

Potential effect of date pits fortified bread on diabetic rats

Mona S. Halaby, Mohammed H. Farag, Attyat H. Gerges

Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Cairo-Egypt

Email address:

monahalaby03@yahoo.com(M. S. Halaby)

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Abstract: The present work was conducted to evaluate the effect of fortified pan bread with 5%, 10%, and 15% date pits powder on the chemical constituents, the nutritional value, and rheological characteristics of different dough samples using farinograph and on its sensory properties. The rheological properties of dough referred that there was an increase in water absorption in all blends compared to the control sample, and there was an increase in dough development, dough stability and degree of softening at 15% compared with 5% or 10% only. Pan bread fortified with 15% date pits showed the highest score in overall acceptability when compared to control pan bread and other various concentrations of pits. Twenty four adult male albino rats were divided into four groups. The first group (6 rats) fed on basal diet as a (negative control group). The second main group (6 rats) was injected with alloxan to induce hyperglycemia (positive control group) and then the rats fed on the same basal diet. The other 12 rats after being injected with alloxan were divided into 2 groups (3 & 4) and received basal diets containing fortified bread with 10% and 15% date pits powder for 45 days respectively. Biological evaluation was carried out by determination of body weight gain and food intake. At the end of the experimental period rats were sacrificed, blood samples were collected from the aorta then separate serum to determine glucose, insulin, HbA1c, total cholesterol and other lipid, and also to determine each of kidney and liver functions of experimental rats. Liver and Pancreas were removed to demonstrate histopathological observation. Our results showed significant reduction in the glucose, HbA1c, TC, TG, LDL-C and VLDL-C, as well as reducing hazards on liver and kidney functions compared with positive control group. Histopathological observation proved that the last group diet (with 15% date pits) looked like the negative control group. So date pits powder fortified bread is recommended to gain nutritional and healthy benefit to decrease the risk of diabetic diseases.

Keywords: Diabetes, Albino Rats, Date Pits Powder, Bread, Sensory Quality, Lipid Profile, Liver & Kidney Functions, Histopathology

1. Introduction

Type 2 diabetes mellitus has become a global epidemic in the recent decades; it's a leading cause of blindness, non-traumatic amputation and end-stage renal disease as well as a major risk factor for cardiovascular disease. Diabetes is a disease of worldwide significance and increasing their prevalence without any plateau (Raman et al., 2012 and Spanou & Tziomalos 2013). There is an increasing demand for healthy products, natural and high quality, among Egyptian consumers. Therefore, one recent trend is to enrich the components in food products to overcome health problems and many of them are known to be effective against diabetes. In fact bread wheat plays an important role in the human diet. It is one of the most important crops in the world as well as in Egypt (Halaby et al., 2006 and

Litwinek et al., 2013).

The date has always played an important role in the economy and social life of the people of various regions of the world. Egypt is considered to be one of the date-producing countries (Ahmed et al., 2008). World production of dates reaching 9 million tons in 2007, from these approximately 960 thousand tons of date seeds are produced according to FAO (2010). Thus, utilization of such waste is very important to date cultivation and to increase the income to the responsible sector (Al-Farsi et al., 2010 and Raman et al., 2012).

Date pits could be regarded as an excellent source of food ingredients with interesting technological functionality that could also be used in food as an important

source of dietary fiber (Besbes *et al.*, 2009 and Bouaziz *et al.*, 2010). The seed powder is used in some traditional medicines and has been investigated for human potential health benefits, and for addition to animal feed to enhance growth, in addition, date pit extract shows an ability to protect against hepatotoxicity on the liver in rats (Sabah *et al.*, 2010).

Bread wheat is an Egyptian product that represents the main diet component for rich and poor Egyptian consumers. In Egypt, there is a big gap between wheat production and its consumption, where the total production of wheat grains covers only about 55% of the total needs one of the ways, to overcome this problem is to search for other native cereal sources or else which could be used with wheat flour bread making (Eissa *et al.*, 2007 and Litwinek *et al.*, 2013). Few researches have been published regarding incorporation of date pit powder with wheat flour to prepare bread with higher degree of acceptability. The aim of our study is to find the effect of bread supplemented with date pits on the metabolic control of diabetes through biological and histopathological evaluations.

2. Materials and Methods

2.1. Materials

Date palm pits variety "Semi- dry" and wheat flour (82% extraction) variety "Triticum aestivum L." were obtained from Agriculture Research center Giza, Egypt. Casein, cellulose, vitamins and minerals ingredients were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. Corn oil and starch were obtained from the local market. Twenty four adult male albino rats (Sprague Dawley strain) were obtained from the Helwan breeding farm, Cairo - Egypt. Weight an average of 120 ± 10 g.

