Effect of Yogurt Supplemented with Date Fruit, Seeds and Leaves on Blood Sugar of Diabetic Rats

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Abstract: Nowadays, tend to use synthetic drugs to lower serum glucose in diabetic patients is gradually decreased because of their related side effects, as well as a progression of drug resistance. In this regard, tend to use of medicinal plants has been doubled. Therefore, this work was conducted to investigate the effect of date fruit, leaves and seed at the level of 10% on diabetic rats. Thirty five adult male albino rat of Sprague Dawley strain, weighing (176.5±1.20 g) were divided in two main groups. The first main group (n=7) was kept as negative control group, the second main group (n=28) was injected interperitonial with Streptozotocin (STZ) to induce diabetes, then these rats were divided into four subgroups. Subgroup one was fed on the basal diet and served as a positive control group, subgroups from 2 to 3 were fed on basal diet and supplemented with yogurt and date fruit, seed, and leaves at the level of 10% respectively. At the end of the experimental period (8 weeks), rats were sacrificed and blood samples were collected to obtain serum. The results indicated that, STZ treated rats showed significant reduction ($P<0.05$) in serum insulin concentration and, increased glucose levels compared to normal rats. Supplementation with yogurt and date fruit, seed, or leaves in the diet caused significant ($P<0.05$) increase in the concentration of insulin while glucose level was significantly ($P<0.05$) decreased compared to the positive control one. It was also observed that, liver and kidney functions and lipid profile of the treated rats was improved compared to the positive control group. In conclusion, diet supplemented with yogurt and date fruit, seed, and leaves caused an improvement of the biochemical results from diabetes, therefore yogurt and date fruit, seed, or leaves could be used as a suitable supplementation therapy for diabetic patients.

Keywords: Diabetes, Date fruit, Date seed, Date leaves, glucose, serum lipid profile, fortification.

Introduction

Diabetes mellitus is an endocrine dysfunction resulting from insulin deficiency or incapability of peripheral tissues to respond to insulin **(Singh *et al*., 2008)**. The cumulative effects of metabolic disorders of diabetes lead to cell damage, circulatory changes and eventually to cardiovascular disorders including increased plasma lipoproteins, atherosclerosis, hypertension and cardiomyophathy. Other clinical consequences of diabetes include nephropathy, neuropathy, retinopathy and liver dysfunction **(Vidro *et at*., 1999)**. Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels **(Susman and Helseth, 1997)**.

The world prevalence of diabetes among adults (aged 20–79 years) was 6.4%, affecting 285 million adults, in 2010, and will be increased to 7.7%, affecting 439 million adults by 2030. It has been estimated that by the year 2030, there will be 8.6 million adults with diabetes in Egypt, making it the country with the tenth largest population of diabetics in the world **(Shaw *et al*., 2010)**. Reasons for this rise includes an increase in sedentary lifestyle, the consumption of energy-rich diet, obesity, and a higher life span, etc. **(Yajmk, 2001)**.

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of the disease increases so we need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes **(Kesari *et al*., 2005)**. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed **(Tiwari and Madhusudanarao, 2002)**. Most of the oral drugs are costly and have a lot of side-effects. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes (**Lo *et al*., 2011**). Simple and inexpensive diet strategies should aid in achieving and maintaining optimal control of diabetes and diabetic complication (**Xuemei *et al*., 2012**).

Date fruits are a significant component of the diet in the majority of the Arab countries with low cost. For Muslims, dates are of religious value and have been mentioned several times in the Quran. They are usually breaking their long day fasting with dates in the month of Ramadan. Fruits of the date palm (*Phoenix dactylifera* L.) are very commonly consumed in many parts of the world and a vital component of the diet and a staple food in most of the Arabian countries **(Al-farsi and lee, 2008**).

Egypt is considered one of the major producers of dates in the Middle East with 17% of the world production **(Saleh *et al*., 2011)**. The importance of the date in human nutrition comes from its rich composition of carbohydrates (70–80%), salts and minerals, dietary fiber, vitamins, fatty acids, amino acids, protein **(El-Beltagy *et al*., 2009)** and phytochemicals **(Vayalil, 2011)**.

Research proves that when dates are eaten alone or as mixed meals with yoghurts they have low glycemic indexes **(Gad *et al*., 2010)**. Unlike most other fruits, dates can be consumed at any of the three major stages of maturity such as khalal or besser (fresh, hard ripe, color stage), rutab (crisp to succulent or ripe stage), or tamr (soft and pliable, fully ripe stage) **(Saleh *et al*., 2011)**. The date fruit is used in folk medicine to treat the different infectious diseases probably because of their antibacterial ability, immuneomodulatory activity and antifungal property **(Baliga *et al*., 2011)**. Date pits can be used to improve the nutritional value of incorporated food products. Also, extract shows hepatoprotective and antimicrobial activity in rat **(Jassim and Naji, 2010)**.

In literature, it is well known that date fruits and its seeds, in the form of powder, pulp and infusion, are widely used against atherosclerosis, cancer, asthenia, pulmonary diseases, and throat diseases. In addition, date fruits and seeds are used as hypoglycaemic, expectorant, tonic, aphrodisiac, mouth hygiene and antidiarrheic **(Bnouham *et al*., 2002)**. The weight of the seeds is 5.6-14.2% of the date. Moreover, several saturated and unsaturated fatty acid are present in the flash and seeds of date

(Azmat *et al*., 2010). Date seeds contain high levels of valuable bioactive compounds **(Al–Farsi and Lee, 2011)**.Date seeds can be also used as a functional food ingredient because they are a good source of dietary fiber, phenolic compounds and antioxidant activity. In addition, date seeds contain a considerable amount of food ingredients such as protein and minerals **(Golshan *et al*., 2017)**.

