Effect of date palm (*Phoenix dactylifera* L.) flavonoids on hyperglycemia

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ABSTRACT

Phytochemical screening of date palm inflorescence (Zaghloul cv.) was performed to determine active constituents of source (*in vivo*) and callus (*in vitro*) tissue. Flavonoids, carbohydrates and/or glycosides, sterols and/or triterpenes were present at high concentrations. Flavonoids were selected to study their effect on hyperglycemia disease. Serum glucose level for alloxan-induced diabetic rats was decreased from 325.48 mg/100 ml in positive control rats to 288.39 mg/100 ml in rats administrated with 40 mg ethanolic extract / 100 g diet, after 4 weeks. Results of liver function tests showed that feeding normal rats with ethanolic extract induced a statistically significant increase in the levels of Serum glutamate oxaloacetate transaminase (sGOT) and serum glutamate pyruvate transaminase (sGPT) compared to the control negative group. On the other hand hyperglycemia rats fed with ethanolic extract showed a significant decrease in the sGOT and sGPT compared to the positive control. Feeding rats diet including ethanolic extract decreased the total lipids content and total cholesterol. Kidney's function tests for hyperglycemia revealed that rats treated with ethanolic extract induced a decreasing level of Urea, Creatinine and Alkaline phosphatase to the positive control. Flavonoids compounds were extracted from date palm tissue are often added as an extract to many convenience foods protect from hyperglycemia and some other diseases.

INTRODUCTION

Flavonoids are a widely distributed group of polyphenolic compounds with health-related properties, which are based in their antioxidant activity. These properties have been found to anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility, and an ability to inhibit human platelet aggregation **(Benavente-Garcia *et al*., 1997**).

It is generally assumed that the active dietary constituents contributing to these protective effects are the antioxidant nutrients, although more recent work has highlighted the additional role of the polyphenolic components of the higher plants **(Hertog *et al.*, 1993),** which may act as antioxidants or agents of other mechanisms that contribute to their anticarcinogenic or cardioprotective actions. These compounds have applications in food stabilization due to their ability to protect against peroxidation of oxygen sensitive foods. Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-y-pyrone structure, which have been reported to act as antioxidants in various biological systems **(Morel *et al*., 1993; Salah *et al*., 1995; Wang and Zheng, 1992).** Flavonoids present in wide varieties of edible plants, especially in Citrus species. Four types of flavonoids (flavanones, flavones, flavonols, and anthocyanins, the last only in blood oranges) occur in Citrus **(Horowitz and Gentili, 1977)**, and more than 60 individual flavonoids have been identified.

The preliminary photochemical screening of date palm inflorescence (Zaghloul cv.) showed the presence high concentrations of flavonoids and carbohydrates (**Abo-El-Soaud, 2003**). In addition to other active constituents such as tannins, saponins, sterols and/or triterpenes, alkaloids and/or nitrogen bases and coumarins which present in different *in vivo* and *in vitro* date palm tissues (shoot tip, pollen grain, leaves, fruits and callus **(Mohamed *et al.*, 2001)**. In this paper we study mainly the health-related property of inflorescence-date palm flavonoids, which are based in their antioxidant activity (hyperglycemia).

MATERIALS AND METHODS

The experimental work was carried out at the period of 2000-2003 in the laboratory of plant tissue culture, Agriculture Development System project (ADS), Ministry of Agriculture and the Central Laboratory of Chemistry, Faculty of Agriculture, and Cairo University. Plant materials were secured from inflorescence cultures in tissue culture part (part I, micropropagation).

Preliminary phytochemical screening:

The dried powdered samples of *in vivo* (source tissue) and *in vitro* (callus) tissues derived from the female *Phoenix dactylifera* L. inflorescence and their extracts were subjected to preliminary phytochemical screening as follows:

1. **Test for carbohydrates and/or glycosides:**

One gram of the powdered sample was extracted with 10 ml of 50% ethanol. About 5 ml of the ethanolic extract was mixed with 0.5 ml ethanolic α-naphthol, then 1ml sulphuric acid was carefully poured on the wall of the test tube to form a lower layer. A violet ring forming at the interface, indicating the presence of carbohydrates and/or glycosides; **(Lewis and Smith, 1967).**

2. **Test for tannins:**

About 5 gram of the powdered sample was extracted with 50% ethanol and filtered. Upon the addition of ferric chloride reagent, (10% ferric chloride ($Fe\ Cl_3$) solution in ethanol), to the filtrate, a green color formed which changed to a blush black color or precipitate, indicating the presence of tannins; **(Shellard, 1957).**

3. **Test for flavonoids:**

Ten grams of the powdered sample was macerated in 50 ml of 1% hydrochloric acid overnight, then filtered, and the filtrate was subjected to the following tests:

a) About 10 ml of the filtrate was rendered alkaline with NaOH; formation of a yellow color, indicating the probable presence of flavonoids.
b) Shinoda test:

About 5 ml of the filtrate was mixed with 1 ml HCl, and magnesium metal was added. A red color formation indicates the presence of flavanones and/or flavonols; **(Geissman, 1961).**

4. **Test for saponins:**

About 5 gram of the dried powder was macerated in 20 ml water and the filtrate was shaken vigorously. A persisting froth for about 30 minutes indicates the possible presence of saponins; **(Shellard, 1957).**

5. **Test for sterols and/or triterpenes:**

About 1 gram of the powdered material was extracted with 10 ml petroleum ether (40-60˚C). The filtrate was evaporated to dryness. The residue was dissolved in 5 ml anhydrous chloroform and filtered. To the filtrate, 0.3 ml acetic anhydride was added, followed by few drops of sulphuric acid down the side of the tube. A reddish violet ring is forming at the junction of the two layers indicating the presence of unsaturated sterols and/or triterpenes; **(Hanson, 1972)**.

6. **Test for alkaloids and/or nitrogenous bases:**

The residue of 50 ml of the alcoholic extract was dissolved in 20 ml diluted HCl (20%), and filtered. The filtrate was rendered alkaline with NH_4OH and extracted with successive portions of chloroform. The combined chloroformic extracts were evaporated to dryness, the residue was dissolved in 2 ml diluted HCl and tested with Mayer's reagent (mercury $^{+2}$, potassium iodide, hydrochloric acid, zinc chloride, ammonia solution) and Dragendorff's reagent (bismuth^{+3}, carbonate basic, sodium iodide, glacial acetic acid, ethyl acetate, 0.1 N sulphuric acid). If no precipitate was formed, that indicates the absence of alkaloids and/or nitrogenous bases; **(Farnsworth *et al*. 1964).**

7. **Test for coumarins:**

The powdered sample (5gm) was subjected to a sublimation, and a filter paper moistens with NaOH solution was exposed to sublimate and then examined for any fluorescence under UV light; **(Feigl, 1960)**.