2.2. Methods

Pits were extracted from the date, washed and dried in an oven at 30°C for 48 hours. Pits were crushed using pestle and mortar followed by high speed laboratory blender and then sieved to obtain finely divided powder. Seeds powder was stored under refrigeration until processing and analyses.

2.2.1. Dough Preparation

Pan Bread (control) was prepared according to the common method described by Khorshid *et al.*, (1989).

2.2.2. Types of Pan Bread were Classified into

Control pan bread was made from 100% wheat flour. Different formulas were made from a mixture of wheat and date pits powder at various ratios (5%, 10% & 15%), respectively. Then according to the panel test we used the best two concentrations on the experimental animals.

2.2.3. Physical Properties of Pan Bread

Weight before and after baking for each treatment was recorded (gm). The volume (cm^3) was determined by rapeseeds displacement method as described by Keskin *et*

al., (2004). While, bread fermented time (min.), was determined (twice), height (cm), and weight (by using sensitive balance) were estimated individually and the specific volume was calculated according to the method of A.A.C.C. (2002). Specific volume (cm^3/g) of bread was calculated by dividing volume by weight in grams. All measurements were done on duplicates and the mean value was calculated.

2.2.4. Rheological Characteristic

Different dough samples including: water absorption, arrival time, dough development time, dough stability and degree of softening were carried out using Farinograph according to the method of A.A.C.C. (1994).

2.2.5. Chemical Constituents of Raw Materials and Bread

Moisture, ash, protein, fat, dietary fiber, were determined according to the method outlined in A.O.A.C. (2002). Total carbohydrates were calculated by difference. Lignin content was measured according to the method described by Tanaka *et al.*, (1985). Cellulose and hemicelluloses contents were determined as described by Chahal *et al.*, (1979).

Unsaturated and saturated fatty acids were analyzed by gas chromatography according to the method described by IUPAC (2000). Minerals contents including (Ca; Mg; Na, K; Cu, Fe, Mn & Zn) were assayed as recommended by A.O.A.C. (2005) using atomic absorption Spectrophotometer.

2.2.6. Sensory Evaluation of Pan Bread

The scoring scheme was established according to A.A.C.C. (2002) by 15 trained panelists (staff members and students) from department of Nutrition and Food Science, Faculty of Home Economics, Helwan University.

2.3. Diet Composition

The basal diet was prepared according to Campbell (1963) and Reeves *et al.*, (1993).

2.4. Animal Groups

Male rats were housed in well aerated cages (six rats for each group) under hygienic laboratory conditions, in the animal house of Faculty of Home Economics, Helwan University. They were fed on the basal diet for two weeks for adaptation. After this period, the rats were randomly classified into 4 groups as follows: Group (1) as negative control group fed on basal diet. Group (2): as positive control group, injected with Alloxan and then fed on basal diet. Groups (3 & 4) after being injected with Alloxan they were fed fortified bread with the two most acceptable concentrations of date pits which detected by the panel test. After forty five days rats were fasted over night before sacrificing. Blood samples were collected from the aorta. They were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C .

2.5. Biochemical Analysis of Serum

Glucose (Trinder 1959), insulin (Burgi et al., 1988), HbA1c Sudhakar & Pattabiraman (1981), total cholesterol (Allain et al., 1974), triglycerides (Fassati & Prencipe 1982), HDL-C (Lopes et al., 1977), LDL-C and VLDL-C were calculated by using the method of Friedewald et al., (1972). Uric acid, urea nitrogen and creatinine were estimated according to the methods described by Fossati et al., (1980), Patton & Crouch (1977) and Bartels et al., (1972) respectively. AST and ALT were determined according to Retiman & Frankel (1957).

2.6. Histopathological

Tissues from liver and pancreas of the sacrificed rats were examined as described by Bancroft et al., (1996).

2.7. Statistical Analysis

Results are expressed as mean \pm SD. Data were statistically analyzed for variance using one-way analysis of variance "ANOVA" according to (Armitage & Berry, 1987). Computer software system SPSS (version 15) was used for these calculations.

3. Results and Discussion

Chemical composition of soft wheat flour (SWF) and date pits powder (DPP) were determined in Table (1). The tabulated results it could be noticed that the highest moisture, protein, carbohydrate and hemicelluloses contents were that of the SWF while, the lowest content was that of DPP. Results of ash, fat, and dietary fiber including lignin and cellulose contents in DPP had the highest values (1.01, 7.12, 64.24 (13.67 & 36.32% DW) while, in soft wheat flour the amounts of these contents were lowest (0.80, 1.79, 40.79 (5.35 & 8.01% DW), respectively. The present results are in agreement with those Ardekani et al., (2010) and Najafi (2011) who published that the date seeds contain high levels of dietary fiber (78–80g/100g), and the insoluble dietary fibers in date seeds are those components that are insoluble in water and includes cellulose, hemicelluloses and lignin confirmed by

Amany et al., (2012).