The utilization of the seeds as by-product of date industry in medicine would be of great interest **(Alkhateeb , 2008)**. Date seeds have been used successfully in the folk medicine to treat diabetes mellitus for many years without scientific basis. The seed powder is also used in some traditional medicines **(Sabah *et al*., 2007)**. Date seed extract, has protective effect against toxicity caused by carbon tetrachloride, possibly due to antioxidant's effects of date seed that can inhibit radicals created by carbon tetrachloride **(Siahpoosh *et al*., 2012)**. In addition, one study demonstrated that date seed extract has cerebroprotective role in male rats **(Kalantaripour *et al*., 2012)**.

Therefore, this work was aimed to investigate the biological effects of dried date fruit, seeds and leaves on blood glucose level of diabetic rats.

Materials and Methods:

Materials:

Plants: Palm date fruits, seeds (*Phoenix dactylifera*) at tamr stage and date leaves were obtained from the Agriculture Research Center, Giza.

Rats: Thirty five adult male albino rat of Sprague Dowley strain, weighing (176.5±1.20 g) were purchased from Helwan Farm for Experimental Animals, Cairo, Egypt.

Chemicals: Streptozotocin was obtained from Sigma Company. Kits were purchased from Gama Trade Company, Egypt. Casein, vitamins, minerals, cellulose and choline were obtained from El-Gomhoria Company, Cairo, Egypt.

Methods:

Preparation of dried date fruit, seeds and leaves: Date fruit, seeds were cut into small pieces with a sharp knife and ground into a fine powder. The leaves were subject to dryness process under vacuum at the National Research Center, Dokki, Giza, and then were ground to fine particles.

Chemical composition: Moisture, protein, ash, crude fiber and fat content of date fruit, leaves and seed were determined according to the method described in **A.O.A.C, (2005).** Total carbohydrate was calculated by the following equation: Total carbohydrate = 100 – (protein% + fat% +ash% +fiber).

Phytochemical analysis of date fruit, seed and leaves: Total phenolic and total flavonoid content were expressed as mg of gallic acid equivalent (GAE) and catechin equivalent (CE) per g of sample, respectively and determined according to the procedure of (**Zilic *et al*., (2012)**.

Fortification: Ten gram of each date fruit, seed and leaves was added separately to one cup of prepared yogurt (75g) and was added to the basal diet as one cup of fortified yogurt/kg diet.

Induction of Diabetes: Diabetes was induced to rats by single intraperitoneal injection of freshly prepared Streptozotocin (60 mg/kg b.wt.). Three days after STZ administration, serum glucose level of each rat was measured. Rats with fast serum glucose (≥200 mg/dl) were considered diabetic **(Sarkar, *et al*., 1996)**.

Biological study:

Thirty five rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. The basal diet was consisted of 14% protein (casein), 4% oil, 0.25% choline, 1% vitamin mixture, 3.5% mineral mixture, 10% sucrose, 5% cellulose, 0.3% DL-methionine and the remainder was starch. The diet was formulated according to **(Reeves et al., 1993)**. After this week rats were divided into two main groups as follows:-

The first main group (7 rats) was fed on basal diet (as a negative control). The second main group: (diabetic rats, n=28) was divided into (4 subgroups, 7 rats each) as follows: Subgroup (1): was fed on basal diet (as a positive control group). Subgroup (2): was fed on basal diet supplemented with one cup of yogurt fortified with dried date fruit. Subgroup (3): was fed on basal diet supplemented with one cup of yogurt fortified with dried date seeds. Subgroup (4): was fed on basal diet supplemented with one cup of yogurt fortified with dried date leaves.

Each rat was weighted at the beginning and at the end of experiment and feed intake was also recorded daily. At the end of experimental period (8 weeks), rats were sacrificed after overnight fasting and blood of each rat was taken from the abdominal aorta under anesthesia by diethyl ether. The serum was separated by leaving the blood samples 15 minutes at room temperature then centrifuged at 3000 rpm for 20 minutes, and then kept in plastic vials at -20°C until biochemical analysis.

The biological effect of date fruit, leaves and seeds were assessed by the determination of body weight gain percent (BWG%) and feed efficiency ratio (FER) according to the method of **Chapman et al., (1959)** .

Biochemical analysis of serum:

Insulin activity was estimated using enzyme linked immunosorbent assay ELISA method as described by **Clark and Hales, (1994)**. Glucose level was determined according to **Asatoor and King, (1954).** Calorimetric determination of total cholesterol and triglycerides were carried out according to the method of **Richmond**, **(1973)** and **Fossati and Praneipe, (1982)** respectively**.** Determination of HDL-c level was carried out according to the method of **Richmond, (1973)**. VLDL-c and LDL-c were calculated according to the equation of **Friedewald *et al*., (1972).** Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to method of **Reitman and Frankel, (1957)**. Serum creatinine, urea and uric acid level were determined by the method of **Tietz, (1999), Wills and Savory, (1981)** and **Patton and Crouch, (1977**) respectively.

Statistical analysis:

The results were expressed as mean ± SE. The statistical analysis was carried out by using SPSS, PC statistical software (Version 18) using the Duncan' test multiple range post-hoc test. Data was analyzed by one way analysis of variance (ANOVA). The values were considered significantly different at ($P<0.05$) **(SPSS, 1986)**.

Result and Discussion:

The concerned chemical composition of dried date fruit, seeds and leaves in Table (1) indicated that, Siwa date (fruit, seed and leaves) is rich in total carbohydrates(84.25,81.32 and 79.88 %), Protein (4.2 , 3.78 and 2.98%).Total fiber was (8.5, 10.1 and 15.23%), but low in fats (2.0, 3.6 and 0.94%) respectively. Regarding the minerals content, it is cleared that, contains a high level of Zn (0.94, 2.3and 4.1mg) and Mg (1.43,1.88 and 0.680 mg) %, respectively. These results are agreed with **(Myhara *et al*., 1998; Al-farsi *et al* 2008 and Safi *et al*., 2008)** who mentioned that Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrate content (70–80%). Most of the carbohydrates in dates are in the reducing sugars form of (fructose and glucose) which are easily absorbed by the human body. These results are agreed with **Assirey *et al*., (2015)** who reported that date flesh samples also had a very low fat content.