Pharmacological study:

pharmacological studies of the ethanolic extract of flavonoids were carried out on rats to evaluate their effects on different animal organs as to detect their toxicity on humans.

Experimental animals:

The effect of natural extracts on liver and kidney's functions were evaluated using adult male albino rats, 60-80 g. The animals were obtained from Helwan Station for Experimental Animals, Helwan, Cairo, Egypt. The animals were grown in the animal house of Biochemistry Department, Faculty of Agriculture, Cairo University. The animals were kept under normal laboratory conditions (temperature remain 25± 2^0C) for 2 weeks before the initiation of the experiments and were fed on a standard commercial diet consisting of casein (12%) corn seed oil (10%) salt mixture (4%), vitamin mixture (1%), equal proportion of sucrose and corn starch (63%) and crude fiber (10%), according to (**Ibrahim, 1982)** with some modifications.

Composition of the salt mixture(g/Kg of diet):

Element	Quantity
Ca	**4.0**
K	**2.4**
Na	**1.6**
Mg	**0.4**
Fe	**0.12**
Mn	**0.032**
Cu	**0.005**
Zn	**0.018**
Co	**0.00004**
I	**0.00002**

This composition is according to **Bouziane *et al*., (1994)**.

Composition of vitamin mixture (mg/Kg of diet):

Vitamin	Quantity
Thiamine	**40.0**
Riboflavin	**30.0**
Nicotinic acid	**140.0**
Cyanocobalamine	**0.1**
Alfatocopherol	**340.0**
Biotin	**0.6**
Retinal	**12.0**
Cholecalciferol	**0.125**

This composition is according to **Bouziane *et al*., (1994)**.

Preparation of the Ethanolic extract for the bioassay:

About 50 gram of the air-dried powdered of each samples was exhaustively extracted with ethanol 70%. The ethanolic extract was concentrated under vacuum, and the residue was dissolved in distilled water (250ml). **(Mabry *et al.,* 1970)**.

Experimental design:

Rats were administrated the ethanol extract of date palm inflorescence orally by a stomach tube at the dose levels of 10, 20, 30 and 40 mg/100 g of diet (daily food intake 14 g/day for each rat), for 4 weeks. All rats were fed with the previous supplemented diet until the end of the experiment. Food consumption and body weight were recorded daily for each animal.

The rats were divided into 6 groups and each group consists of 9 rats as follows:

Group I : Rats served as untreated control, they fed with the basal diet (negative control).

Group II : Hyperglycemia rats, which treated with alloxan (positive control).

Group III: Rats fed with the basal diet supplemented with 10 (mg/100g diet) ethanolic extract of callus tissues

Group IV: Rats fed with the basal diet supplemented with 20 (mg/100g diet) extract of callus tissues

Group V : Rats fed with the basal diet supplemented with 30 (mg/100g diet) ethanolic extract of callus tissues.

Group VI: Rats fed with the basal diet supplemented with 40 (mg/100g diet) ethanolic extract of callus tissues.

Blood sampling:

Blood samples were obtained every week from the orbital plexus by means of a fine capillary glass tubes in accordance with the method of (Schermer, 1967). Each sample was placed in a dry clean centrifuge tube. The blood was centrifuged for 10 minutes at 3000 r.p.m to separate the serum from the cells. The clean non-haemolyzed supernatant serum was then dispensed into epindorff tubes and kept in the deep freezer (-20 °C) until analysis. Glucose level, total lipids, total cholesterol, creatinine, urea, the activity of alkaline phosphatase, sGOT and sGPT were determined.

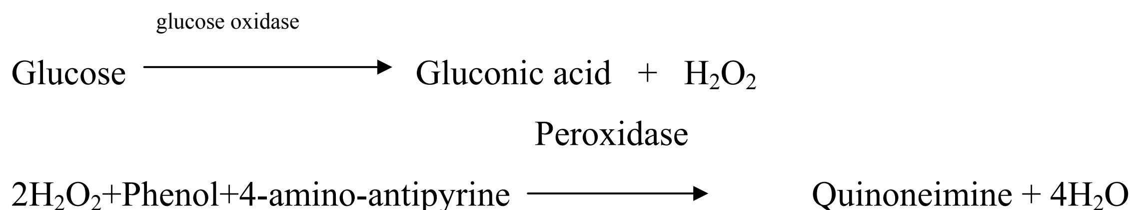
Induction of hyperglycemia:

Biochemical analysis:

Determination of serum glucose:

Principle :

Blood glucose was determined according to the procedure of **Trinder (1969)**. The glucose presents in the sample was determined according to the following reactions:



The produced quinoneimine was colourimetrically determined at wavelength 505 nm.

Reagents:

1. **Phosphate buffer solution:**

Sodium dihydrogen phosphate (8.9g) were dissolved in distilled water and adjusted the PH value to 6.8 with 0.1M of NaOH solution and then diluted up to one liter with distilled water to give final concentration of 75mM.

2. **Phenol reagent:**

Phenol (0.94g) was dissolved in one liter of phosphate buffer, to obtain a phenol solution 10mM concentration.

3. **Enzymatic reagent:**

Mixture of 4-amino-antipyrine (8.13mg), peroxidase (30 IU) and glucose oxidase (1000 IU) were dissolved in (100ml) of phenol reagent.

4. **Standard glucose solution:**

Pure glucose (100mg) was dissolved in enough volume of distilled water and the solution was diluted to (100ml) with distilled water.

Procedure:

The following tubes were prepared for enzymatic determination of glucose:

Test tube	Sample	Standard	Blank
Serum (ml)	0.01	___	___
Standard glucose (ml)	___	0.01	___
Enzyme reagent (ml)	1.0	1.0	1.0

The solution was incubated at 37 °C for 10 minutes and the developed color was stable for 30 minutes. The absorbance of developed color was measured at 505 nm against blank.

Calculation:

The glucose concentration was calculated using the following equation:

$$\text{Serum glucose (mg / 100ml)} = \frac{\text{A Sample}}{\text{A Standard}} \times 100 \text{ (wt. of standard)}$$

Since A: absorbance at 505 nm.

Determination of serum total lipids:

Principle:

Serum total lipids were reacted with vanillin in the presence of sulphoric and phosphoric acids mixture. The produced colored complex was measured at 530nm according to the method described by **(Knight *et al*., 1972).**

Reagents:

1. **Standard lipids:**

Stearin (1g) was dissolved in (100ml) of chloroform: methanol (2:1, v\v).

2. **Phospho-vanillin:**

Vanillin (0.6g) was dissolved in (10ml) absolute ethanol and diluted to (100ml) with distilled water. Concentrated H_3PO_4 (400ml) was added with constant stirring and the mixture stored in a dark bottle at room temperature.

