molina *et al.* (2003).

Also, polyphenols are effective antioxidants in a wide range of chemical oxidation systems, being capable for example scavenging peroxyl radical, alkyl radicals, superoxide, hydroxyl radicals, nitric oxide and peroxynitrite in aqueous and organic environments Duthie and Crozier (2000).

Nowadays the consumption of fruit and vegetables is regarded as important and good for health. Indeed, recent epidemiological studies have indicated that a high intake of fruit, vegetables and tea is associated with reduced risk for a number of chronic disease Kenkt *et al.* (2000); Duffy *et al.* (2001) and Augar *et al.* (2004). Also, at the same time the date palm fruit possesses antioxidant and antimutagenic properties *in vitro* Proteggente *et al.* (2002).

Also, epidemiological studies suggest, the antioxidant effects of flavonoids including oxidative damage of low density lipoprotein and several studied showed an inverse association of flavonols intake and subsequent resulting in a reduction of cardiovascular disease (CVD) Kris and Keen (2002).

The aim of the present study was to explore the potential antioxidant activity using 2,2-diphenyl-1-picrylhydryzyl (DPPH˙) test and nitroblue tetrazolium (NBT) reduction method for different flavonoids extracts of Egyptian date palm tip shoot, record the phenolic profile of palm tissue ethanolic extract by the HPLC technique. Also, biological effects of this extract on lipid pattern, liver and kidney functions of normal and hypercholesterolemic rats were investigated.

MATERIALS AND METHODS

1- Preparation of the date palm tip shoot ethanolic, ethylacetate and flavonoids aglycon extracts for the bioassay.

Date palm (*Phoenix dactylifera* L.) c.v. Zagleol tip shoot samples were obtaind from trees of Experimental Station of Faculty of Agricultural, Cairo University, Giza,. Egypt during 2004 and about 50g of the fresh tip shoot samples were exhaustively extracted with ethanol 80%. The ethanolic extract was concentrated under vacuum and the residue was weight then dissolved in distilled water (250ml). the aqueous solution was successively extracted with chloroform to remove the pigments and fatty materials and then with ethylacetate. The ethylacetate extract was evaporated to dryness under vacuum and weighted (EA-1). The aglycon was extracted from ethanolic extract by taken a few mgs and partially hydrolyzed by refluxing with 0.1N HCl for 3h. The acidic aqueous solution was neutralized, evaporated to dryness, then the aglycone was extracted with ethylacetate and the extract was evaporated to dryness and weighted (EA-2) Mabry *et al.* (1970).

2- Determination of flavonoids in different extracts of palm shoot tip.

Total flavonoids were determined in date palm tip shoot samples as (mg flavonoids/g dry weight) according to the method of Zhuang *et al.* (1992).

3- Fractionation of polyphenols and flavonoids by high performance liquid chromatography (HPLC).

Preparation of ethanol extract.

Ten grams of tip shoot sample were mixed with 100 ml ethanol (80%) and shaken at room temperature for 3 days to obtain ethanol extract. The resulting ethanol extract was subsequently filtered and centrifuged to obtained clear filtrate which evaporated in rotary evaporator at 45°C.

Separation conditions by HPLC.

Technique HPLC [Hewlett Packard series 1100 (HP 1100)]. Column hyperis BDS 5µm. Detector UV 254nm. Flow rate 0.3ml/min. Mobile phase **A:** (0.5ml acetic acid / 99.5ml distilled water), **B:** (0.5ml acetic acid / 99.5ml acetonitrile). Temperature ambient 25°C Merfort *et al.* (1997).

4- Evaluation of antioxidant activity using the DPPH$^{\cdot}$ method.

The antioxidant activity of different flavonoids extracts of date palm tip shoot were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH$^{\cdot}$ according to Brand-Williams *et al.* (1995). The decrease in the absorbance was determined at 515nm when the reaction reached the plateau. Using an Hp 8452A diode array spectrophotometer in a 10mm quartz cuvette and the inhibition percent of the DPPH$^{\cdot}$ by the samples was calculated according to the formula of Yen and Duh (1994) as follows:

$$\% \text{ Inhibition} = [(Ac\ (0) - Ac\ (t) / Ac\ (0)] \times 100$$

Where: Ac (0) was the absorbance of the control at t = 0 min and Ac (t) was the absorbance of the antioxidant at t, which varied with the different content.