Table (1): The crude chemical composition of dried date fruit, seeds and leaves.

Nutrients (100 g sample)		Siwa date	Siwa seed	Leaves
Nutrients	**Proteins (g)**	4.2	3.78	2.98
	Fats (g)	2.0	3.6	0.94
	Carb. (g)	84.25	81.32	79.88
	Total fiber (g)	8.5	10.1	15.23
	Ash (g)	1.05	1.2	0.97
Minerals	**Zinc (mg)**	0.94	2.3	4.1
	Mg (mg)	1.43	1.88	0.680

Total phenols and total flavonoids content of Date (fruit, seed and leaves) were recorded in Table (2). It contains have total phenols and total flavonoids in the following concentrations (233.8, 430.00 and 580mg GAE) and (66, 142 and 125.64mg CE), respectively. **Baliga *et al*., (2011)** reported that phytochemical investigations have revealed that the fruits contain anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids, compounds known to possess multiple beneficial effects. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities.

Many studies have showed that date fruit is rich in phenolic acids (Al-**Farsi *et al*., 2005a and Mattila *et al*., 2006)**. Differences in date fruit phenolic acid concentration are attributed to date cultivar, environmental conditions, etc. **Mansouri *et al*., (2005)** found that the main phenolic acids in seven varieties of date fruit were *p*-coumaric acid, ferulic acid, sinapic acid, some cinnamic acid derivatives and three different isomers of 5-o-caffeoyl shikimic acid. The main phenolic acids date fruit were ferulic acid, caffeic acid, *p*-coumaric acid and o-coumaric acid (**Al- Farsi *et al*., 2008 and Farsi *et al*., 2005b)**.The total concentration of these phenolic acids varied from 0.0261 to 0.1227 g kg−1 and from 0.0606 to 0.1477 g kg−1 in fresh and dried dates respectively. **Chaira *et***

***al*.,(2009)** found the lowest phenolic acid concentration in Mermella cultivar (0.0573 g kg−1) and the highest in Korkobbi cultivar (0.5466 g kg–1). The main phenolic acids were ellagic acid, gallic acid and *p*-coumaric acid. Caffeic acid was not detected.

It was observed that date–pits contained high levels of phenolic compounds (21.0– – 62.0 mg gallic acid equivalents, GAE/100 g date–pits) and antioxidants (580–929 mL Trolox equivalents/g) **(Al–Farsi *et al*. 2007and Suresh *et al*., 2013)**.

Flavonoids are important phenolic compounds that include proanthocyanidins, flavanoid glycosides and anthocyanins (**Gu et al., 2003; Farsi *et al*., 2005a** and **Mansouri *et al*., 2005**). **Hong *et al*., (2006)** identified 13 flavonoid glycosides of luteolin, quercetin and apigenin in date fruit at the khalal stage. **Chaira *et al*., (2009)** found the highest concentration of flavonoids dates (544.6 g kg−1). **Biglari *et al*., (2008)** reported that the flavonoid concentration in date fruit varied from 0.0162 to 0.8179 g kg−1.

Table (2): Total phenols and total flavonoids of dried date fruit, seeds and leaves.

Sample (100 g) / Parameters	Date	Date seeds	Leaves
Total phenols	233.8 mg GAE	430.00 mg GAE	580.6 mg GAE
Total flavonoids	66 mg CE	142 mg CE	125.64 mg CE

GAE: Gallic acid equivalent, CE: Catchin equivalent.

Table (3): Effect of date fruit, seeds and leaves on serum glucose level and insulin activity on diabetic rats.

Parameters / Groups	Glucose (mg/dl)	% of glucose reduction	Insulin (mIU/ml)
Control (-ve)	122.22±2.42 [d]	-	3.0225±.29 [a]
Control (+ve)	277.40±3.63 [a]	-	1.3750±.11 [c]
Date seeds (10%)	153.52±2.96 [c]	44.65	2.4500±.25 [b]
Date Fruit (10%)	169.72±2.74 [b]	38.81	2.0700±.05 [b]
Leaves Date (10%)	157.95±2.59 [c]	43.06	2.2750±.17 [b]

Values were expressed as Means ± SE. Values at the same column with different letters are significantly different at P<0.05.

Rats injected with STZ had significantly (P<0.05) higher glucose level and significantly (P<0.05) lower insulin concentration, compared to the control negative group Table (3). Feeding diabetic rats on diet supplemented with date fruit, leaves, or seed at the level of 10% caused a significant decrease (P<0.05) in the elevated serum glucose level, compared to the control positive group. It was clear that, there were no significant differences in glucose level between the treated groups with date seeds and date leaves. The percent of glucose reduction as a result of supplementation with date seed, fruit or leaves are (44.65%, 38.81% and 43.06%) respectively, as compared to the value of glucose level in the positive control group. Supplementation with date seeds or leaves caused the highest reduction in glucose level.

These results are in agreement with A study carried out by **Abuelgassim (2010)** who investigated the effect of date-palm leaves at (2%) on glucose level among diabetic rats, which found that extract of date-palm leaves had a hypoglycemic and hypoepidemic properties. **Mard *et al*., (2010)** found that hydroalcoholic extract of Phoenix Dactylifera Palm Leaves exhibits antidiabetic and antilipaemic effects in alloxan-induced diabetic rats**.**

The antidiabetic effect of date seeds, leaves or fruit may be due to the effect of active flavonoids, phenols, steroids, and saponins; these compounds may scavenge free radicals liberated by alloxan in diabetic rats (**Jurgoński *et al*., 2008** and **Rauter *et al*., 2009)**.