The same Table (1) illustrated the macro & micro-elements including calcium (Ca); magnesium (Mg); sodium (Na); potassium (K); copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in soft wheat and date pits. The present results indicated that date pits contained the highest levels of macro and micro elements such as (Ca); (Mg); (K); (Cu); (Fe); (Mn) and (Zn) with an average of 84.80; 1857.00; 143.25; 8.27; 43.10; 9.07 and 16.82 (mg/100g), respectively, comparing with wheat flour which had the lowest values of mineral contents. These results were in agreement with El-Kassas (2004) who reported that date seeds are considered as a good source of the macro-elements such as K, P, Ca and Mg.

The data presented in Table (2) showed the fatty acid composition of total lipids extracted from the wheat flour and date pits under this study using gas chromatography (GC), eight saturated fatty acids were recognized in date seeds powder variety (Semi- dry), besides six unsaturated fatty acids were identified in the same variety. These results were in agreement with Al-Hooti et al., (1998); Walid & Richard (2003) and Akbari et al., (2012). It could be noticed that DPP (100%) associated with the increasing of unsaturated and saturated fatty acids than that of wheat flour.

The results presented in Table (3) showed the difference between soft wheat flour and different blends in wet and dry gluten content. Soft wheat flour had the highest wet and dry gluten content (25.10% and 9.12%) respectively. According to Reda (2006) showed that there is a relation between wet and dry gluten content of wheat and its protein content. The blend that contains 15% date pits powder had the lowest content of gluten. In all bread making experiments, as the percentage of weight gluten and gluten index decreased, it could result from the interaction between gluten and fiber material (Chen et al., 1988). The values of weight gluten and gluten index reached to 23.80% & 94.07% for dough with 5% date pits powder and 19.24% & 93.94% for dough with 10% date pits and it reached 14.21% & 90.21% for dough with 15% date pits flour compared with dough prepared from soft wheat flour without any supplementation, which reached to 25.10 % & 96.60 % respectively.

Table (1). Chemical composition of wheat flour and date pits powder (g/100g dry weight basis)

Component %	Material				
	SWF (100%)	DPP (100%)	Macro-elements	SWF (100%)	DPP (100%)
Moisture	11.61	04.28	Calcium (Ca)	20.7	84.80
Ash	00.80	01.01	Magnesium (Mg)	22.6	1857.0
Protein	10.82	05.79	Sodium (Na)	04.20	02.3
Fat	01.79	07.12	Potassium (K)	112.9	143.25
Carbohydrate	34.19	17.56	Micro-elements		
Total fiber	40.79	64.24	Copper (Cu)	00.18	08.27
Lignin	05.35	13.67	Iron (Fe)	01.02	43.10
Cellulose	08.01	36.32	Manganese (Mn)	00.71	09.07
Hemicelluloses	27.43	14.25	Zinc (Zn)	01.45	16.82

SWF: Soft Wheat flour. _____ DPP: Date pits flour.

Table (2). Fatty acids content of wheat flour and date pits powder (mg/100g dry weight basis)

Fatty acids	Material	
	SWF (100%)	DPP (100%)
Unsaturated FA		
Hexadecenoic acid C 16:1 ω 7	-	10.619
Heptadecenoic acid C 17:1	0.006	12.152
Oleic acid C18:1 ω 9	0.046	14.035
Linoleic acid C 18:2 ω-6	0.048	14.886
Linolenic acid C 18:3 ω 3	0.803	15.068
Ecosenic acid C 20:1 ω 9	0.002	17.242
Saturated FA		
Lauric acid C 12:0	0.002	06.206
Myristic acid C 14:0	0.004	07.879
Palmitic acid C16:0	0.013	10.181
Margarinic acid C 17:0	0.011	11.682
Stearic acid C 18:0	0.022	13.556
Arachidic acid C 20:0	0.004	16.864
Docosanoic acid C ₂₂ H ₄₄ O ₂	0.029	20.281
Ligoceric acid C ₂₄ H ₄₈ O ₂	0.150	24.686

Table (3). Wet, dry gluten contents and gluten index (GI) in wheat flour for different blends with date seed powder