5- Evaluation of antioxidant activity using nitroblue tetrazolium reduction (NBT) method.

Superoxide radicals were generated by xanthine / xanthine oxidase (XO) and measured by the nitroblue tetrazolium (NBT) reduction method according to Chung *et al.* (2004). A test sample was mixed in a 100 *M*m phosphate buffer solution (pH 7.0) containing (XO) (1.65×10^2 unites ml^{-1}) and NBT (133 µ*M*) at 25°C in well flat-bottomed microassay plates. The measurement was started with adding xanthine (164µ*M*). Production of superoxide radical was followed spectrophotometerically at 560nm at 25°C for 10 min. superoxide savenging activity was calculated according to the following formula.

$$\text{Superoxide scavenging activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Where: Absorbance control and Absorbance sample represent the increased absorbance in the absence of samples.

6- Biological experiment.

Male albino rats (80- 100g) were obtained from the Helwan, Cairo, Egypt. Rats raised in the Animal House of Toxicology Department, at Central Agricultural Pesticide Laboratory, Agriculture Research Center. The rats were kept at normal laboratory condition for one week before the commencing the experiments. During this period the animals were allowed free access of

water and basal diet. Food consumption and body weight of each animal were monitored in the beginning and in the end of the experiment.

After feeding on basal diet, rats were divided into five groups. The first group (6 rats) was fed on the basal diet for another ten weeks which considered as normal control group (negative control). The second group (24 rats) was fed on diet high cholesterol content for 6 weeks (1% cholesterol + 0.25 bile salts), it was then divided into 4 subgroups. The first subgroup (6 rats) which considered as positive control or hypercholesterolemic rats. The second subgroup (6 rats) took 50mg palm tissue ethanolic extract / kg body weigh. The third subgroup (6 rats) took 75mg ethanolic extract / kg body weigh. The fourth subgroup (6 rats) took 100mg ethanolic extract / kg body weigh. All concentrations of ethanolic extract were dissolved in 10% dimethyl sulfoxide (DMSO) and taken orally for 30 days during this period diet and water were supplied libitum to the different rats groups. Each rat was weighed every two days and feed intake was also daily recorded. At the end of experimental period rats were fasted for 18 hrs and killed by decapitation.

Animal diet.

The basal diet consisted of corn starch 63%, casein 12%, corn oil 10%, salt mixture 4%, vitamins mixture 1% and crude fiber 10% according to Zulet *et al.* (1999).

7- Biochemical parameters.

The following biochemical parameters were analyzed in the serum to evaluate the effect of ethanolic extract of palm tissue on lipid pattern, liver functions and kidney functions by the methods given bellow:

Lipids pattern.

Serum total lipids were determined according to Knight *et al.* (1972). Serum triglycerides were determined according to the methods of Fossati and Prencipe (1982), Serum cholesterol was determined according to the enzymatic method of Allain *et al.* (1974). Serum HDL-cholesterol was determined by the method of lopez-Virella *et al.* (1977), serum LDL-cholesterol was determined by the method of Steinberg (1981).

Liver functions.

Activities of aspartate amino transferase (AST), alanine amino transferase (ALT) were determined according to Reitman and Frankel (1957).

Kidney functions.

Blood urea was estimated by the enzymatic method of Patton and Couch (1977), serum creatinine was determined according to the method described by Faulkner and King (1976).

8- Statistical analysis.

Statistical analysis for each of the collected data was done following procedure outlined by Gomez and Gomez (1984). The treatment means were compared using the least significant difference test (LSD) at the 5% and 1% levels of probability as outlined by Waller and Duncan (1969). Using the SAS institute program.

RESULTS AND DICUSSION

1- Flavonoids contents of different extracts of date palm tip shoot.

Results are shown in Table (1) show that the yields of different extracts, ethanol 80%, (Et) ethylacetate and ethylacetate (EA-1) after hydrolysis with HCl (EA-2) were 3.87%, 1.73% and 1.03%, respectively. These results may be due to ethanol extract both polar and non-polar compounds such as amino acids, proteins, carbohydrates and phenolic compounds but ethylacetate extract neutral glycosides and ethylacetate after hydrolysis extract free aglycon only.

