

Influence of Fortification of Biscuits with *Hyphaene thebaica* Flour on Quality Attributes, Biochemical Parameters and Histological Examination of Pancreas in Diabetic Rats

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Abstract:

With the widespread use of functional foods, the worldwide looking for new healthy food products with suitable proportions of bioactive ingredients such as fiber, phenolic and flavonoid compounds. Doum is a rich source of fiber and antioxidants. The aim of this research was to use doum flour (DF) in biscuits preparation to increase its nutritional value. Wheat flour was substituted by three levels of DF 10, 20 & 30% to prepare biscuits. Chemical and sensory properties of prepared biscuits were studied. Also, the effect of feeding diabetic rats with doum biscuits on biochemical examinations and histological changes of pancreas was assessed. Results showed that increasing the ratio of DF in biscuits led to a significant increase ($P<0.05$) in fiber and ash values in prepared biscuits as compared to control. Results recorded that significantly increased gradually ($P<0.05$) in phenolic and flavonoid levels as well as antioxidant activity when the level of DF increased in biscuits compared to control. Sensory evaluation showed that biscuits fortified with DF up to 30% were acceptable by panelists compared to control biscuits. A significant decrease ($P<0.05$) in blood glucose, TG, TC, LDL-C and VLDL-C values was noticed after feeding diabetic rats with doum biscuits at different levels of DF, while, insulin and HDL-C levels significantly increased ($P<0.05$) comparing with diabetic rats. Findings demonstrated that a significant reduce ($P<0.05$) in AST and ALT as well as ALP levels of diabetic rats treated with biscuits containing DF comparing to diabetic rats. Also, kidney functions were significantly improved ($P<0.05$) for diabetic rats fed on doum biscuits, whereby both creatinine and urea values in serum were significantly reduced ($P<0.05$) compared to diabetic rats. Moreover, feeding diabetic rats with doum biscuits showed marked improvement in pancreatic tissues and these results are consistent with findings obtained above. Results confirmed that the best improvement for all parameters was found in diabetic rats group fed on doum biscuits (30% DF) as compared to

diabetic rats where parameters reached to be near that of the healthy control group. Furthermore, doum biscuits (30% DF) have the highest values of fiber and ash as well as phenolic and flavonoid compounds and this associated with the lowest energy value as compared with control biscuits. Accordingly, it can be concluded that doum biscuits which were produced by low cost have potential benefits in the preventing and treating of diabetes and play a role in its management as well as reduce the risk of diabetes complications. Also, doum biscuits could be useful for people suffering from obesity and hyperlipidemia. Thus, this will aid in the promotion of consuming doum biscuits and management problems related to nutrition in Egypt. Therefore, the current study recommends increasing the consumption of bakery products fortified with doum flour, increase consumers awareness about benefits of consuming food products made from doum because its great importance in the preventing and treating of diabetes and some other diseases via the audio-visual media and health centers.

Key words: *Hyphaene thebaica*, biscuits, fortification, antioxidant activity, alloxan, sensory evaluation and diabetes.

Introduction:

Diabetes is one of the most common problems challenging the physicians in 21st century (**Bennet, 2004**). Diabetes mellitus is considered as one of the chronic diseases, it results from the pancreas gland does not produce insufficient insulin or the human body cannot effectively utilize the insulin it produces (**WHO, 2006**). Diabetes mellitus causes dangerous complications such as kidney failure, stroke, blindness, lower limb amputation and heart attacks (**Tripathi and Srivastava, 2006**). There has been an increase in the prevalence of diabetes at an alarming rate and this prevalence reached to be epidemic in some of the world's population (**Williamson, et al., 2013**). Diabetes is one of the biggest health challenges in Egypt, and diabetics in adults (20–79 years) reached to 8.9 million patients in 2019. Egypt is ranked ninth globally in terms of the prevalence of diabetes. It is expected to reach the seventh rank globally by double the number of 16.9 million diabetics by 2045 if it is not well controlled. Middle- and low-income countries has the highest prevalence in diabetes (**IDF, 2019**).

Diabetes is the third human killer after cancer and cardiovascular diseases, is one of the most challenging diseases facing health care

professionals today. So, there is a trend to find natural products that lower blood sugar, especially from plant sources.