Sample	SWF (100%)	SWF 95% + DPP 5%	SWF 90% + DPP 10%	SWF 85% + DPP 15%
Wet gluten %	25.10	23.80	19.24	14.21
Dry gluten %	09.12	07.30	06.50	05.90
Gluten Index (GI)	96.60	94.07	93.94	90.21

Table (4). Farinograph measurements of soft wheat flour and different blends with date seed powder

Sample	Water absorption %	Arrival time (min)	Dough development time (min)	Dough stability time(min)	Degree of softening (B.U)
SWF (100%)	51.6	1.5	2.5	5.0	90
SWF 95% + DPP 5%	54.6	1.5	2.0	6.5	100
SWF 90% + DPP 10%	67.6	1.5	3.0	7.5	130
SWF 85% + DPP 15%	70.0	1.0	5.5	9.0	150

Table (5). Physical properties of pan bread fortified with date pits powder at various ratios

Parameters	Control	Date pits powder		
		5%	10%	15%
Fermentation time (min)	20	25	25	26
Weight before baking (g)	300	300	300	300
Weight after baking (g)	290±0.2	284±0.3	275±0.2	258±0.5
Change in weight after baking (%)	3.33	5.33	8.33	14.00
Weight relative to control (%)	100	98	95	89.33
Loaf volume (cm ³)	1150	850	825	750
Volume relative to control (%)	100	73.91	71.73	65.21
Loaf height (cm)	7.2±0.2	6.9±0.2	6.2±0.2	5.4±0.2
specific volume (cm ³ /g)	3.97	2.99	3.00	2.91
Blank Density (g/cm ³)	0.25	0.33	0.33	0.34

Each value represents the mean of 3 replications and expressed as mean ± SD.

Table (6). Sensory evaluation of pan bread fortified with date pits powder at various ratios

Types of bread	Taste 20	Odor 20	Volume 15	Crust color 15	Crumb color 15	General Appearance 15	Overall acceptability 100
Control pan bread	18.30 ^{ab} ±0.26	18.00 ^b ±0.72	13.35 ^a ±0.23	13.31 ^b ±0.24	12.18 ^c ±0.73	13.20 ^{bc} ±0.35	88.34
Bread+5% DPP	18.40 ^{ab} ±1.06	19.00 ^a ±0.42	13.05 ^{ab} ±0.26	14.70 ^a ±0.41	13.42 ^b ±0.92	14.27 ^b ±0.16	94.14
Bread+10% DPP	19.00 ^a ±1.06	19.40 ^a ±0.63	12.92 ^b ±0.97	14.80 ^a ±0.74	13.93 ^b ±0.72	14.89 ^a ±0.63	95.24
Bread+15% DPP	19.30 ^a ±1.02	19.60 ^a ±0.13	12.88 ^b ±0.23	14.84 ^a ±0.56	14.89 ^a ±1.48	14.96 ^a ±1.09	97.74

The average of total score was converted to a descriptive category as follows: V.good: 90-100, Good: 80-89, Acceptable: 70-79, Poor: less than 70.

Table (7). Effect of date pits fortified bread on food intake, body weight gain, glucose, insulin and HbA1C of diabetic rats

Parameters Groups	FI (g/day)	BWG (%)	Glucose (mg/dl)	Insulin U/ml	HbA1c %
Control negative group	14.65	63.642 ^a ± 3.05	66.05 ^d ±3.49	18.90 ^a ± 3.25	15.05 ^d ± 3.25
Control positive group	13.14	53.556 ^c ± 2.73	152.59 ^a ± 3.41	8.50 ^d ± 1.09	20.40 ^a ± 4.99
Bread + 10% DPP	14.73	60.023 ^b ± 5.30	119.85 ^b ± 4.73	16.40 ^{bc} ± 4.02	18.70 ^b ± 5.03
Bread +15% DPP	15.97	64.184 ^a ± 4.16	105.60 ^c ± 4.15	17.10 ^b ± 4.60	17.50 ^{bc} ± 3.79

All results are expressed as mean ± SD. BWG%: Body Weight Gain %. FI: Food Intake (g/day). Values in each column which have different letters are significantly different (p<0.05).

Table (8). Effect of date pits fortified bread on lipid profile of diabetic rats

Parameters Groups	mg/dl				
	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C
Control negative	88.72 ^b ±7.02	45.75 ^d ± 4.09	68.50 ^a ±3.57	11.07 ^d ±2.35	9.15 ^d ±0.92
Control positive	90.63 ^a ± 5.58	76.13 ^a ±11.85	44.86 ^d ±5.19	30.54 ^a ±4.99	15.23 ^a ±1.81
Bread + 10% DPP	83.42 ^c ± 6.84	70.13 ^b ±5.26	50.75 ^c ±6.47	18.64 ^b ±3.94	14.03 ^b ±0.45
Bread + 15% DPP	88.96 ^b ± 5.28	57.29 ^c ±7.56	61.71 ^b ±4.46	15.79 ^c ±2.39	11.46 ^c ±0.48

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different (p<0.05).