3. **Concentrated sulphuric acid 98% (analytical grade).**

Procedure:

The following tubes were prepared for determination of total lipids:

Test tubes	Sample	Standard	Blank
Serum (ml)	0.1	---	---
Standard (ml)	---	0.1	---
Sulphuric acid(ml)	3	3	3
The tubes were mixed well and plugged with apiece of cotton wool, then the lipid was hydrolyzed by heating in a boiling water bath for 10 minutes. After cooling the hydrolyzed lipid was used as in the following reactions:			
Phospho-vanillin reagent (ml)	3	3	3

After mixing, the tubes were left at room temperature for 30 minutes in a dark place. The intensity of obtained color was measured at 530nm against blank.

Calculation:

The concentration of total lipids in serum was calculated using the following equation:

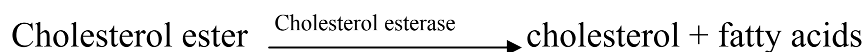
$$\text{Total lipid conc. (mg/100ml)} = \frac{\text{A Sample}}{\text{A Standard}} \times 1000 \text{ (conc. of standard)}$$

Since A: absorbance at 530 nm.

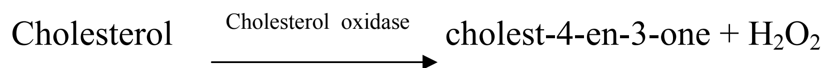
Determination of serum cholesterol:

Principle:

Cholesterol was determined according to the enzymatic method of **(Allain *et al*., 1974).** Cholesterol was determined according to the following reaction:



Cholesterol ester $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acids



Cholesterol $\xrightarrow{\text{Cholesterol oxidase}}$ cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + phenol + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ quinoneimine + $4H_2O$

Quinoneimine was colourimetrically determined at 500 nm.

Reagents:

1. **Phosphate buffer solution:**

Sodium dihydrogen phosphate (11.9g) was dissolved in distilled water and pH value was adjusted to 7.0 with NaOH solution (0.1M). Phenol (1.4g) and sodium cholate (1.6g) were added to phosphate solution and diluted to one liter with distilled water to give a final concentration of (0.1M).

2. **Enzymatic reagent solution:**

These reagent was prepared by dissolving of 4-amino-antipyrine (0.1g), peroxidase (1000 IU), cholesterol oxidase (200 IU) ,and cholesterol esterase (125 IU) in one liter of the previous phosphate buffer

3. **Standard cholesterol solution:**

Cholesterol (2.0g) was dissolved in one liter of isopropanol to give a concentration of (0.2% w/v).

Procedure:

The following tubes were set up :

Test tubes	Sample	Standard	Blank
Serum (ml)	0.01	---	---
Standard cholesterol (ml)	---	0.01	---
Enzymatic reagent (ml)	1.0	1.0	1.0

The tubes were incubated at 370C for 5 minutes and the intensity of the developed color was measured at 500nm against blank.

Calculation:

The cholesterol concentration was calculated by using the following equation:

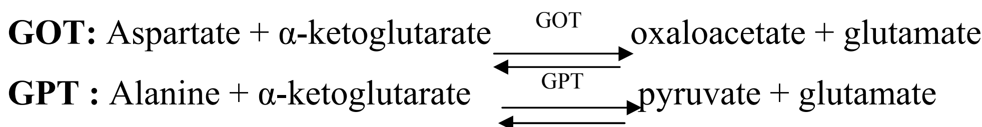
$$\text{Serum cholesterol (mg/100ml)} = \frac{A\ \text{Sample}}{A\ \text{Standard}} \times 200\ \text{(Conc. of standard)}$$

Whereas A = the absorbance at 500nm

Determination of serum transaminases:

Principle:

Serum glutamate oxaloacetate transaminase (sGOT) or serum aspartate transferase (sAST) and serum glutamate pyruvate transaminase (sGPT) or serum alanine transferase (sALT) activities were measured according to the method described by **(Reitman and Frankel, 1957).** The activity of GOT and GPT were determined colourimetrically according to the following reactions:



The produced pyruvate or oxaloacetate reacted with 2,4-dinitrophenylhydrazine and the obtained color was colourimetrically measured at 505nm.

Reagents :

1. **Phosphate buffer solution:**

Sodium dihydrogen phosphate (11.9 g) was dissolved in distilled water and pH value was adjusted to 7.4 with 0.1M of NaOH solution and completed to one liter with distilled water to give 0.1M concentrate.

2. **GOT substrate solution :**

dL-aspartic acid (2.66g) and α-ketoglutaric acid (29.2mg) were dissolved in aminimum volume of sodium hydroxide solution (1M). The solution made up to 100ml with phosphate buffer (pH 7.4).

3. **GPT substrate solution :**

A mixture of dL-alanine (1.78g) and α-ketoglutaric acid (29.2mg) were dissolved in aminimum volume of sodium hydroxide solution (1M). The solution made up to 100ml with phosphate buffer (pH 7.4).

4. **2,4-dinitrophenylhydrazine (2,4-DNP) solution:**

2,4-dinitrophenylhydrazine (19.8mg) was dissolved in 100ml hydrochloric acid solution (1M).

5. **Standard pyruvate solution:**

Sodium pyruvate (22.0mg) was dissolved in 100ml of phosphate buffer (pH 7.4).

6. **Sodium hydroxide solution:**

Pure NaOH (1.6g) were dissolved in 100ml distilled water to give (0.4M) concentrate.

Procedure:

1. **Preparation of standard curve of both GOT and GPT:**

The following tubes were prepared as follow:

Reagent	Tube number					
	1	2	3	4	5	6
Distilled water (ml)	0.2	0.2	0.2	0.2	0.2	0.2
GOT or GPT substrate (ml)	1	0.9	0.8	0.7	0.6	0.5
Standard pyruvate (ml)	---	0.1	0.2	0.3	0.4	0.5
2,4-DNP reagent (ml)	1.0	1.0	1.0	1.0	1.0	1.0
All tubes were mixed well and left at room temperature for 20min.						
NaOH solutin (ml)	10	10	10	10	10	10
The tubes were mixed again and left for 5 minutes. The intensity of developed color was measured at 505nm.						
GOT units/ml	0	22	55	95	150	215
GPT units/ml	0	25	50	83	126	---

2. **Measurement:**

GOT and GPT activities were measured as follow :

Test tubes	GOT	GPT
GOT substrate (ml)	1.0	---
GPT substrate (ml)	---	1.0
Incubation was carried out at 37^{0}C for 5minutes.		
Serum (ml)	0.2	0.2
After mixing, the tubes were incubated at 37^{0}C for:	Exactly 1 hour	Exactly 30 minutes
2,4-DNP reagent (ml)	1.0	1.0
The tubes were mixed well and left at room temperature for 20 minutes.		
NaOH solutin (ml)	10	10

The tubes were mixed well and left at room temperature for 5 minutes. The developed color was measured at 505nm against blank.