Table (1): Yield percentage of different extracts of palm shoot tip (based on dry weight).

Extract	Yield %
Ethanol 80% (Et)	3.87
Ethylacetate (EA-1)	1.73
Ethylacetate (EA-2)	1.03

Total flavonoids of palm tissues were extracted and determined as rutin was shown in (Table, 2). It was found to be noticed that 0.062mg/g in ethanolic extract, but 0.0044mg/g in ethylacetate (EA-1) extract, at the same time 0.0036mg/g in ethylacetate extract (EA-2), this may be due to (EA-1) extract content of flavonoids in natural glycosides and (EA-2) extract content of free flavonoids or aglycone. These results are in agreement with found by Al-Baker (1972) who showed that stem of date palm contained low content of flavonoids compared with those leaf and root.

Table (2): Flavonoids content of different extracts of palm shoot tip (based on dry wt.).

Extract	Total flavonoids
Ethanol 80% (Et)	0.0620
Ethylacetate (EA-1)	0.0044
Ethylacetate (EA-2)	0.0036

2- Polyphenols and flavanoids fractions in ethanolic extract of data palm tissue.

Data in Table (3) reveal that ethanolic extract of data palm contained 18 compounds; relatively high amount of myricetin 14.48%, coumarin 13.66, , pyrogallic acid 8.21, %, ferulic acid 7.43%, gallic 6.85%, rutin 6.56%, chlorogenic acid 6.35%, cinnamic acid 6.01% and catechin 5.33%. Some of components were found at low amounts such as protocatechuic acid 3.55% and quercetin 2.31%.

There data are parallel with those obtained by Mansouri *et al.* (2005) who found that all the varieties of data palm fruit (*Phoenix dactylifera* L.) contain mainly p-coumaric, ferulic and some cinnamic acid derivatives as flavonoids compounds.

3- Antioxidant activity of different extracts from shoot tip according to the DPPH$^{\cdot}$ radical scavenging method.

The results in Table (4) show that the decrease absorbance of DPPH$^{\cdot}$ radical was due to its reduction by different antioxidants. Rutin (standard) is superior inhibitor of DPPH$^{\cdot}$ compared to palm shoot tip extracts and gave high percentage inhibition 91.64% and 94.75% for 50, 100µg/ml, while (Et) extract, showed increasing inhibition effect for all concentration than other extracts because ethanolic extracts contained high content of antioxidant compounds compared with (EA-1) and (EA-2). (EA-1) contained neutral flavonoids which have high antioxidant effect than free aglycone in (EA-2).

Table (3): Polyphenols and flavanoids contents of ethanolic extract of data palm tip shoot (%).

Compound	*Rt	%
Pyrogallic acid	5.297	8.21
Gallic	7.790	6.85
Resrocenol	9.565	1.12
Protocatechuic acid	11.142	3.55
Hydroxy benzoic acid	14.420	1.17
Chlorogenic acid	16.380	6.53
Catechin	16.814	5.33
Phenol	18.399	0.95
Vanillin	19.156	1.16
p-coumaric acid	21.025	6.36
Ferulic acid	22.465	7.43
Salicylic acid	22.972	1.08
Rutin	24.006	6.56
Coumarin	25.718	13.66
Myricetin	27.510	14.48
Cinnamic acid	31.406	6.01
Quercetin	32.430	2.31
Kaempferol	37.540	6.79

***Rt= Retention time.**

Table (4): Free radical scavenging activity of various concentrations from palm shoot tip-extracts.

Sample (µg/ml)	DPPH radical decoloration %		
	10	50	100
Rutin (Standard)	45.33	91.64	94.75
Ethanol 80% (Et)	43.12	85.85	90.12
Ethylacetate (EA-1)	40.24	78.99	85.33
Ethylacetate (EA-2)	31.22	60.22	71.09

*** Comparison of antioxidant activity of different extracts of palm shoot tip with rutin expressed as (%) inhibition.**

**** All values are means of 3 replicates.**

These results are in disagreement with those obtained by Noroozi *et al.* (1998) who found that free flavonoids are higher protective than the conjugated flavonoids (e.g. quercetin compared with its conjugated quercetin-3-glucoside) and antioxidant activity of free flavonoids is related to the number and position of hydroxyl group. Also, Zheng and Wang (2001) who reported that natural mixture of phenolic compounds has high antioxidant activity. Based on these experimental it is suggested that palm shoot tip extracts comprise effective potential source of natural antioxidant.