Table (9). Effect of date pits fortified bread on kidney and liver functions of diabetic rats

Parameters Groups	mg/dl			(u/l)	
	Uric acid	Urea nitrogen	Creatinine	AST	ALT
Control negative group	1.40 ^d ± 0.13	56.87 ^d ± 8.16	0.55 ^{cd} ± 0.09	28.75 ^d ± 3.89	08.38 ^{cd} ± 1.24
Control positive group	2.46 ^a ± 0.13	90.43 ^a ± 8.74	1.09 ^a ± 0.08	67.71 ^a ± 3.89	24.57 ^a ± 6.35
Bread + 10% date pits	1.63 ^b ± 0.13	64.29 ^b ± 4.22	0.71 ^b ± 0.05	41.29 ^b ± 6.31	11.25 ^b ± 1.84
Bread + 15% date pits	1.59 ^c ± 0.17	60.03 ^c ± 4.22	0.58 ^c ± 0.039	31.75 ^c ± 4.26	09.29 ^c ± 1.61

All results are expressed as mean ± SD.

Values in each column which have different letters are significantly different (p<0.05).

The rheological analyses (Table 4) were realized on samples from the SWF as a control sample and variety of blends at 5%, 10% and 15% from DPP. Our results indicated that Farinograph absorption expresses the amount of water (%) needed to form from 100g of flour the standard dough, addition of date pits flour at 5%; 10% & 15%

into bread dough systems increased dough water absorption rate, the ratios reached to 54.6%, 67.6% and 70.0% respectively, comparing with bread wheat flour gave 51.6% only, while, there were reduced in an arrival time. In terms of success of new varieties in approval process, Farinograph absorption represents a very important

criterion and the high values are expected which predicts high absorption ability of wheat proteins. Results indicated that chemical composition of date pits powder percent in blend and a particle size of these products effect on the water absorption of the blend and properties of dough, these results are in agreement with those of Abang *et al.*, (2008) and Ktenioudaki *et al.*, (2010). According to Bojňanská *et al.*, (2013) revealed that the process of dough formation from the initial water addition to flour up to forming of compact dough with desired qualities (consistency, resistance to deformation, stability) goes through different phases during which fluidity, firmness and elasticity gradually change.

Our results indicated that the incorporation of various levels of DPP into wheat flour significantly increased in the development of dough, time stability and degree of softening, in fact, incorporation of the date pits powder at 10% & 15% produced dough that was more stable compared with control dough (wheat only). These results are in agreement with Skendi *et al.*, (2009) and Voicu *et al.*, (2012), confirmed by Bojňanská *et al.*, (2013) they showed that dough development time depends on amount and quality of gluten, flour granules and degree of milling and dough stability indicates the time interval during which dough maintains maximal consistency, and the high dough stability are considered of good quality from the point of view of further baking use.

Physical properties of pan bread: Results of the parameters for control bread with wheat flour and fortified bread with various levels of date pits powder at (5%, 10% & 15%) are illustrated in Table (5). There was a delay in fermentation time with all fortified bread with date pits as compared to that of control bread. It was found that percent of weight relative to control value decreased upon increasing the level of date pits powder by (98, 95 & 89.33% respectively. At the same time, percent of volume relative of control bread compared to the bread fortified with date pits at different ratio decreased by 73.91, 71.73 & 65.21% respectively, this reduction in volume could be related to lower gluten content in bread fortified with date pits which had high water absorption, therefore less water is available for gluten formation.

Density as a percentage of control value increased with increasing level of date pits powder (0.25, 0.33, 0.33 & 0.34 g/cm³) respectively, may be related to the increase in relationship between volume and density, as the volume decrease, the density increases. The present results are agreement with those obtained by Titcomb & Juers (1996) who found that addition of cereal bran to formulations of baked products may result in loss of baked volume. Jinshui & Benedito (2002) mentioned that fibers, increased water absorption and adversely affect volume and specific volume in bakery products. Sangnark & Noomhorn (2003) and Bouaziz *et al.*, (2010) revealed that volume and specific volume of white pan bread decreases as dietary fiber increased. The loaf volume of fortified breads with date pits was lower than the bread control, also in terms of

specific bread volume (cm³/g), date pits at 5%, 10% & 15% promoted a significant decrease compared with control bread without any supplementation.