Prolong treatments with the extract of seed extracts of Ajwa/ Sukkari date (100g/L in dosage of 10ml/day/rat.) restores the function of liver and kidney and balance the oxidative stress condition in diabetic treated rats **(Hasan and Mohieldein, 2016)**.

Date fruit has been reported as a good source of fibre, sugars and minerals **(Ali *et al*., 2009),** which exhibit many effective benefits to health such as antimutagenic **(Vayalil, 2002),** antioxidant **(Mrabet *et al*., 2016)**. A study by **Zangiabadi *et al*., (2011)** also reported that date fruit extracts are deliberated as an effective treatment in preventing diabetic diseases and in enhancing the pathological parameters concerned with diabetic neuropathy.

In regarding to insulin concentration, the level of insulin activity was significantly ($P<0.05$) higher in the treated groups date fruit, leaves, or seed, compared to the control diabetic group. Moreover, there were no significant differences in insulin activity level between the treated groups. Biochemical results suggested an increase in endogenous insulin secretion in the case of type 1 diabetic rats treated with date seed extract, which might be the cause of its hypoglycemic effect (**El Fouhil *et al*., 2013)**.

Results illustrated in Table (4) shows the effect of date fruit, seeds and leaves on lipids profile of diabetic rats. STZ injection to rats caused a significant increase ($P<0.05$) in serum lipid profile, however, serum HDL-C was significantly lowered, compared to the healthy rats. Diet supplemented with date fruit, seeds and leaves at the level of 10% significantly decrease ($P<0.05$) the mean value of serum TC, TG, VLDL-C and LDL-C, however, serum HDL-C level was increased significantly ($P<0.05$), compared to the positive control group. It was obvious that, the treatments with date fruit, seeds or leaves at the level of 10% gave the highest beneficial effect in improving lipid profile in diabetic rats.

The most common lipid abnormalities in diabetics are hypertriglycaemia and hypercholesteraemia **(Suba *et al*., 2004)**. Previous studies have reported that some phytocomponents, particularly saponins and steroids elicit anti-hyperlipidaemic action by inhibiting intestinal lipid absorption via resin-like action and inhibition of lipase activity **(Adeneye *et al.*, 2010 and Juárez-Rojop *et al.*, 2012)**. Saponins are one of main component of date seed extract (**Hasan M and Mohieldein A (2016)**.

Insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis because it inhibits the activity of hormone-sensitive lipases in adipose tissue and suppresses the release of free fatty acids. In diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into circulation (**Riyad *et al*., 1998**).

Table (4): Effect of date fruit, seeds and leaves on serum lipids profile of diabetic rats.

Parameters / Groups	TC	TG	HDL-C	VLDL-C	LDL-C
	(mg/dl)				
Control (-ve)	77.60±0.62 [c]	58.42±3.26 [c]	54.50±1.44 [a]	11.68±0.65 [c]	11.41±1.59 [c]
Control (+ve)	117.82±3.43 [a]	100.45±1.88 [a]	36.25±0.85 [b]	20.09±0.37 [a]	61.48±3.92 [a]
Date seeds (10%)	92.82±2.49 [b]	80.40±1.99 [b]	48.50±2.32 [a]	16.08±0.39 [b]	28.24±2.83 [b]
Date Fruit (10%)	91.82±2.20 [b]	86.42±2.44 [b]	45.75±2.30 [a]	17.28±0.48 [b]	28.79±0.34 [b]
Leaves Date (10%)	88.75±2.32 [b]	79.20±1.73 [b]	51.75±5.52 [a]	15.84±0.34 [b]	21.16±3.52 [b]

Values were expressed as Means ± SE .Values at the same column with different letters are significantly different at P<0.05.

Table (5): Effect of date fruit, seeds and leaves in serum urea, uric acid and creatinine on diabetic rats

Parameters / Groups	Urea	Uric acid	Creatinine
	(mg/dl)		
Control (-ve)	25.72±1.11 [d]	2.52±0.08 [c]	0.37±0.02 [c]
Control (+ve)	45.82±1.72 [a]	3.57±0.21 [a]	0.90±0.04 [a]
Date seeds (10%)	31.05±1.75 [bc]	2.80±0.09 [bc]	0.56±0.05 [b]
Date Fruit (10%)	35.67±1.93 [b]	2.95±0.06 [b]	0.56±0.03 [b]
Leaves Date (10%)	29.79±1.67 [cd]	2.75±0.13 [c]	0.57±0.05 [b]

Values were expressed as Means ± SE .Values at the same column with different letters are significantly different at P<0.05

Table (5) illustrates the effects of date fruit, seeds and leaves at the level of 10% in serum kidney functions on diabetic rats. Injection with STZ significantly increase (P<0.05) the level of urea, uric acid and creatinine, compared to the control normal group (control –ve). Feeding diabetic rats on diet supplemented with date fruit, seeds or leaves at the tested level caused a significant decrease (P<0.05) in the mean values of uric acid, creatinine and urea as compared to the positive control group. There were no significant differences in serum urea and uric acid between the groups fed either date seeds or date fruit, and also between the rats fed on date seeds or date leaves. Moreover, there was no significant differences in serum creatinine among the three treated groups.

In line with agreement with **El Arem *et al*., (2014)** attributed the nephroprotective effect of the aqueous extracts of palm date against the renal damage induced by dichloroacetic acid to its richness in antioxidant compounds such as ferulic, caffeic and p-coumaric acids. Previous studies demonstrated significant increase in plasma serum urea levels (**Dewanjee *et al*., 2011)**, creatinine and uric acid (**King and Loeken, 2004)** in diabetic rats compared to normal. The elevated levels of serum lipids in DM cause the risk of development of diabetic nephropathy **(Vaziri, 2006)**.