5- Antioxidant activity of different extracts of palm shoot tip according to NBT reduction method.

Data in Table (5) show the antioxidant activity of different extracts of palm tissues to scavenging superoxide radical which generated by xanthine / xanthine oxidase. It is clear that rutin (standard) is superior inhibitor of xanithine / xanthine oxidase compared to different extracts of palm tissues and gave high percentage as superoxide scavenging activity 90.12% and 96.54% for 50 and 100µg/ml, while (Et) extract showed good free radical scavenging activity depending on the concentration used compared with (EA-1) and (EA-2). This may be due to ethanolic extract found to contain significant amount of natural phenolic compounds such as quercetin and ferulic acid as described in Table (3).

Table (5): Superoxide radical scavenging activity of various concentrations from palm shoot tip flavonoids extracts.

Sample (µg/ml)	Superoxide radical scavenging %		
	10	50	100
Rutin (Standard)	70.22	90.12	96.54
Ethanol 80% (Et)	66.34	79.38	91.15
Ethylacetate (EA-1)	60.88	68.24	82.55
Ethylacetate (EA-2)	49.24	60.88	73.66

These results are in agreement with those found by Parr and Bolwell (2000) who showed that some enzymes such as various cytochrome P_{450} iso-forms, lipoxygenases, cyclooxygenases and xanthine oxidase are potentially pro-antioxidant and can generate radicals. Certain flavonoids and phenylpropanoids are effective inhibitors of these enzymes such as certain flavonoids and purpurogallin derivatives were found to inhibit xanthine oxidase.

6- Biological experiment.

6.1- Body weight gain and feed efficiency.

Rats fed on basal diet (Table, 6) showed the highest gain (99.02g) in body weight, while rats feed on diet with high cholesterol content showed a significantly lowest in body weight gain (44.83g).

Treatment of hypercholesterolemic rates with different concentration from palm tissues ethanolic extract (50, 75, 100mg/kg body weight) significantly increased body weight gain from 44.38g in control positive to 57.28, 64.13 and 75.93g, respectively.

6.2- Feed intake.

Feed intake results by rats fed on basal diet and high cholesterol content and / administration with various concentration of ethanolic extract are shown in Table (6). Feed intake by rats fed on hypercholesterolemic and hypocholesterolemic diets was significantly lower than those of negative control, this may be due to the digestion of food constituents inhibited with addition bile salts Hamama *et al.* (1988).

6.3- Feed efficiency.

Feed efficiency (Table, 6) also followed the same trend where it was increased from (0.1401) in diet with high cholesterol content to (0.1618, 0.1650, and 0.1827) in treatment with different concentration of ethanolic extract. It is clear that treatment with ethanolic extract of palm tissues to hypercholesterolemic rates had a beneficial effect in daily gain in body weight and feed efficiency as compared with positive control.

Table (6): Effect of administration with various concentrations of palm tip shoot ethanolic extract on body weight gain, feed intake and feed efficiency on hypercholesterolemic rats.

Treatment	Final body weight (g)	Body weight gain (g)	Feed intake (g/weeks)	Feed efficiency (B.Wt gain/ feed intake)
Control (negative)	199.88 ±12.62	99.02 ±6.20	431.14 ±24.20	0.2297 ± 0.011
Control (Positive)	140.11 ±11.61	44.83 ±3.20	320.01 ±19.58	0.1401 ± 0.007
Hyper + 50mg Et /kg B.Wt	155.01 ± 9.86	57.28 ±4.26	354.04 ±26.12	0.1618 ± 0.006
Hyper + 75 mg Et /kg B.Wt	163.22 ±10.84	57.28 ±4.26	388.68 ±21.19	0.1650 ± 0.010
Hyper + 100mg Et/kg B.Wt	175.17 ±13.78	75.93 ±5.80	415.59 ±31.43	0.1827 ± 0.0128
L.S.D. $_{(0.05)}$	21.505	9.038	45.213	0.018

Each value represents the mean ± S.E.; B.Wt.: Body weight; Hyper: Hyper-cholesterolemic rats.