Hyphaene thebaica is a kind of palm tree which is considered as a member of family Arecaceae and its common name is doum. Doum has been cultivated since ancient times in Egypt as well as found along the Nile River in both Egypt and Sudan where the presence of groundwater. Doum is considered as one of the beneficial plants in worldwide (**Fletcher, 1997**). Doum fruit is one of nutrients that maintain life in desert areas, specifically during dried periods (**Hsu et al., 2006**). Doum fruit is rich in minerals including calcium, sodium, potassium, phosphorus and magnesium, moreover, it provides B complex vitamins, carbohydrate and fiber essential for improving human nutrition and health (**Aboshora et al., 2014**). Also, *Hyphaene thebaica* a good source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds. *Hyphaene thebaica* is considered as antioxidant (**Gharb and Fadhel, 2018**), anticancer (**Mohammed et al., 2019**), antimicrobial (**Mohamed et al., 2010**), hypotensive (**El-Gendy et al., 2008**), anti-inflammatory (**Farag and Paré, 2013**) and hypolipidemic (**Elhaj and El-Bagir, 2016**). Doum tea is popular in Egypt and is believed to be beneficial for diabetics (**Salib et al., 2013**). Alcoholic and aqueous extracts of *Hyphaene thebaica* improved the carbohydrate metabolism of diabetic rats (**Abdel-Rahim et al., 2011 and Bayad, 2016**). Doum powder was used in some foods as a source of fiber (**Aboshora et al., 2019**), minerals and stabilizer (**Abdelrashid and Hassan, 2005**).

The demand for healthy, natural and high-quality products is increasing for Egyptian consumers. So, one of the recent trends is to fortify food products with some nutrients to overcome health problems in particular against diabetes (**Abul-Fadl et al., 2016**). Biscuits are considered as a common food consumed by a large percentage of the population, due to their long shelf life, varied taste and low cost (**Agarawal, 1994**).

There is an urgent need to increase the abundance and consumption of healthy foods that appeal to consumers in order to counteract the diet-related diseases such as obesity and diabetes mellitus (type 2). Furthermore, doum is available at a cheap price in Egypt. So, this study aimed to investigate the effect of adding *Hyphaene thebaica* to biscuits in different concentrations on sensory evaluation, nutritional value, antioxidant content as well as study biological

effects of feeding diabetic rats on prepared biscuits at various concentrations of doum.

Materials and Methods:

Materials:

Hyphaene thebaica (doum) fruit was purchased from local herbal shop in Sharkia Governorate, Egypt. Wheat flour (72% extraction), sugar, salt, shortening, sodium bicarbonate, baking powder and ammonium bicarbonate were obtained from local markets in Sharkia Governorate, Egypt. Casein, cellulose, minerals, vitamins were purchased from El-Gomhoria Company, Sharkia Governorate, Egypt. Purchasing of Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl, gallic acid and alloxan were from Sigma Chemical Company, USA. Kits for blood analysis were purchased from Alkan-Medical Division Biocon, Germany. All chemicals were of the analytical reagent grade.

Methods:

Preparation of doum flour:

Doum flour was obtained after grinding the pulp using an electric mill. The milled flour was stored in air-tight polyethylene bags at 4°C until used.

Biscuit Preparation:

Biscuits were prepared by substituting wheat flour (72% extraction) with 10, 20 and 30% doum flour (DF) as described by **Manohar and Rao, (1999)** with modification in amount of sugar which was reduced from 30 to 20g/100g flour. Control sample was prepared without using of doum flour. All the other ingredients were added at the same ratio percentages as follows: wheat flour (100g), shortening (20g), salt (1g), baking powder (0.3g), sodium bicarbonate (0.4g) and ammonium bicarbonate (1g). The basic recipe for biscuits preparation was as follows: sugar and fat were whipped to a white cream for 3-4 min in a mixer. Sodium bicarbonate, ammonium bicarbonate and salt were dissolved in (20-22 ml water), then the solution was added to the above white cream and mixed for 5 min to obtain a homogenous cream. Wheat flour or mixtures of wheat flour with doum flour were sieved twice with baking powder and were added to the above cream then all were mixed for 3 min. Biscuits dough was sheeted to a thickness of 3.5 mm, sheets were cut using circular shape (45 mm diameter), placed on a tray and baked at 160°C for 15

min, then allowed to cool for 1 hr. Biscuits were packaged in polyethylene bags and stored in air-tight containers for further analyses.

Animals preparation:

Thirty-six adult male wistar strain albino rats weighing between 130 - 140g were used in this study. Rats were purchased from the Faculty of Veterinary Medicine, Zagazig University, Sharkia Governorate, Egypt. Rats were lived separately in stainless steel cages under hygienic conditions (under standard conditions of temperature, humidity and a light/dark cycle). Animals were allowed to acclimatize for a period of one week before the experiment with free access to food and water *ad libitum*. Rats were fed on a standard diet according to **Reeves *et al.*, (1993)**.