Sensory evaluation of pan bread: Most of panelists preferred the odor and taste of pan breads made from 10% and 15% of the date pits powder Table (6). They commented that the odor and taste of 5% of date pits with wheat flour was like the control bread. However, pan bread made with 10% and 15% of date pits powder received the highest overall score than pan bread containing wheat flour without any supplementation. These results are in agreement with Besbes *et al.*, (2009) and Bouaziz *et al.*, (2010) they published that by-product of date-processing industries could be regarded as an excellent source of food ingredients with interesting technological functionality that could also be used in food as an important source of dietary fiber. However, Najafi (2011) showed that bread containing 10% date seed had higher dietary fiber content and similar sensory properties to the wheat bran control, but lower color, flavor, odor, and overall acceptability sensory scores.

Replacing up to 10% & 15% of the date pits with wheat flour reduced volume. Reductions in volume were probably related primarily to the overall decrease in gluten content, confirmed by Kawka *et al.*, (1999) and Reda (2006). The crust and crumb colors were significantly different in pan bread containing 5% & 10% compared with the control bread, while, in pan bread containing 15% date pits there was a significant increase than that of control (El-Porai *et al.*, 2013). The results of crust, crumb colors and general appearance showed that there were significant changes between control pan bread (100% SWF) and pan bread containing date pits powder, confirmed by Mirghani *et al.*, (2012) who evaluated taste, texture, aroma, appearance and overall acceptability for pan breads fortified with date pits which can be a source of dietary fibers, without any negative impact on sensory quality of end-products. Pan bread supplemented with 15% date pits, showed significant high score in overall acceptability when compared to control pan bread and other various concentrations of pits. Generally, changes in dough properties were greater using different concentrations of date pits with significant differences for overall acceptability and had good acceptance to most members compared to control pan bread.

Data presented in Table (7) showed the mean value of food intake and body weight gain of hyperglycemic groups. The mean value of food intake decreased in the positive control group (injected with alloxan) than that of the negative control group (13.14 vs. 14.65 g/day, respectively). At the same time, our results indicated that rats fed on fortified bread with (10% & 15% DPP) showed gradual increase in food intake in diabetic rats. From the data presented in the same Table (7) it could be observed that, there was a significant decrease ($p < 0.05$) in body weight gain for control positive group, as compared to the negative control group. BWG% of all treated diabetic groups with fortified bread (10% & 15% DPP) increased significantly ($p < 0.05$), as compared to the positive control group. On the

other hand all treated groups showed non-significant changes, as compared to the negative control group.

The effect of different levels of date pits fortified bread on concentrations of glucose; insulin and HbA1c of diabetic rats are illustrated in Table (7). The serum glucose and HbA1c levels were increased as expected in alloxan injected rats (positive group) to reach 152.59 mg/dl and 20.40%, than that of the control negative group since alloxan causes a massive reduction in insulin 8.50 U/ml, release by the destruction of the β -cells of the islets of Langerhans and inducing hyperglycemia. Confirmed by Prabu & Natarajan (2013) who showed that HbA1c concentration is proportionately increased in diabetic patients with ambient hyperglycemic and reflects the extent as well as management of diabetic condition.

Oral administration to diabetic groups of date pits fortified bread at 10% & 15% after 45 days resulted in a significant reduction in the blood glucose levels. ($P < 0.01$), it reached 119.85 & 105.60 mg/dl, the reduction is less by 21.46% and 30.79%, respectively compared with the positive control group. At the same time, ratios of HbA1c decreased by 8.33% and 14.21%, respectively, our results agree with Bouaziz et al., (2010) they observed that high content in fibers in two varieties of date pits (Deglet Nour and Allig), could be considered as an ingredient in dietetic food formulations. Moreover, treatment with bread fortified with date pits may increase the activity of the enzyme glucose 6- phosphate dehydrogenase, via increased secretion of insulin which increases the influxes of glucose into pentose monophosphate shunt in an attempt to reduce high blood glucose levels revealed by Akbari, et al., (2012); Hussein et al., (2013) and Nandhagopal et al., (2013).

The increase in insulin reached to highly levels by 92% & 101% after feeding bread fortified with 10% & 15% date pits powder than that fed on positive control group, it could be due to increased pancreatic secretion from existing β -cells. It is well documented that adherence to a healthy diet can improve glycemic control which may reduce glycosylated hemoglobin (HbA1c) levels confirmed by Lee et al., (2003) and Prabu & Natarajan (2013).