Statistical analysis corresponding to hypercholesterolemic rats group.

The obtained results are in agreement with Kahlon *et al.* (1993) who found that feed efficiency was affected by diets containing hypocholesterolemic agents.

7- Effect date palm ethanolic extract on lipids pattern.

Total cholesterol, total lipids and triglycerides were determined in serum to evaluate the role of flavonoids compounds in ethanolic extract as hypocholesterolemic agent (Table, 7) the amount of lipids, cholesterol and triglycerides were calculated compared to the original control groups (value in control group equal to 100). Data of total cholesterol showed that it was significantly increased to be 92.10mg/dl in negative control to 288.13mg/dl in positive control. On the other hand; serum total lipids and triglycerides were also significantly increased in positive control group by 51.33% and 55.37%, respectively (Table, 7) as compared with negative control. The effect of

ethanolic extract on hyper-cholesterolemic rats showed that significant decreases in serum total cholesterol were 19.92%, 30.22% and 32.93%, respectively compared with positive control.

Also, a significant decrease in total lipids by 4.42%, 11.31% and 22.92% and significant decrease of triglycerides by 11.81%, 22.34% and 28.19%, respectively compared with positive control.

The effect of ethanolic extract of palm tissues on high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and the ratio of LDL/HDL (Risk ratio) were studied and results are shown in Table (8). The risk ratio is a good indication for hypercholesterolemia. This ratio was increased to 5.961 mg/dl in positive control group as compared with negative control (0.736mg/dl). It was significantly decreased to 3.665, 2.913 and 2.160 mg/dl in rats treated with 50, 75 and 100mg/kg B.Wt. from ethanolic extract. The result in Table (8) showed that LDL-cholesterol was significantly lowered and HDL-cholesterol was significantly increased in rats groups treated with different concentrations of ethanolic extract.

Table (7): Serum total cholesterol, total lipids and total triglycerides of normal and hypercholesterolemic rats.

Treatment	Total Cholesterol		Total lipids		Total Triglycerides	
	mg/dl	%	mg/dl	%	mg/dl	%
Control (negative)	92.10 ±6.12	100.00	350.36 ±19.51	100.00	117.96 ±8.48	100.00
Control (Positive)	288.13 ±13.16	312.84	530.21 ±14.63	151.33	183.27 ±12.94	155.37
Hyper + 50mg Et /kg B.Wt	230.72 ± 11.26	250.51	506.78 ±10.32	144.64	161.61 ±9.82	137.00
Hyper + 75 mg Et /kg B.Wt	201.06 ±10.63	218.31	470.24 ±13.61	134.22	142.32 ±10.62	120.65
Hyper + 100mg Et/kg B.Wt	193.26 ±10.78	209.83	408.66 ±17.61	116.64	131.61 ±18.56	111.57
L.S.D. (0.05)	19.367		28.143		22.912	

Each value represents the mean ± S.E.
Statistical analysis corresponding to hypercholesterolemic rats group.

Conclusively decreasing trend in lipid patterns was caused by several antioxidants such as polyphenols and flavonoids which found in ethanolic extract. On the other hand, antioxidants decrease LDL-cholesterol (LDL/HDL) ratio but caused an increase in (HDL-C). In this respect Steinberg *et al.* (2003) and Tatiana and Stanley (2006) showed that flavonoids can inhibit LDL oxidation *in-vitro* by scavenging radicals. This action protects the LDL particles and theoretically, flavonoids may have protective action against atherosclerosis .

Also, orange juice and grapefruit juice are rich sources of flavonoids the principal citrus flavonoids hesperetin from orange and naringenin from grapefruit are structurally similar to genistein, which decreased serum LDL-cholesterol by 43% in hyper-cholesterolemic rabbits Kurowska *et al.* (2000). In addition, liver cholesterol esters were decreased by 42% but fecal excretion of cholesterol or bile acids did not increase, suggesting that orange

juice components, possibly flavonoids, might affect cholesterol metabolism directly in the liver.