Induction of diabetes:

Diabetes was induced in overnight fasted rats by single intraperitoneal injections of a freshly prepared solution of alloxan monohydrate 150 mg/kg BW (**Buko *et al.*, 1996**) dissolved in physiological saline according to **Famakin *et al.*, (2016)**. Animals were allowed to drink 5% glucose solution for 24 h to avoid the drug-induced hypoglycemia. Rats were considered as diabetic, if their blood glucose values were above 250 mg/dl after three days of alloxan injection (**Zheng *et al.*, 2011**) and severe diabetic rats were selected for current study.

Experimental design:

Animals were divided into (6) groups (6 rats for each group). The first group (G1) normal rats serving as normal control, received standard diet and considered as negative control. Diabetic groups (G2), (G3), (G4), (G5) & (G6) fed as following. The second group (G2) consumed standard diet and considered as positive control. The third group (G3) consumed standard diet after replacement 30% of the starch in the standard diet by control biscuits. The fourth (G4), fifth (G5) and sixth (G6) groups were fed on the standard diet containing 30% doum biscuits at different levels 10, 20 and 30%, respectively.

Body weight of rats was recorded at the beginning of the test and then every week and food consumption was recorded daily for twelve weeks. After the end of twelfth weeks of the experiment, rats were anesthetized with diethyl ether after 12 hours fasting. Blood samples were taken via cardiac puncture and centrifuged at 3000 rpm for 20 min to obtain the serum, which were kept at -

20°C until analysis. Pancreas was immediately removed from rats to be examined microscopically.

Chemical composition:

Moisture, protein, ash, fiber and fat were estimated according to **AOAC, (2012)**. Total carbohydrate was calculated by the equation as follows:

Total carbohydrate = 100 - (protein% + fat% + ash% + fiber%).

Calculation of energy (Kcal/100g sample):

The energy value (EV) was calculated for different biscuits samples according to **Chaney, (2006)** as follows:

Energy value (Kcal/100 sample) = (Carbohydrates% x 4) + (proteins% x 4) + (fats% x 9).

Biochemical Analysis:

Blood glucose estimation was done by using (**Trinder, 1969**) method. Insulin level was determined by the method of (**Temple et al., 1992**). Lipid profile [total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C)] were estimated by using (**Richmond, 1973**), (**Fossati and Prencipe, 1982**) and (**Burstein et al., 1970**) methods, respectively. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated by equations as described by (**Friedewald et al., 1972**). Liver functions [alanine aminotransferase (ALT) and aspartate-aminotransferase (AST)] estimations were carried out by (**Reitman and Frankel, 1957**), while, alkaline phosphatase (ALP) was estimated as described by **Klein and Kaufman, (1967)**. Kidney functions including urea was determined according to (**Patton and Crouch, 1977**) method, whereas, creatinine was measured by the method of (**Larson, 1972**).

Free radical scavenging activity Determination:

Free radical scavenging activity of raw materials and biscuits was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by **Brand-Williams et al., (1995)**.

Total phenolic determination (TP):

The Folin–Ciocalteu method was used and the measurement was performed at 765 nm with gallic acid as the standard as described by **Singleton *et al.*, (1999)**. Phenolic values were expressed as mg of gallic acid equivalents (GAE) per g of sample.

Total flavonoid determination (TF):

Flavonoids was measured at 510 nm with known quercetin concentration as a standard and expressed as milligrams of quercetin equivalents per g of sample (**Zhishen *et al.*, 1999**).

Sensory evaluation:

Organoleptic quality was carried out for biscuits by using 20 semi trained panelists from the staff members of the Department of Food Science, Faculty of Agriculture, Zagazig University, Egypt, to evaluate color, taste, odor, crispness and appearance and overall acceptability (**Saleh *et al.*, 2012**).

Histological examination:

After 3 months, rats were euthanized and the pancreas was removed. Samples were washed with normal saline and were fixed in 10% formalin. The histopathological examination was done as described by **Suvarna *et al.*, (2013)**.

Statistical Analysis:

Results were analyzed using SPSS program (version 25) and presented as mean \pm standard deviations (SD). The one-way analysis of variance (ANOVA) and Duncan's multiple comparison test were used to determine the most significant effect of feeding by doum biscuits. P-values were considered as significant when ($p < 0.05$) (**IBM SPSS, 2017**).

Results and Discussion:

Chemical composition of doum and wheat flour:

Chemical characteristics of doum and wheat flour in Table (1), revealed that DF showed higher levels of fiber (14.90 %) and ash (5.48%) than wheat flour, conversely, wheat flour indicated higher values of moisture (11.70%), protein (10.05%), fat (1.50%) and carbohydrate (75.33%) than DF. Fat content of doum flour was found to be 0.91% and this result is in agreement with the result reported by **Siddeeg *et al.*, (2019)**. It can be noticed that carbohydrate

(65.67%) and protein (3.63%) contents of doum flour in the present study were similar to that found by **Abubakar *et al.*, (2017)** who estimated that carbohydrate and protein levels were 65.90 and 3.34%, respectively, in doum. **Mohammed and Zidan, (2018)** reported that moisture and fiber values were 7.81 and 15.50%, respectively, in Egyptian doum. Protein content of wheat flour is close to that determined by **Kefale and Yetenayet, (2020)** who found that protein reached 10.66% in wheat flour. These results are in line with **Aniess *et al.*, (2015)** who found that ash and fat levels in wheat flour were 0.42 and 1.52%, respectively.