Effect of date pits fortified bread on lipid profile of diabetic rats: Diabetes is a key factor in the predictive equations for cardiovascular disease. There are few studies assessing the effects of date pits in diabetes, which may be part of vegetarian diet. In combination, these plant food components may have a very significant impact on blood lipids and cardiovascular disease, which appeared to be complications of diabetes (Reda 2006). Type II diabetic patients in combination with hypercholesterolemia, increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation was proposed to be the mechanisms responsible for the hypocholesterolemic properties Yao & Chiang (2006a) and Prabu & Natarajan (2013).

To evaluate the effect of fortification of bread with (10% & 15% date pits) components in this respect, total cholesterol (TC), triglycerides (TG), low and very low

density lipoprotein cholesterol (LDL-C & VLDL-C) and high-density lipoprotein cholesterol (HDL-C) in diabetic rats is presented in (Table 8). It could be noticed that the control positive group fed on basal diet has shown a significant increase $p < 0.05$ in the mean values of TC, TG, LDL-C and VLDL-C (90.63 ± 5.58 ; 76.13 ± 11.85 ; 30.54 ± 4.99 & 15.23 ± 1.81 mg/dl) respectively, compared with those of the control negative group (healthy rats) fed the same diet (48.29 ± 7.02 ; 45.75 ± 4.09 ; 11.07 ± 2.35 & 9.15 ± 0.92 mg/dl) respectively. Concerning the mean value of serum HDL-C, the control positive group exhibited a markedly significant decrease as compared to the negative control group (68.50 ± 3.57 vs 44.86 ± 5.19 mg/dl). All tested rats fed on bread fortified with date pits at 10% & 15% improved serum lipid fraction, in comparison with alloxanized control group (positive). The best results in lipid fractions for all treated groups was noticed in diabetic group fed on basal diet containing the date pits used in fortification of bread at 15%.

The effect of date pits used in fortified bread with (10% & 15%) on kidney functions (uric acid, urea nitrogen and creatinine) of diabetic rats is presented in Table (9). Diabetes is among the leading causes of kidney failure. Ten to twenty percent of people with diabetes die of kidney failure (Singh et al., 2012). It could be observed that the control positive group fed on basal diet has shown a significant increase ($p < 0.05$) in serum uric acid, urea nitrogen and creatinine, compared with those of the control negative group fed on basal diet (2.46 ± 0.13 vs. 1.40 ± 0.13 ; 90.43 ± 8.74 vs. 56.87 ± 8.16 and 1.09 ± 0.08 vs. 0.55 ± 0.09 mg/dl) respectively, that confirmed previously by Shebl et al., (2013) they referred that the chronic elevation of plasma glucose causes many of the major complications of diabetes including nephropathy, retinopathy, neuropathy, macro- and micro vascular damages.

Our results indicated also that, all treated groups fed on fortified bread with 10% & 15% levels of date pits induced significant decrease $p < 0.05$ in all parameters of kidney functions, as compared to the positive control group. The highly significant reductions of all parameters were observed in the group fed on date pits fortified bread with 15%. Ratios of parameters were 1.59 ± 0.17 ; 60.03 ± 4.22 and 0.58 ± 0.039 mg/dl as compared with control negative group 1.40 ± 0.13 ; 56.87 ± 8.16 and 0.55 ± 0.09 mg/dl, respectively. Results in the same table indicated that, feeding diabetic rats on basal diet resulted in significant increase in serum AST & ALT, as compared to healthy rats fed on basal diet (67.71 ± 3.89 and 24.57 ± 6.35 vs. 28.75 ± 3.89 and 08.38 ± 1.24 u/l), respectively. Moreover, the mean values \pm SD of serum AST & ALT for the positive control groups of diabetic rats showed significant increase $p < 0.05$, compared with other groups fed on different levels of bread fortified with date pits at 10% and 15%.

The best results of liver function recorded with group rats fed on bread fortified with date pits at 15%, because this group showed non-significant changes in AST & ALT enzymes activity, compared to control negative groups. The

results of the present study agree with Reda (2006) and Najafi (2011) who revealed that date seed extract shows an ability to restore the normal functional status and also to protect liver in rats against poisoning. Moreover, Hadrami & Al-Khayri (2012) published that improving diabetic diet may help to prevent a variety of diseases. In addition, individuals with type 2 diabetes have a higher incidence of liver function test abnormalities than individuals who do not have diabetes. Mild chronic elevations of transaminases often reflect underlying insulin resistance (Salih, 2013).