In support of this hypothesis, serum cholesterol was decreased and *in-vitro* activities of hydroxyl methyl glutaryl-COA reeducates and sterol O-acyltransferrase, 2 key enzyme in cholesterol metabolism were inhibited in cholesterol-fed rats supplemented with mixtures of principal citrus flavonoids Bok *et al.* (1999). Also, Zang *et al.* (2000) found that oral administration of coumaric acid (317mg/day) for 30 days significantly inhibited LDL oxidation.

Table (8): Serum lipoprotein cholesterol fraction of normal hyper-cholesterolemic rats.

Treatment	HDL-cholesterol		LDL- cholesterol		Risk ratio (LDL- Cholesterol / HDL- cholesterol)
	mg/dl	%	mg/dl	%	%
Control (negative)	51.82 ±3.14	100.00	38.12 ±2.66	100.00	0.736 ± 0.029
Control (Positive)	35.79 ± 1.86	69.06	213.34 ±7.63	559.65	5.961 ± 0.357
Hyper + 50mg Et /kg B.Wt	38.99 ±2.61	75.24	142.92 ±2.01	374.92	3.665 ± 0.165
Hyper + 75 mg Et /kg B.Wt	42.18 ±3.18	81.40	122.88 ±1.37	322.35	2.913 ± 0.175
Hyper + 100mg Et/kg B.Wt	46.29 ±2.99	89.33	99.99 ±0.97	262.30	2.160 ± 0.151
L.S.D. $_{(0.05)}$	5.093		6.911		0.372

Each value represents the mean ± S.E
Statistical analysis corresponding to hypercholesterolemic rats group.

8- Effect of palm tissues ethanolic extract on activities of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes.

Data in Table (9) revealed an increase in the activities of ALT and AST enzymes in hypercholesterolemic rats but a non significant decrease in the activities when treatment with different concentrations of flavonoids extract these results are in agreement with Beutner *et al.* (2001) who showed that flavonoids and carotenoids appear to be involved in protection against both singlet and triplet oxygen, singlet oxygen is known to be capable damaging DNA.

Table (9): Effect of date palm ethanolic extract at different concentration on activities of liver marker enzymes.

Treatment	AST (IU/L)	ALT (IU/L)
Control (negative)	31.20 ± 1.24	20.36 ± 1.02
Control (Positive)	34.26 ± 3.14	23.91 ± 2.13
Hyper + 50mg Et /kg B.Wt	32.98 ± 2.01	23.01 ± 1.51
Hyper + 75 mg Et /kg B.Wt	30.48 ± 1.45	20.53 ± 2.64
Hyper + 100mg Et/kg B.Wt	31.02 ± 1.97	21.09 ± 1.76
L.S.D. $_{(0.05)}$	3.766 ^{a}	3.445 ^{a}

Each value represents the mean ± S.E; ^{a}: Non-significant differences at P < (0.05).
Statistical analysis corresponding to hypercholesterolemic rats group.

9- Effect of ethanolic extract on serum urea and creatinine content.

Data in Table (10) show the kidney function tests for normal and hypercholesterolemic rats treated with ethanolic extract that induced a non-significant decreasing effect on urea and creatinine respectively to compared to the positive control. The same results were obtained by Devi and Shyamala (1999) who remarked the cytoprotective effect of flavonoids (quercetin) on induced nephrotoxicity in rats and the protection can be attributed to a decreased in lipid peroxide formation restoration of glutathione status and the activities of antioxidant.

Table (10): Effect of date palm ethanolic extract at different concentration on urea and creatinine contents in the serum.

Treatment	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Control (negative)	29.89 ± 1.36	1.21 ± 0.098
Control (Positive)	33.62 ± 2.61	1.39 ± 0.10
Hyper + 50mg Et /kg B.Wt	31.18 ± 1.95	1.30 ± 0.89
Hyper + 75 mg Et /kg B.Wt	29.88 ± 2.12	1.28 ± 0.93
Hyper + 100mg Et/kg B.Wt	27.70 ± 1.19	1.23 ± 0.10
L.S.D. $_{(0.05)}$	3.745 ^{a}	1.057 ^{a}

E.S value represents the mean ± S.E; ^{a}: Non-significant differences at P < (0.05). Statistical analysis corresponding to hypercholesterolemic rats group.