Calculation:

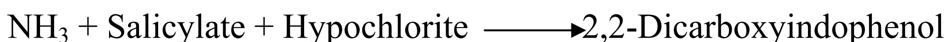
GOT and GPT activities were calculated using a standard curve.

Determination of blood urea:

Principle:

Blood urea was estimated by the enzymatic method of **Patton *et al.*, 1977** according to the following reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + 2CO_2$$



$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{2,2-Dicarboxyindophenol}$$

Urea in the sample is hydrolyzed by the enzyme urease, to yield ammonia and carbon dioxide. The ammonium ions react with the salicylate and hypochlorite to form a green colored indophenols .The intensity of colour was read at 630nm.

Reagents:

1. **Phosphate buffer solution:**

Sodium dihydrogen phosphate (14.3g) was dissolved in distilled water and pH value was adjusted to 8.0 with 0.1M of NaOH solution and completed to one liter with distilled water to give 120mM concentrate.

2. **Enzymatic reagent solution:**

It was prepared by dissolving sodium salicylate (6.0mmol), sodium nitroprusside (3.2mmol), EDTA (1mmol) and urease (10KU) in one liter of the previous phosphate buffer solution.

3. **Colour reagent solution:**

It was prepared by dissolving sodium hydroxide (130mmol) and sodium hypochloride (6mmol) in one liter of phosphate buffer solution (120mmol, pH 8)

4. **Standard urea solution :**

It was prepared by dissolving (30mg) urea in 100ml of distilled water.

Procedure:

The following tubes were prepared as follow:

Test tubes	Sample	Standard	Reagent blank
Enzyme reagent (ml)	1.5	1.5	1.5
Serum (ml)	0.01	---	---
Standard (ml)	---	0.01	---
All tubes were mixed well and incubated for 3 minutes at 37^0C.			
Colour reagent (ml)	1.5	1.5	1.5

All tubes were mixed well and incubated for 5 minutes at 37^0C. The intensity of colour was measured at 630nm against blank.

Calculation:

The urea concentration was calculated by using the following equation:

$$\text{Blood urea (mg/dl)} = \frac{\text{A Sample}}{\text{A Standard}} \times 30 \text{ (Conc. of standard)}$$

Whereas A = absorbance

Determination of serum creatinine:

Principle:

Serum creatinine was determined according to the method described by **(Faulkner and King, 1976)**.Excess of picric acid used for deproteinization. This technique avoids preparation of conventional tungestic acid protein- free filtrate or supernatant. Creatinine in a picric acid protein-free solution reacts with added alkali to form a reddish-brown complex. The absorbance of this compound at 520nm is proportional to creatinine concentration.

Reagents:

1. **Picric acid solution :**

This reagent was prepared by dissolving of picric acid (14g) in one liter of distilled water to give a final concentration (1.4% w/v).

2. **Sodium hydroxide solution :**

It was prepared by dissolving of sodium hydroxide (30g) in one liter of distilled water to give a final concentration (3.0% w/v).

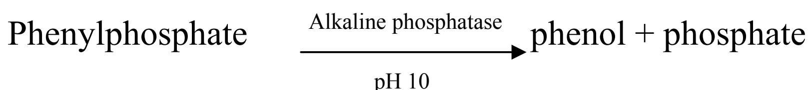
3. **Standard creatinine solution :**

It was prepared by dissolving of creatinine zinc hydrochloride (25mg) in one liter of picric acid solution (1.4%w/v).

Determination of serum alkaline phosphatase:

Principle:

The activity of alkaline phosphatase in serum was determined according to the method of **(Kind and King, 1954)**. Colorimetric determination of alkaline phosphatase activity was performed according to the following reactions:



The liberated phenol was measured in the presence of 4-amino- antipyrine and potassium ferricyanide. Sodium arsenate was added to stop the enzymatic reaction.

Reagents:

1. **Substrate buffer:**

   Five m mol disodium phenylphosphate, (6.36g) anhydrous sodium carbonate and (3.36g) sodium bicarbonate were dissolved in distilled water and adjusted the pH value to 10 and then diluted up to one liter with distilled water.

2. **Standard phenol:**

   Phenol Solution is equivalent to 30 U/L.

3. **Blocking reagent:**

   (0.6g) 4-amino-antipyrine and (2.4g) sodium arsenate were dissolved in 100ml distilled water.

4. **Colour reagent :**

   150 m mol potassium ferricyanide was dissolved in 1.0 liter distilled water.

Procedure:

The following tubes were prepared to determine the activity of alkaline phosphatase in serum:

<table>
<tr><th>Test tubes</th><th>Sample</th><th>Blank 1</th><th>Standard</th><th>Reagent blank 2</th></tr>
<tr><td>Substrate buffer (ml)</td><td>2.0</td><td>2.0</td><td>2.0</td><td>2.0</td></tr>
<tr><td colspan="5">The solutions were incubated at 37^0C for 5 minutes.</td></tr>
<tr><td>Serum (ml)</td><td>0.05</td><td>---</td><td>---</td><td>---</td></tr>
<tr><td>Standard phenol (ml)</td><td>---</td><td>---</td><td>0.05</td><td>---</td></tr>
<tr><td colspan="5">All tubes were incubated for exactly 15 minutes at 37^0C</td></tr>
<tr><td>Blocking reagent (ml)</td><td>0.5</td><td>0.5</td><td>0.5</td><td>0.5</td></tr>
<tr><td colspan="5">All tubes were mixed well.</td></tr>
<tr><td>Colour reagent (ml)</td><td>0.5</td><td>0.5</td><td>0.5</td><td>0.5</td></tr>
<tr><td>Serum</td><td>---</td><td>0.05</td><td>---</td><td>---</td></tr>
<tr><td>Distilled water (ml)</td><td>---</td><td>---</td><td>---</td><td>0.05</td></tr>
</table>

All tubes were mixed well and incubated in the dark for 10 minutes. The intensity of colour was read at 510nm against reagent blank.

Calculation:

Activity of serum alkaline phosphatase (~~U/L~~) = $\frac{\text{A serum sample} - \text{A serum blank}}{\text{A Standard}} \times 30$

Whereas A = absorbance at 510 nm.

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

In vivo (source tissue) and *in vitro* (callus tissue) explants secured from the female inflorescence of date palm Zaghloul cv. were subjected to varies tests to detect some chemical constituents such as carbohydrates and/or glycosides; tannins; flavonoids; saponins; sterols and/or triterpenes; alkaloids and/or nitrogenous bases ,and coumarins, as shown in Table (1).