4. Histopathological Results

Microscopical examination of control negative group revealed normal hepatic tissues (Figure 1), and normal pancreatic acini, islets, and ducts (Figure 2). Regarding histopathological examination of control positive group, the liver showed dilated and congested blood sinusoids and fatty hepatocytes with signet ring appearance (Figure 3), and pancreas revealed hyperplasia of the pancreatic islets (Figure 4). Considering the initial treated group (bread fortified with 10% date pits powder), there were dilated liver blood sinusoids only with disappearance of the fatty cells infiltration (Figure 5), and pancreas revealed minute blood vessel congestion (Figure 6). Referring the final treated group (bread fortified with 15% date pits powder), there were marked improvement and normal hepatic parenchyma (Figure 7), and normal pancreatic acini (Figure 8).

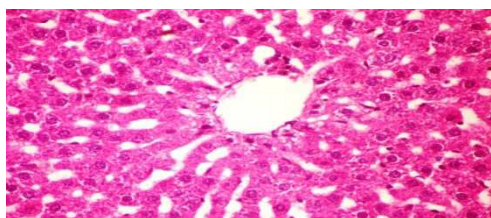


Fig (1). Section through liver of control untreated rat showing normal histological findings (H&E X 400).

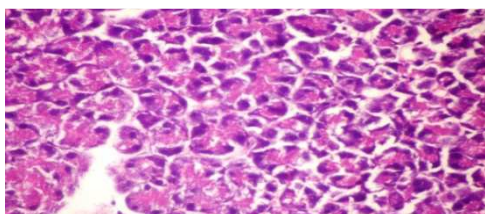


Fig (2). Pancreas of control untreated rat showing normal pancreatic acini (H&E X 400).

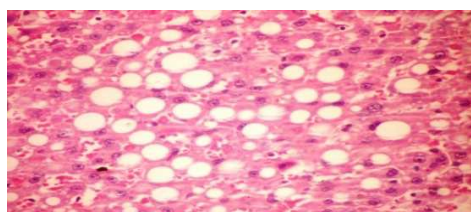


Fig (3). Liver of rat from group fed on positive diet showing dilated and congested blood sinusoids (arrow), and fatty hepatocytes with signet ring appearance (arrow head) (H&E X 400).

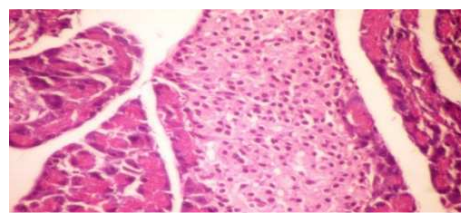


Fig (4). Pancreas of rat from group fed on positive diet showing hyperplasia of the pancreatic islets (arrow) (H&E X 400).



Fig (5). Fortified bread with 10% date pits: Liver showing dilated blood sinusoids (arrow), and congested central vein (c) (H&E X 400).

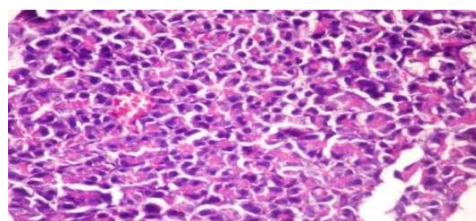


Fig (6). Fortified bread with 10% date pits: Pancreas showing minute blood vessel congestion (c) (H&E X 400).

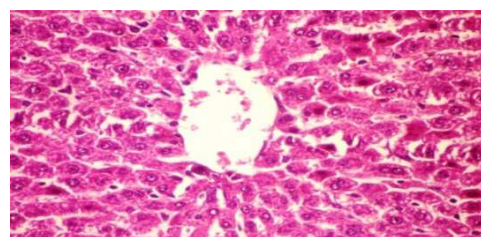


Figure (7). Fortified bread with 15% date pits: Liver showing normal histological findings (H&E X 400).

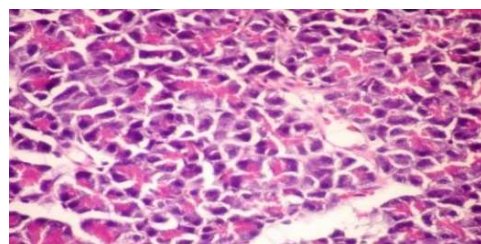


Figure (8). Fortified bread with 15% date pits: Pancreas showing normal pancreatic acini (H&E X 400).

5. Conclusion

The current investigation concludes that bread fortified with date pits powder had improving effects on the quality and nutritive values of pan bread as well as it has a hypoglycemic effect and could have a protective effect against diabetes complications as well as improvement of lipid profile.

Nutrition education programs should be designed to inform the public about the nontraditional fortification of bread with date pits and different baked products might decrease the risk of diabetic diseases.

Recommendation

- The study may encourage plant chemists, pharmacologists, biochemists and molecular biologists to combine their efforts in a search for natural agents that can limit with better therapeutic management of diabetic diseases.

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