The obtained results were also in the line with (**El-Mousalamy *et al*., 2016**) who mentioned that, aqueous and methanolic fruit and seed extracts of palm date protect kidneys from diabetic nephropathy in rats which might be due to their antioxidant properties. **El Fouhil *et al*., (2011)** demonstrated the safety of date seed extract administration on liver and kidneys of rats, and showed that a date seed extract-insulin combination minimizes the diabetic toxic effects on the liver and kidneys of rats, compared to insulin administration as a single drug.
The results in Table (6) revealed the effect of date fruit, seeds and leaves on liver function of diabetic rats. The activities of serum ALT and AST significantly increased (P<0.05) in the diabetic group, compared with the corresponding value of normal control group.

The elevation of serum AST and ALT level might be due to the release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (**Dunsford *et al*., 1989**). High concentrations of serum transaminases are considered to be an index of hepatic injury where elevation of ALT is regarded as a more sensitive indicator and is usually accompanied by a rise in AST (**Ha *et al*., 2001**). Previous studies reported increased activities of liver enzymes such as AST and ALT in cases of insulin resistance (**Marchesini *et al*., 2001**), metabolic syndrome and type 2 diabetes **(Wannamethee *et al*., 2005)**.

Supplementation with date fruit, seed and leaves at the level of 10% significantly decreased (P<0.05) the elevated levels of both serum ALT and AST compared to the negative control group. Moreover, there was no significant difference in serum ALT among the three treated groups, however, serum AST was significantly different (P<0.05) among the treated groups. These findings suggest hepatic injury in type 2 DM and the hepatoprotective effect for date fruit, seed and leaves.

Liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This key function of liver makes it vulnerable to diseases in subjects with metabolic disorders, particularly diabetes **(Levinthal and**

Tavill, 1999). Date fruit and seed extracts have hepatoprotective effect in type 2 diabetic rats which might be due to their phenolics and flavonoids contents **(Hussein *et al*., 2015)**. Moreover**, Chukwugozie *et al*., (2014)** reported that the hepatoprotective effect for palm date extracts could be attributed to its contents such as quercetin which has a strong antioxidant effects. **Al-Qarawi *et al*., (2004)** revealed a significant reduction in elevated ALT, AST, and ALP activities due to CCl_4 in rats subjected to both pre- and post-treatments with the aqueous extracts of P. dactylifera flesh and seeds.

Table (6): Effect of date fruit, seeds and leaves on liver function of diabetic rats.

Parameters / Groups	ALT	AST
	(μ/L)	
Control (-ve)	53.60±3.59 [c]	119.00±3.16 [c]
Control (+ve)	90.92±3.26 [a]	166.12±1.78 [a]
Date seeds (10%)	73.97±2.52 [b]	141.25±3.59 [b]
Date Fruit (10%)	80.02±3.39 [b]	123.00±2.61 [c]
Leaves Date (10%)	75.45±2.00 [b]	103.00±2.58 [d]

Values were expressed as Means ± SE . Values at the same column with different letters are significantly different at $P<0.05$

Regarding to changes in body weight status, Table (7) illustrated the changes of body weight, feed intake and FER in the diabetic rats fed on diet supplemented with of date fruit, seeds and leaves. The initial body weight of rats was (176.5±1.20 g), there were no significant differences in IBW among all groups. Diabetic rats had significant decrease ($P<0.05$) in the FBW compared to the negative control group. It was observed that STZ induced diabetic in rats caused significant decrease ($P<0.05$) in FBW compared to the healthy rats.

The supplementation with date seeds, fruit or leaves significantly ($P<0.05$) increased the lowered FBW compared to the positive control group. There were significant differences ($P<0.05$) in FBW among the three treated groups. Date seeds caused the highest increase in FBW compared to other treatments. In regarding to BWG% and FER, diabetic rats had significantly ($P<0.05$) lowered BWG% and FER compared to the negative control group. However, the supplementation with the tested materials caused a significant increase ($P<0.05$) in BWG% and FER compared to the positive control group. Date seeds caused the highest increase in BWG% and FER compared to other treatments.

Zangiabadi et al., (2011) demonstrated that aqueous date extract improved body weight and fasting blood glucose in rat model of diabetic peripheral neuropathy.

Table (7): Changes of body weight, feed intake and FER in the diabetic rats fed on diet supplemented with of date fruit, seeds and leaves.

Parameters / Groups	Initial Body weight (g)	Final Body Weight (g)	BWG%	Feed intake (g/day/rat)	FER
Control (-ve)	175.00±1.77 [a]	204.33±1.89 [b]	16.80±1.30 [a]	16.60	0.029±0.12 [b]
Control (+ve)	176.83±1.57 [a]	140.00±1.23[e]	-20.78±1.13 [d]	19.00	-0.032±0.12 [d]
Date seeds (10%)	178.16±1.01 [a]	210.33±1.14 [a]	18.08±1.17 [a]	14.50	0.036±0.13 [a]
Date Fruit (10%)	176.00±1.75 [a]	191.33±2.09 [d]	8.71±0.66 [c]	11.70	0.021±0.10 [c]
Leaves Date (10%)	177.16±1.10 [a]	198.50±2.45 [c]	12.03±1.18 [b]	12.50	0.028±0.16 [b]

Values were expressed as Means ± SE. Values at the same column with different letters are significantly different at $P<0.05$.

References

Abuelgassim, AO. (2010): Effect of Flax Seeds and Date Palm Leaves Extracts 011 Serum Concentrations of Glucose and Lipids in Alloxan Diabetic Rats. *Pak. J. Biol. Sci.*, 13(23): 1141-1145.

Adeneye A.; Adeyemi O. and Agbaje E. (2010): Anti-obesity and antihyperlipidaemic effect of Hunteriaumbellata seed extract in experimental hyperlipidaemia. J Ethnopharmacol.;130(2):307-14.