Carbohydrates and/or Glycosides: source and callus tissues of female inflorescence contained high amounts of carbohydrates.

Tannins: on the addition of Fe Cl3 to the clear filtrate of source and callus tissues, green color was observed, that indicating the presence of catechol tannins in both tissues in different amounts. Since, they were markedly present in the in vivo tissue (++) more than *in vitro* (+) ones. Either turning the green color into blush black color was indicating the presence of free gallic acid.

Flavonids: data in Table (1) showed the high amount of flavonoids in source tissue while a moderate amount in callus tissue. Two test were performed to determine the total flavonids, as well as to detect flavonones and/or flavonols, which present in a high amounts in the acidic solution of inflorescence extracts as indicated by red color. These results are agreement with **Mohamed *et al*., (2001)** who demonstrated that the flavonids are present only in pollen grains in high amount and shoot tip in moderate amount (*in vivo* tissues). Concerning flavonols and/or flavonones, they are present in some date palm explants in low amounts.

Saponins: A persisting froth for about 30 minutes in aqueous solution indicates the possible presence of saponins, but in low amounts in source and callus - inflorescence tissues.

Sterols and/or Triterpenes: there are moderate amount of sterols. Since, the two samples of both tissues of female inflorescence gave positive reaction to Hanson test, indicating the presence of unsaturated sterols and/or triterpenes.

Alkaloids and/or Nitrogenous bases: were detected in source tissue as well as inflorescence callus tissue in low amounts.

Coumarins: no any fluorescence under UV light was detected indicating the absence of coumarins in both samples.

One can conclude that, carbohydrates and flavonoids present in female inflorescence date palm in high amounts in both tissues. Also, it has got almost moderate amounts of tannins and sterols. Whereas, saponins and alkaloids are detected in low amounts. But, no presence of coumarins was observed in samples.

The preceding results are in harmony with **Mossa *et al*., (1986)** in their phytochemical analysis of date palm seeds in Saudi Arabia **(Mohamed *et al*., 2001)** who reported that carbohydrates; steroids; flavonoids and tannins are mostly found in all tested samples, either in vivo shoot tips; pollen grains; leaves and fruits tissues or *in vitro* callus; embryos and leaves tissues. Also, they found that they were markedly present in the in vivo tissues more than the *in vitro* ones. They suggested that, it is of interest to do trials to increase such compounds of biological values. In this study, we consider with increase the flavonoids which have antioxidant effects as well as a principal purpose in this study.

Table (1): Preliminary phytochemical screening of dried powdered of date palm- inflorescence callus, cv. Zaghloul.

Test	Source tissue	Inflorescence callus tissue
Carbohydrates and/or Glycosides	+++	+++
Tannins	++	+
Flavonoids	+++	++
Saponins	±	±
Sterols and/or Triterpenes	++	++
Alkaloids and/or Nitrogenous bases	±	±
Coumarins	–	–

(+++) high amount, (++) moderate amount, (+) low amount, (±) trace, (–) absent.

1. Effect of flavonoids on hyperglycemia:

1.1. Daily gain in body weight:

Data in Table (2) represents gain in animal body weight every day, during 30 days (period of experiment). Rats were subjected to 40 mg ethanolic extract incorporated into 100 g of a diet gained 1.544 g/day in comparison of normal rats (control negative) where the gain in body weight was 1.914 g/day. Whereas rats of positive control a gain in body weight was 0.973 g/day, after 30 days and the body weight was increased gradually by increasing the amount of ethanolic extract of date palm tissue from 1.020 g/day to 1.544 g/day. Data presented in Table (2) showed that date palm ethanolic extract administration caused slight increases of body weight as a result of arising the health of hyperglycemic rats.

1.2. Daily feed intake:

Data in Table (2) indicated that feed efficiency was declined considerably in hyperglycemic rats (control positive) where it was 12.604 g/day in comparison with 14.033 g/day in normal rats. Feed intake in hyperglycemic rats treated with ethanolic extract was found more efficient than hyperglycemic rats (12.604 g/day) since it was in between 12.887 and 13.656 (g/day), after 30 days.

Table (2) : Gain in body weight, food intake and food efficiency in (Hyperglycemia) rats for alloxan-induced diabetic rats after 4 weeks.

Parameters / Group	Initial body weight(g*)	Final body weight(g)	Gain in body weight(g)	Daily gain-body weight(g/day)	Feed intake (g)	Daily feed intake (g/day)
Control negative	101.21	158.63	57.42	1.914	421.36	14.033
Control positive	105.52	134.71	29.19	0.973	378.13	12.604
Group (1)	107.61	138.22	30.61	1.020	409.64	13.656
Group (2)	103.32	140.69	37.73	1.245	401.61	13.387
Group (3)	104.69	147.54	42.85	1.428	398.37	13.279
Group (4)	101.68	148.00	46.32	1.544	386.61	12.887

* gram(s). Each value represents the mean of 9 rats. Group 1 contained 10; 20; 30 and 40 mg l^{-1} ethanolic extract of flavonoids.

1.3. Organs weight:

Data presented in Table (3) showed the effect of addition of flavonoids extract in diabetic rat's diet and reflective effect on different organs weight. Indeed, different organs ratios indicated that all organs weight were increased because of the organs inflation and didn't work correctly. Since, the weight ratios of liver; kidney; heart; brain and tests in control positive were 0.0518; 0.0114; 0.0061; 0.0114 and 0.0178 respectively, out of the initial weight, in compare to control negative (normal rats) where they were 0.0384; 0.0075; 0.0045; 0.0088 and 0.0124, respectively. These ratios of positive control were gradually decreased by adding flavonoids in feed diet. Due to the effect of the date palm flavonoids to raise the efficiency of these organs in overcome of hyperglycemia dangerous.

From previous data in Tables (2, 3) we can extract that the health of control positive rats when the diet contained the hyperglycemic agent (alloxan) was decreasing strongly in addition to organs weight and the rats feed intake decreased too, as mentioned previously. This led to decreasing in body weight, since it was 134.71 g compared to 158.63 g in normal rats. These obstacles could be overcome by adding ethanolic extract of date palm tissue which includes flavonoids that reduce the effect of this hyperglycemic agent where it has got antioxidant effect. As subsequently the body weight of alloxan-diabetic rats which treated with flavonoids extract staidly was increased by increasing the flavonoid content (from group-1 to group-4), indicated that they were returning into a normal case. From our point of view using tablets of flavonoids powder may be effective in the hyperglycemia disease remedy. As well as, flavonoids inclusion into foods and cosmetics industry will reduce the danger of the oxidative factors in these substances. Adding natural antioxidants is more preferable than using artificial antioxidants that affect in human health. This concept received some support nowadays from using natural compounds pathway in medicate diseases, but before using the inflorescence-date palm flavonoids, it should be study the effect of date palm flavonoids on a human public

health, for this purpose we need to study the liver and kidney functions on rats before recommendation with safe usage in varies human life aspects.