Conclusion

As a results of this study ethanolic extracts (Et) of date palm tip shoot give a high effect in free radical and superoxide radical scavenging activity than other extracts (EA-1 and EA-2) and it has hypocholesterolemic effect on hypercholesterolemic rats.

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التأثيرات المضادة للأكسدة والمخفضة لكولستيرول الدم في فئران التجارب المرتفعة الكولستيرول باستخدام فلافونيدات أنسجة القمة النامية لنخيل البلح المصري

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تعتبر الفلافونيدات من المركبات الثانوية الهامة التي لها العديد من الوظائف البيولوجية حيث أنها تعمل كمضادات لعملية الأكسدة داخل الجسم ومضادات للأورام السرطانية وأيضا كمضادات للميكروبات.

وتعتبر أنسجة القمة النامية لنخيل البلح (الجمار) من المصادر الهامة لهذه المركبات ولهذا تم في هذا البحث أخذ أنسجة القمة النامية لنخيل البلح في الحالة الطازجة وتم إجراء استخلاص للمركبات الفلافونيدية الموجودة بها باستخدام مذيبات مختلفة في القطبية مثل الإيثانول ٨٠% و خلات الإيثايل قبل ويعد أجراء عملية التحليل المائي باستخدام حمض الهيدروكلوريك للحصول علي الاجيلكون (الفلافونيدات) في صورة حرة وتم وزن الناتج وتقدير محتوي الفلافونيدات بعد أجراء عملية الاستخلاص السابقة وقد وجد أن المستخلص الايثانولي أعطي أعلي ناتج وأعلي محتوي من الفلافونيدات.

ولهذا تم أجراء تفريد للمركبات الفينولية و الفلافونيدية الموجودة في المستخلص الإيثانولي باستخدام جهاز التحليل الكروماتوجرافي عالي الكفاءة فوجد أنه يحتوي علي تركيزات عالية من كل من الميرستين، و الكومارين وحمض البيروجاليك.

ونظرا لأن هذه المواد لها خواص عالية كمضادات أكسدة فتم إجراء تجربة معملية لقياس قدرة المستخلصات المختلفة علي الاتحاد مع الشقوق الحرة والتخلص منها وذلك باستخدام مركب ٢،٢ داي فينايل بكريل هيدرازيل وأيضا تم قياس قدرة هذه المستخلصات علي التخلص من شق السوبر أوكسيد باستخدام صبغة نتروبلوتترازوليم فوجد أن المستخلص الإيثانولي له قدرة عالية علي التخلص من الشقوق الحرة المختلفة مقارنا بمستخلصات خلات الإثيايل المختلفة و أيضا بالمقارنة بالروتين كمادة قياسية.

وأيضا نظرا لأن المركبات الفلافونيدية تعمل كمواد مخفضة لدهون الدم فتم إجراء تجربة بيولوجية باستخدام فئران التجارب من بعد تغذيتها علي عليقه عالية في محتوي الكولسترول لمدة ٧ أسابيع وبعدها تمت المعاملة بثلاث تركيزات وهم ٥٠ ، ٧٠، ١٠٠ ملجم/كجم من وزن الجسم من المستخلص الإيثانولي لمدة ٤ أسابيع فوجد أن المعاملة بالمستخلص الإيثانولي أدي إلي انخفاض مستوي الكولسترول والدهون الكلية و الجليسريدات الثلاثية و انخفاض مستوي الكوليسترول المنخفض الكثافة وزيادة مستوي الكولستيرول عالي الكثافة كذلك وجد أن المستخلص الإيثانولي عمل علي تحسين كل من وظائف الكبد والكلية في الفئران المصابة بارتفاع كوليسترول الدم.

لذلك يوصي البحث باستخدام هذه المواد الطبيعية ومستخلصاتها في الوقاية من العديد من الأمراض الهامة نظرا لقلة أثارها الجانبية مقارن بالأدوية المستخدمة.