Al- Farsi, M.A. and Lee, C.Y. (2008): Nutritional and functional properties of dates: a review. Critical Reviews in Food Science and Nutrition., (48): 877−887.

Al–Farsi M.; Alasalvar C.; Al–Abid M.; Al–Shoaily K.; Al–AmryM. and Al–Rawahy F.(2007): Compositional and functional characteristics of dates, syrups and their by–products. Food Chem., 104, (3): 943–947.

Al-Farsi M.; Alasalvar C,; Morris A.; Baron M. and Shahidi F.(2005a): Comparisonof antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J Agric Food Chem* 53:7592–7599.

Al-Farsi M.; Alasalvar C.; Morris A.; Baron M. and Shahidi, F. (2005b): Compositional and sensory characteristics of three native sundried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J Agric Food Chem* **53**:7586–7591.

Al-Qarawi A.; Mousa HM.; Ali BH.; Abdel-Rahman H. and El-Mougy SA. (2004): Protective effect of extracts from dates (Phoenix dactylifera L.)on carbon tetrachloride-induced hepatotoxicity in rats. Intern J Appl Res Vet Med 2: 176-180.

Ali A.; Al-Kindi YS. and Al-Said F. (2009): Chemical composition and glycemic index of three varieties of Omani dates. *Int. J. Food Sci. Nutr.*, **60**(Sup 4): 51-62.

Alkhateeb, A. (2008): Comparison effects of sucrose and date palm syrup on somatic embryogenesis of date palm (Phoenix dactylifera L). American Journal of Biochemistry and Biotechnology; 4: 19-23.

A.O.A.C., (2005): "Official methods of analysis of AOAC international., 18th ED., AOAC international Gaithersburg, MD, USA.

Asatoor A.M. and King E.J. (1954): Simplified calorimetric blood sugar method . Biochem.J.56:XIIV.

Assirey, AR.E.(2015): Nutritional composition of fruit of 10 date palm(*Phoenix dactylifera* L.) cultivars grown in Saudi Arabia. Journal of Taibah University for Science 9 75–79.

Azmat S.; fzal R.; Rasheed M.; Mohammas F. andAhmad V. (2010): GC-MS Analysis of n-haxan Extract from seeds and leaves of Phoenix dactyliferaL. J chemic soc pak. 32(5):672-77.

Belfield A. and Goldberg L. (1971): D.M-Revised assay for serum phenyl phosphatase activity using 4- amino-antipyrine-enzyme. 12:561-573.

Biglari F.; Alkarkhi AFM. and Easa AM.(2008): Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chem* **107**:1636–1641 (2008).

Bnouham M, Mekhfi H, Legssyer AK, Ziyyat A. (2002): Medicinal plants used in the treatment of diabetes in Morocco. Int J Diabetes & Metabolism.; 10:33-50.

Camplle J.A. (1992): Methodology of protein evaluation., RAG. Nutrition DOC. R. 10/Led 37. June Meeting, New York.

Chaira N.; Mrabet A. and Ferchichi A. (2009): Evaluation of antioxidant activity, phenolics, sugar and mineral contents in date palm fruits. *J Food Biochem* **33**:390–403.

Chukwugozie N.; Theophilus K.; Joshua E.; Augustine C.; Innocent C. and Romanus E. (2014): Hepatoprotective Effect of Methanolic Fruit Extracts of Phoenix dactylifera (Arecaceae) on Thioacetamide Induced Liver Damage in Rats. American Journal of Phytomedicine and Clinical Therapeutics., 2(3): 290-300.

Clark P.M.S. and Hales C.N. (1994): How to measure plasma insulin, Diabet. Metab. Rev., 10: 79-90.

Dewanjee S.; Maiti A.; Sahu R.; Dua TK. and Mandal V. (2011): Effective control of type 2 diabetes through antioxidant defense by edible fruits of Diospyros peregrina. Evi Based Compliment Alter Med 675397.

El Arem A.; Thouri A.; Zekri M.; Saafi EB. and Ghrairi F. (2014): Nephroprotective effect of date fruit extract against dichloroacetic acid exposure in adult rats. Food Chem Toxicol 65: 177-184.

El Fouhil AF.; Ahmed AM.; Darwish HH.; Atteya M. and Al-Roalle AH. (2011): An extract from date seeds having a hypoglycemic effect. Is it safe to use? Saudi Med J; 32(8): 791-796.

El Fouhil A.; Ahmed A.; Atteya M.; Mohamed R.; Moustafa A. and Darwish H. (2013): An extract from date seeds stimulates endogenous insulin secretion in streptozotocin-induced type I diabetic rats Functional Foods in Health and Disease; 3(11):441-446.

El-Mousalamy A.; Hussein A.; Mahmoud S.; Abdelaziz A. and Shaker G. (2016): Aqueous and Methanolic Extracts of Palm Date Seeds and Fruits (Phoenix dactylifera) Protects against Diabetic Nephropathy in Type II Diabetic Rats., Biochem and Physiol 5(2): 205.

FAO (2009): Food and Agriculture Organization of the United Nations. Agro-Statistics Database.

Fossati P. and Praneipe L. (1982): Triglycerides determination after enzymatic hydrolysis. Clin. Chem., 28:2077.

Friedewald W.T.; Levy K.T. and Fredrickson D.S. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 226: 499-504.

Golshan Tafti A.; Solaimani Dahdivan, N. and Yasini Ardakani, S.A. (2017): Physicochemical properties and applications of date seed and its oil. International Food Research Journal 24(4): 1399-1406.

Gu LW.; Kelm MA.; Hammerstone JF.; Beecher G.; Holden J. and Haytowitz D. (2003): Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J Agric Food Chem* 51:7513–7521.