Regarding the human public health, future emphatically studies focusing on the effect of date palm flavonoids on liver and kidney's function should be required in order to recommend the safe usage of such flavonoids in varies human life aspects.

Table (3): Effect of administration of ethanolic extract of date palm inflorescence on organ weight(grams) of diabetic rats, after 4 weeks in culture.

Treatments	Final weight	Liver		Kidney		Heart		Brain		Tests	
		Wt.*	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
Control negative	158.63	6.10	0.0384	1.20	0.0075	0.72	0.0045	1.41	0.0088	1.97	0.0124
Control positive	134.71	6.98	0.0518	1.54	0.0114	0.82	0.0061	1.54	0.0114	2.40	0.0178
Group (1)	138.22	6.77	0.0489	1.31	0.0095	0.79	0.0057	1.48	0.0100	2.12	0.0153
Group (2)	140.69	6.35	0.0451	1.28	0.0090	0.74	0.0052	1.39	0.0098	2.00	0.0142
Group (3)	147.54	6.47	0.0438	1.27	0.0086	0.71	0.0048	1.37	0.0093	1.89	0.0128
Group (4)	148.00	6.29	0.0425	1.24	0.0084	0.68	0.0045	1.32	0.0089	1.88	0.0127

*Weight.

1.4. Liver function test:

1.4.1. Total lipids:

Data presented in Table (4) revealed that, the total lipids in normal rats was 430.61 mg/100 ml of serum, but this amount considerably increased when rats treated with alloxan (a diabetic agent), since it was 760.32 mg/100 ml of serum. This indicates that the liver of diabetic rats was unable to discard the access of lipids. While, the health of these rats were scanty ameliorated when the diet of diabetic rats contained the ethanolic extract of date palm inflorescence. Where, the total amounts of lipids were steadily decreased.

1.4.2. Total cholesterol:

The total cholesterol in normal rats was 112.51, while this amount was increased in hyperglycemic rats, when the rats were treated with alloxan (280.32), as shown in Table (4). On the other side the total cholesterol was decreased when hyperglycemic rats were treated with the ethanolic extract of inflorescence flavonoids. Since, they were 272.58; 250.69; 243.09 and 232.43 for the groups 1;2;3 and 4, respectively.

Table (4) : Effect of administration with Ethanolic Extract of flavonoids of date palm inflorescence on total lipids, cholesterol, SGOT* and SGPT♠ in hyperglycemia rats, after 30 days.

Parameter / Group	Total lipids (mg/100 ml)	Total cholesterol	SGOT	SGPT
Control negative	430.61	112.51	56.67	39.32
Control positive	760.32	280.32	72.16	46.41
Group (1)	742.41	272.58	64.67	45.06
Group (2)	720.56	250.69	62.84	42.68
Group (3)	670.82	243.09	58.79	41.71
Group (4)	610.61	232.43	56.32	38.81

Each value represents the mean of 9 rats.

* serum glucose oxalate trasferase; ♠ serum glucose pyruvate transferase.

1.4.3. Serum Glucose Oxalate Trasferase (sGOT):

Table (4) shows the effect of the enzyme that turns the excess of glucose in serum into other organic acid to discard the increment of sucrose. The normal rate of glucose oxalic acid transferase or in another word aspartate transaminase enzyme (AST) was 56.67 μ/ml of serum. This level of this enzyme increased in hyperglycemic rats, since it was 72.16 μ/ml required to get rid of the high level in serum. Through the way to return to the normal case, the diet of rats contained the flavonoids of date palm, as an ethanolic extract. Hence, the level steadily decreases

by increasing the level of ethanolic extract. The best result occurred when 40 mg l^{-1} of ethanolic extract of date palm was included into the diet which administrated for hyperglycemic rats. Where the enzyme level was 56.32 µ/ml.

1.4.4. Serum Glucose Pyruvate Transferase (sGPT):

The same trend in GOT, or in other word alanine transaminase enzyme (ALT) was found in GPT, as shown in Table (4). Since the amount of the enzyme that turns the glucose into pyruvic acid was 39.32 µ/ml and 46.41µ/ml for control negative and control positive, respectively. Whereas, the level of enzyme was decreased by increasing the level of flavonoids in diet.

1.5. Kidney function tests:

Figure (1) presents the kidney's function tests for normal and hyperglycemia rats treated with ethanolic extract of an inflorescence of date palm that induced a decreasing effect on the urea; creatinine and alkaline phosphatase to the positive control.

1.5.1. serum urea:

Data showed that serum urea level was raised from 28.13 mg/dl to 36.92 mg/dl when the rats were infected with hyperglycemia. After treating hyperglycemic rats with the ethanolic extract of flavonids, serum urea level decreased from 36.92 mg/dl to 32.02 mg/dl.

1.5.2. serum alkaline phosphatase:

The alkaline phosphatase level was considerably increased in serum in positive control treatment, where it was 387.36 mg/dl. Where, it was 216.81 in negative control treatment. The alkaline phosphatase slightly decreased to 316.78 mg/dl when 40 mg ethanolic extract of inflorescence that was added into hyperglycemic rats (group [4]).

1.5.3. serum creatinine:

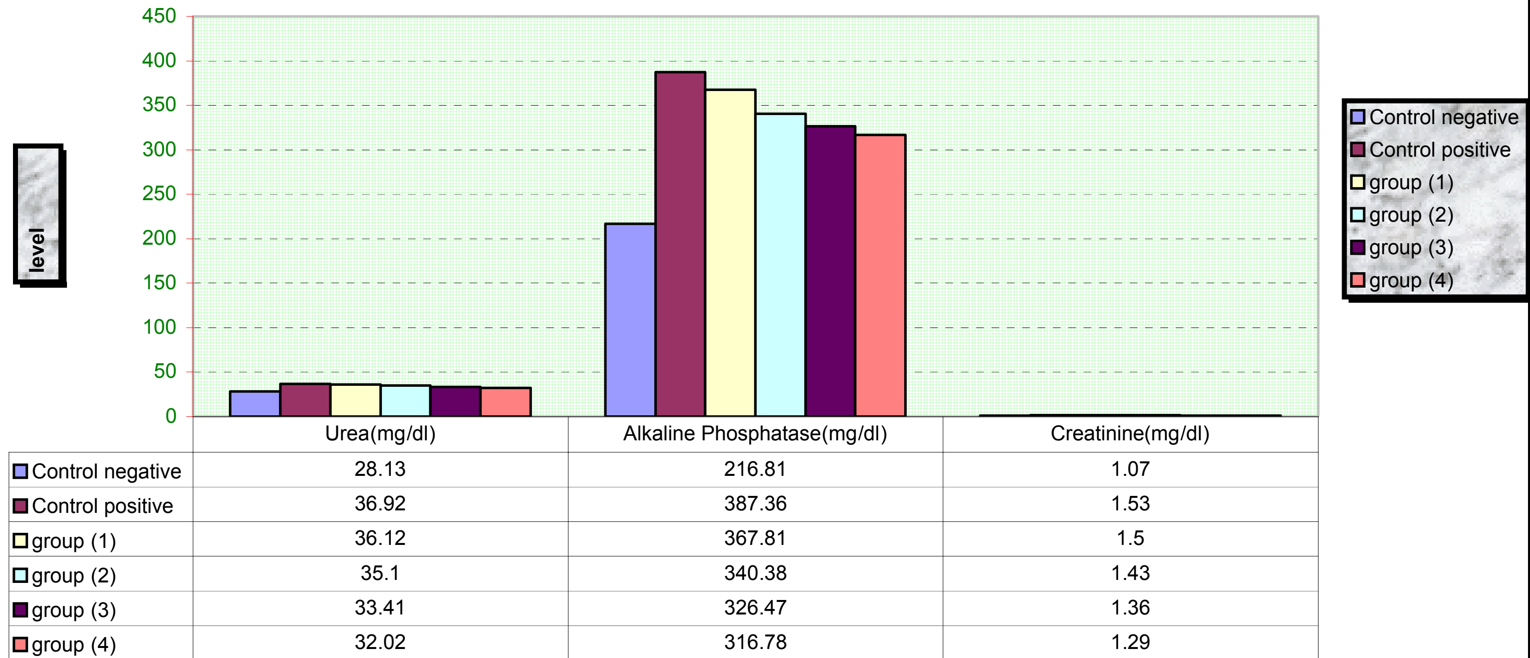
The same trend was observed in serum creatinine level, as mentioned in urea and alkaline phoshpatase. The creatinine level decreased from 1.53 mg/dl to 1.29 mg/dl when hyperglycemic rats were treated with ethanolic extract of date palm flavonoids.

1.6. serum glucose:

Data in Fig. (2) Showed the serum glucose content in the different groups during different times throughout the experiment period. In respect to glucose level in control negative, no change was observed during the experiment period. Wherever, it is ranged between in 100.58 and 106.15 (mg/100 ml). The reverse was found in control positive treatment, so that the glucose level was increased by increasing duration the experiment. Since, it was 160.12; 210.26; 280.37; 310.36 and 325.48 (mg/100 ml) after 2 days; 1 week; 2 weeks; 3 weeks and 4 weeks, respectively. As we have seen, glucose level was considerably raised when the rats were treated with hyperglycemic agent. Whereas, ethanolic extract was used to drop down the high level of serum glucose and its effect on different organ functions, the glucose level reduced by increasing the amount of ethanolic extract of flavonoids, as shown in Fig. (2).

Generally, we can conclude that increasing the activity of liver and kidney and their enzymes to reduce the excess of glucose in serum as a result of hyperglycemia. Whereas, treating with the ethanolic extract of date palm flavonoids was effective in reducing rates of GOT and GPT as an indicator to ameliorate the liver efficiency. As well as, reducing the rates of urea; alkaline phoshpatase and creatinine indicated that the kidney's functions were improved. This provides us a plausible explanation that the flavonoids which have been extracted from date palm inflorescence have a vital role in medicate hyperglycemia.

fig. (1): Effect of administration with Ethanolic Extract of flavonids of date palm tissues on urea, creatinine and alkaline phosphatase in rats, after 30 days.



	Urea(mg/dl)	Alkaline Phosphatase(mg/dl)	Creatinine(mg/dl)
Control negative	28.13	216.81	1.07
Control positive	36.92	387.36	1.53
group (1)	36.12	367.81	1.5
group (2)	35.1	340.38	1.43
group (3)	33.41	326.47	1.36
group (4)	32.02	316.78	1.29