Hasan M. and Mohieldein A. (2016): In vivo Evaluation of Anti Diabetic, Hypolipidemic, Antioxidative Activities of Saudi Date Seed Extract on Streptozotocin Induced Diabetic Rats. J. Clin. Diagn. Res., 10(3): FF06.

Hong YJ.; Tomas-Barberan FA.; Kader AA. and Mitchell AE.(2006): Theflavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). *J Agric Food Chem* 54:2405–2411.

Hussein A.; El-Mousalamy A.; Hussein S. and Mahmoud S. (2015): effects of palm dates (phoenix dactylifera l) extracts on hepatic dysfunctions in type 2 diabetic rat model, world journal of pharmacy and pharmaceutical sciences,4(7): 62-79.

Hwang, E.S. and DO Thi N. (2014). Effects of Extraction and Processing Methods on Antioxidant Compound Contents and Radical Scavenging Activities of Laver (Porphyra tenera). Prev. Nutr. Food Sci., 19: 40-48.

Jiménez-Araujo A. (2016): Quality characteristics and antioxidant properties of muffins enriched with date fruit (*Phoenix dactylifera* l.) fiber concentrates. *J. Food Qual.*, 39(4): 237-244.

Juárez-Rojop IE.; Díaz-Zagoya JC.; Ble-Castillo JL. and Miranda-Osorio PH. (2012): Castell-Rodríguez AE, et al. Hypoglycaemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine.;12:236.

Jurgoński A.; Juśkiewicz J. and Zduńczyk Z. (2008): Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. Plant Foods Hum Nutr.;63(4):176–182.

Kalantaripour TP.; Asadi-Ekkari M. and Najar GM. (2012): Cerebroprotective Effect of Date Seed Extract (Phoenix dactylifera) on Focal Cerebral Ischemia in Male Rats. Journal of Biological Sciences. 12:180-85.

Kesari A.N.; Gupta R.K. and Watal G. (2005): Hypoglycemic effects of Murraya koenigiion normal and alloxan diabetic rabbits. J. Ethnopharmacol., 97: 247–25.

King GL.; Loeken MR. (2004): Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol 122: 333-338.

Levinthal GN. and Tavill AJ. (1999): Liver disease and Diabetes Mellitus. Clin Diabetes; 17: 73.

Lo H. and Wasser S. (2011): Medicinal mushrooms for glycemic control in diabetes mellitus: history, current status, future perspectives, and unsolved problems. Int J Med Mushrooms.; 13(5): 401–426.

Mansouri A, Embarek G, Kokkalou E andKefalas P. (2005): Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chem* **89**:411–420.

Marchesini G, Brizi M, Bianchi G. (2001): Nonalcoholic fatty liver disease. A feature of metabolic syndrome. Diabetes; 50: 1844-1850.

Mard S.; Jalalvand K.; Jafarinejad M.; Balochi H.; Kazem M. and Naseri G.(2010): Evaluation of the Antidiabetic and Antilipaemic Activities of the Hydroalcoholic Extract of Phoenix Dactylifera Palm Leaves and Its Fractions in Alloxan-Induced Diabetic RatsMalaysian J Med Sci.,17(4): 4-13.

Mattila P.; Hellstrom J. and Torronen R. (2006): Phenolic acids in berries, fruits, and beverages. *J Agric Food Chem* **54**:7193–7199.

Myhara, R.M.; Taylor, M.S.; Slominski, B.A. and Al-Bulushi, I. (1998): Moisture sorption isotherms and composition of Omani dates.J. Food Engineering,(37):471–479.

Patton, C.J. and Crouch S.R. (1977): Calorimetric determination of blood urea. Analyt. Chem., 49: 464-469.

Rauter AP.; Martins A.; Lopes R.; Ferreira J.; Serralheiro LM. and Araújo ME. (2009): Bioactivity studies and chemical profile of the antidiabetic plant Genista tenera. J Ethnopharmacol.;122(2):384–393.

Reeves, R.G.; Nielsen, F.H. and Fahey, G.C. (1993): AIN-93 Purified Diets for Laboratory Rodents .J. Nutr., 123(1):1939-1951.

Reitman S. and Frankel S. A. (1957): Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Path. 28:56–63.

Richmond N. (1973): Preparation properties of a cholesterol oxidase from nacardia SP. enzymatic assay of total cholesterol in serum. Clin Chem., 19: 1350-1356.

Riyad MA.; Abdul Ghani; Abdul-Salam S. and Suleiman MS. (1998): Effect of fenugreek and lupin seeds on the development of experimental diabetes rats. Planta Med.;**54(4)**:286–290.

Sabah A. A. Jassim and Mazen A. (2007): Naj; In vitro Evaluation of the Antiviral Activity of an Extract of Date Palm (Phoenix dactylifera L.) Pits on a Pseudomonas Phage, *eCAM* 1-6.

Safi, E.B.; Trigui, M.; Thabet, R.; Hammami, M. and Achour, L. (2008) : Common date palm in Tunisia: chemical composition of pulp and pits. Int. J. Food. Sci. Tech., (43):2033–2037.

Sarkar S.; Pranava M. and Marita R.A. (1996): Demonstration of the hyperglycemic action of Momordica charantia in a validated animal model of diabetes. Pharmacol. Res., 33: 1-4.

Shaw J.E.; Sicree R.A. and Zimmet P.Z. (2010): Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice., 87(1): 4–14.

Siahpoosh A.; Rezaei M. and Azad Bakht Y. (2012): Study of date seed extract (Phoenix dactylifera) varite; Deiri Abadan liver protective effect against carbon tetrachloride toxicity in male rats. Research in Pharmaceutical Sciences (abstract). 7(5):S172.

Sidu, J. S. (2006): Date fruits production and processing. In: Handbook of Fruits and Fruit Processing, Edited by Hui, Y. H. (2006). Pp 391-419.Blackwell publishing, Oxford, UK.

Singh S.K.; Rai P.K.; Jaiswal D. and Watal G. (2008) : Evidence-based critical evaluation of glycemic potential of Cynodon dactylon. Evid. Based Complement Alternat. Med 5 (4): 415-420.

SPSS, (1986): Statistical package for social science, version 19. SPSS Inc., II. U.S.A.

Suba V.; Murugesan T.; Bhaskara-Rao R.; Ghosh L.; Pal M. and Mandal SC. (2004): Antidiabetic potential of Barlerialupulina extract in rats. Fitoterapia.;75:1–4.

Suresh S.; Guizani N.; Al–Ruzeiki M.; Al–Hadhrami A.; Al–Dohani H.; Al–Kindi I. and Rahman M.S.(2013): Thermal characteristics, chemical composition and polyphenol contents of date–pits powder. J. Food Eng., 2013, 119, 668–679.

Susman J. and Helseth L. (1997): Reducing the complications of type II diabetes: a patient centered approach. Am Fam Physician., 56:471-80.

Tietz N.W. (1999): Text book of clinical chemistry. (3rd ed). C.A.Burtis, E.R. Ashwood, W.B. Saunders., P .477-530 to 1241-1245.

Tiwari A.K. and Madhusudanarao J. (2002): Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Curr. Sci., 83: 30–38.

Vayalil PK. (2002): Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). *J. Agric. Food Chem*., 50(3): 610-617.

Vaziri N. (2006): Dyslipidemia of chronic renal failure: the nature, mechanisms and potential consequences. Am J Physiol Renal Physiol., 290(2):F262-72.

Vidro E.; Basu T. and Tsin A. (1999): Insulin dependent diabetes mellitus and vitamin A metabolism. Special issue: Diet nutrition and health". J Clin Biochem. Nutr., 26:155-160.

Wannamethee SG.; Shaper AG.; Lennon L. and Whincup PH. (2005): Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Car; 28: 2913-2918.

Wills M.R. and Savory J. (1981): Biochemistry of renal failure. Ann.Clin.Lab.Sci., 11:292-299.

Xuemei G.; Hai- Lu Z.; Jing G.; Juliana C.N. and Peter, C.Y. (2012): White rice vinegar improves pancreatic beta- cell function and fatty liver in streptozotocin induced diabetic rats. Acta Diabetol.; 49: 185 – 191.

Yajmk, C.S. (2001): The insulin resistance epidemic in India: fetal origins, later lifestyle or both?. Nutr. Rev.,5: 1-9.

Zangiabadi N.; Asadi-Shekaari M.; Sheibani V.; Jafari M. and Shabani M. (2011): Date fruit extract is a neuroprotective agent in diabetic peripheral neuropathy in streptozotocin-induced diabetic rats: A multimodal analysis. Oxid Med Cell Longev , 2011: 1-9.

Zilic S.; Serpen A.; Akýllýoglu G.; Jankovic M. and Gökmen V. (2012): Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. Journal of Cereal Science, 56: 652-658.

Zilic S.; Serpen A.; Akýllýoglu G.; Jankovic M. and Gökmen V. (2012): Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. Journal of Cereal Science, 56: 652-658.

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تأثير الزبادى المدعم بفاكهة البلح والبذور والاوراق على نسبة سكر الدم في الفئران المصابة بالسكر

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الملخص:

في الوقت الحاضر، تهدف الدراسات العلمية الى تقليل استخدام الادوية لتقليل نسبة السكر في الدم في المرضى الذين يعانون من مرض السكر تدريجيا بسبب الآثار الجانبية المرتبطة بها. في هذا الصدد، تسعي الابحاث العلمية الى استخدام النباتات الطبية. لذلك تهدف الدراسة الحالية إلى دراسة تأثير الزيادى المدعم بفاكهة البلح والبذور والاوراق ينسبة 10% على الفئران المصابة بمرض السكر، اجريت الدراسة لمدة 8 اسابيع على الفئران المصابة بالسكر، حبث تم تقسيم عدد 35 من ذكور الفئران البالغة الي مجموعتين رئيسيتين، المجموعة الاولي (7 فئران) وتتغذي علي الغذاء الاساسي فقط، والمجموعة الثانية (28 فأر) تم حقنهم بمادة الاستربتوزوتين (60ملجم/كجم من وزن الجسم) لاحداث السكر، ثم تم تقسيم هذه المجموعة المصابة بالسكر الي 4 مجموعات فرعية وكل مجموعة تغذت علي الغذاء الاساسي والذي تم تدعيمه بالزبادى المدعم بفاكهة البلح والبذور والاوراق بنسبة ال10%. في نهاية التجربة تم أخذ عينات الدم وفصل السيرم. تشير النتائج الي أن تغذية الفئران المصابة السكر علي الغذاء الاساسي المدعم بالزبادي المضاف اليه فاكهة البلح أو البذور أو الاورق في الفئران أدت إلى حدوث انخفاض معنوى ($P<0.05$) في متوسط قيم جلوكوز الدم و زيادة تركيز الانسولين مقارنة بالمجموعه الضابطة الموجبة المصابة بالسكر. وقد لوحظ بأن الزبادى المدعم بفاكهة البلح أو البذور أو الاوراق ادي الى حدوث انخفاض معنوى في مستوى سكر الدم و تحسين صورة دهون الدم ووظائف الكبد والكلى وارتفاع معنوى في نسبة تركيز الانسولين مقارنة بالمجموعة الضابطة الموجبة . وبالتالي يمكن التوصية باستخدام الزبادى المدعم بفاكهة البلح أو البذور أو الاوراق في علاج مرضي السكر .

الكلمات المفتاحية: مرض السكرى، ثمار البلح، بذور البلح، أوراق البلح، جلوجوز، صورة دهون الدم، تدعيم