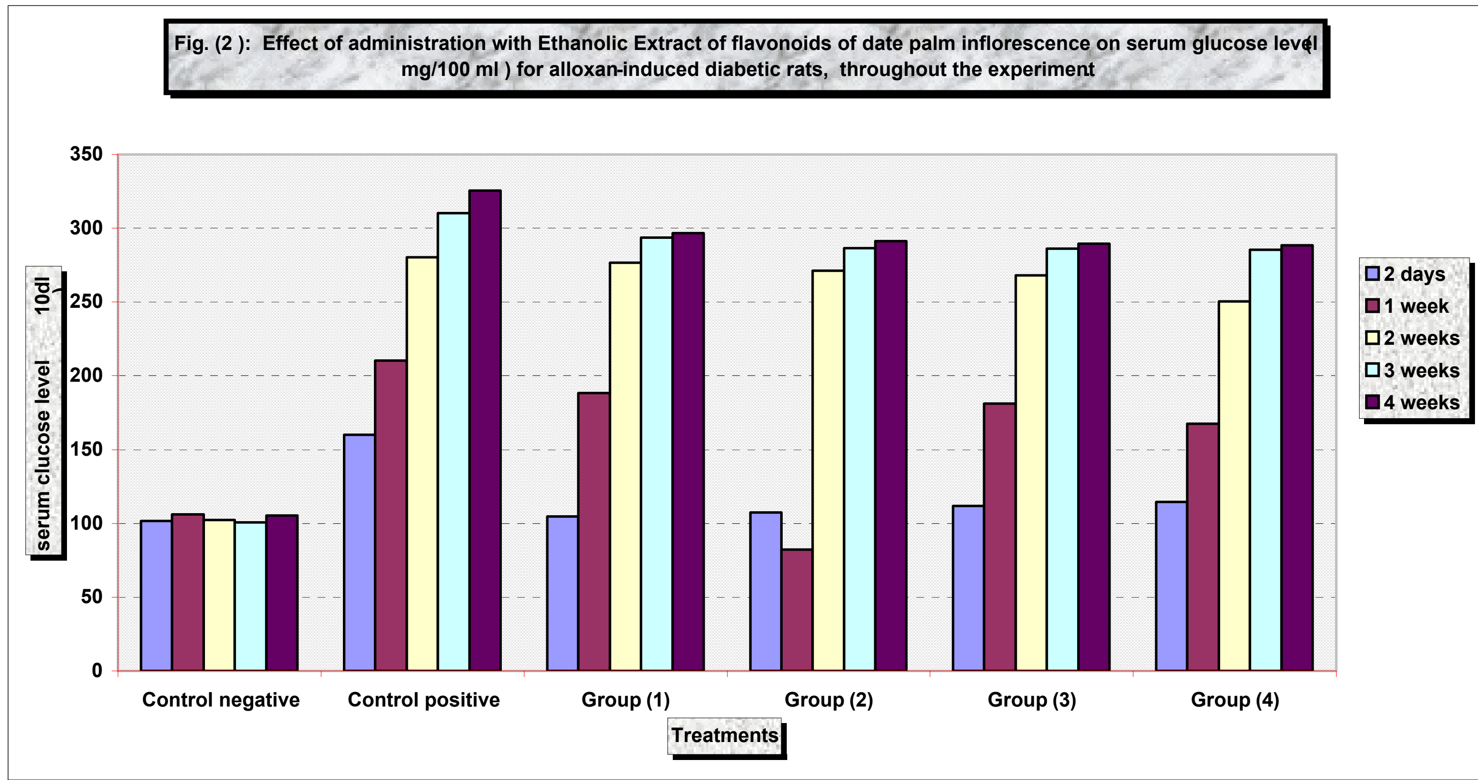


Fig. (2): Effect of administration with Ethanolic Extract of flavonoids of date palm inflorescence on serum glucose level(mg/100 ml) for alloxan-induced diabetic rats, throughout the experiment
350
300
250
200
150
100
50
0
serum clucose level 10dl
Control negative
Control positive
Group (1)
Group (2)
Group (3)
Group (4)
Treatments
2 days
1 week
2 weeks
3 weeks
4 weeks

REFERENCES

Abo-El-Soaud, A. A. (2003), "Biotechnological studies on date palm ". Ph.D. dissertation, Department of Pomology, Faculty of Agriculture, Cairo University, Egypt.

Allain, C.C.; L. S. Poon; C. S. Chan; W. Richamand and P. C. Fu (1974). "Enzymatic determination of total serum cholesterol". Clinical Chemistry, 20 (4):470.

Benavente-Garcia, O.; J. Catillo; F. R. Marin; A. Ortuno and J. A. Del Rio (1997). "Uses and properties of Citrus flavonoids". Journal of Agricultural and Food Chemistry. 45(12): 4505-4515.

Bouziane, M.; J. Prot and J. Bellerile (1994). "Changes in fatty acid composition of total serum and lipoprotein particle in growing rats given protein-deficient diets with either hydrogenated coconut or salmon oils as fat sources".Brit. J. Nutr.,71;345, 1-4.

Farnsworth, R.; W. Loub; R. Blomster; and M. Gorman (1964). "Pericylivine an new *Catharanthus* alkaloid". J. Pharmacology Science. 53; 1558-1564.

Faulkner, N.R. and King, J.W. (1976). "Fundamental of clinical chemistry". 2nd ed., Tietz (ed.), Sannders Philadelphia, Pp.994-998.

Feigl, H. (1960). "Spot tests in organic analysis". Elsevier Puplishing Co., New York, Eth Ed.

Geissman, T.A. (1961). "Chemistry of Flavonoid compounds". Macmillan Company, New York.

Hanson, J.R. (1972). "Chemistry of Terpens and Terpenoids". A.A. Newman (ed.) Academic Press. New York, PP.155-206.

Hertog, M. G. L.; E. J. M. Feskeens; C. H. Hollman; M. B. Katan and D. Kromhout (1993). "Dietary antioxidant flavonoids and risk of coronary heart disease: de Zutphen elderly study". Lancet. 342: 1007-1011.

Horowitz, R. and B. Gentili (1977). “Flavonoids constituents of Citrus”. In: Citrus Science and Technology; S. Nagy; P. E. Shaw and M. K. Vedhuis (eds.); AVI Publishing; Westport; CT. pp 397-426.

Ibrahim, M. (1982). “Biological studies on the interaction between certain agents and antibilharzial compound in the liver function in animals”. Ph.D. dissertation, Faculty of Agriculture, Cairo University.

Knight, J. A.; S. J. Anderson and J. M. Rawle (1972). “Chemical bases of the sulfo-phosphovanillin reaction for estimating serum total lipids”. Clinic Chemistry. 18 (3): 723.

Kind, R.N. and King, E.J. (1954). “Practical Clinical Biochemistry”. J. Clin. path., 7, 322. C.f varley. H, Gowenlock, A.H. and Bell, M. (1980). 5th ed Vol.1 p892. William Heinemann Medical Books Ltd. London.

Lewis, H. and C. Smith (1967). “Sugar alcohols in fungi and green plants, Methods of de4tection and estimation”. New phytol., 66, 185-204. Refered to Harbone, J. B. (1998). Phytochemical Methods, A guide to modern techniques of plant analysis, p54, New York, USA.

Mabry, T. J.; K. R. Markham and M. B. Thomas (1970). “The systematic identification of flavonoids. Springer-Verlag.

Mohamed, S. M.; H. A. Bosila; S. F. E. Sharabasy I. A. Ibrahim and K. A. Refay (2001). “Phytochemical screening of some in vivo and *in vitro* date palm tissue”. Al-Ain, United Arab Emirates, March 25-27.

Morel, I.; G. Lescoat; P. Cogrel; 0. Sergent; N. Pasdecoup; P. Brissot; P. Cillard and J. Cillard (1993). “Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures”. Biochemistry Pharmacology. 1: 13-19.

Mosa, J. S.; M. S. Hifnawy and A. G. Mekkawi (1986). “Phytochemical and biological investigations on date palm seeds (*Phoenix dactylifera* L.) produced in Saudi Arabia Arab”. Gulf Journal of Scientific Research. 4:(2), 495-507.

Patton , C.J. and Couch, S.P. (1977). Spectrophotometric and kinetics investigation of the bertholot reaction for determination of amonia. Anal. Chem., 49: 464-469.

Reitman, S. and S. Frankel (1957). "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyrivic transaminases. American Journal of Clinical Pathology, 28 (56): 56.

Salah, N.; N. J. Miller; G. Paganga; L. Tijburg; G. P. Bolwell and C. Rice-Evans (1995). "Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain-breaking antioxidants". Arch. Biochemistry Biophysics. 2: 339-346.

Shellard, E.j. (1957). "Practical Plant Chemistry". London Ritman Medical Publishing Co. Ltd.

Trinder, P. (1969). "Determination of blood glucose using an oxidation peroxidase system with a non carcinogenic chromogene". Annals Clinic Biochemistry. 6: 24.

Wang, P. and R. L. Zheng (1992). "Inhibitions of the autoxidation of linoleic acids flavonoids in micelles". Chemistry Phys. Lipids. 63: 37-40.

الملخص العربى

تأثير فلافونويدات نخيل البلح علي إرتفاع سكر الدم

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(positive control) **325.48** **100 /** **288.39** **/**

100 **40**

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(sGOT) serum glutamate oxaloacetate transaminase serum

glutamate pyruvate transaminase (sGPT)

(negative control). ,

sGOT sGPT positive

control .

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, , alkaline phosphatase

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