Table 1. Chemical composition of doum and wheat flour (as fresh weight).

Constituents %	Raw materials	
	Wheat flour	Doum flour (DF)
Moisture	11.70±0.52	9.41 ± 0.30
Protein	10.05±0.65	3.63 ± 0.20
Fat	1.50 ±0.12	0.91 ± 0.02
Fiber	1.01±0.06	14.90 ± 0.32
Ash	0.41±0.03	5.48 ± 0.20
Carbohydrate	75.33 ±1.30	65.67 ±1.20

All values are means of 3 determinations ± SD.

Phenolic and flavonoid compounds & DPPH radical scavenging activity of DF:

From the Table (2) results showed that total phenolic compounds were 32.20 GAE/g sample in DF, whereas, total flavonoid was 21.40 QE/g sample. **Taha *et al.*, (2020)** estimated that flavonoids in doum fruit was 20.40 mg QE/g sample. **Atito *et al.*, (2019)** stated that total phenolic was 40.65 GAE/g sample in doum (mesocarp). In addition, DPPH radical scavenging percentage of doum flour was 64.22%. This result is in agreement with **Mohamed *et al.*, (2010)** who investigated that DPPH scavenging activity was 64.55% in *H. thebaica*.

Table 2. Phenolic and flavonoid compounds & DPPH radical scavenging activity of doum flour.

Raw material	TP mg GAE/g	TF mg QE/g	DPPH %
Doum flour	32.20±0.80	21.40±0.40	64.22±1.30

All values are means of 3 determinations ± SD.

Chemical composition for all types of biscuits:

Moisture, fiber and ash values (as fresh weight) significantly increased ($P<0.05$) in made biscuits with increasing the DF level, whereas, protein, carbohydrate, fat and energy values significantly decreased ($P<0.05$) comparing with control biscuits (Table 3). Moisture level of biscuits significantly increased ($P<0.05$) from 2.71% in control biscuits to 3.25% in biscuits fortified with 30% DF. This result is in line with **Aboshora *et al.*, (2016)** who found that fortified bread with doum flour led to a significant increase in moisture level. The gradual increase in moisture content probably is due to the increase in water retention ability of fibers, also, increase the amount of water required to make the dough of biscuits which have more level of fiber (**Choudhury *et al.*, 2015**). Results indicated that protein and fat values of biscuits decreased from 7.63 to 6.48 % and from 14.53 to 14.20%, respectively, with increasing of the amount of DF perhaps this is due to low values of protein (3.63%) and fat (0.91%) in DF as compared with wheat flour. Similar result was found by **Aboshora *et al.*, (2016)** who stated that doum flour-wheat flour bread contained less protein than the control bread. Furthermore, current findings showed that fiber content in control biscuits (0.77%) was the lowest as compared with other prepared biscuits with different proportions of DF. The amount of fiber in doum biscuits with various concentrations of DF (10, 20 and 30%) was 2.17, 3.67 and 5.22%, respectively. Results indicated that fiber content in doum biscuits (30% DF) was approximately sevenfold that of the control biscuits. These results perhaps are due to DF has higher amount of fiber (14.9%) than wheat flour (1.01%). The amount of ash in control biscuits was reported to be 0.36%, while, ash level was significantly increased ($P<0.05$) in doum biscuits prepared with different concentrations of DF (10-30%) from 0.96 to 2.00%. Findings revealed that ash level in biscuits containing 30% DF was approximately sixfold that of the control biscuits. This increase in ash value of doum biscuits perhaps is attributed to higher ash content in DF (5.48%) than wheat flour (0.41%). Biscuits fortified with 30% doum flour had the lowest carbohydrate (68.85%) and energy (429.12%) values as compared with control biscuits. The same findings were found for moisture, protein, fiber, ash and carbohydrate in doum toast bread by **El-Hadidy and El-Dreny, (2020)** and for fat in doum bread by **Aboshora *et al.*, (2016)**.

TP and TF & antioxidant activity of biscuits samples are also clarified in Table (3). Replacement of wheat flour with DF had caused significantly

increased ($P < 0.05$) in phenolics content as compared to control biscuits. Phenolic values in biscuits samples were gradually increased with increasing doum flour level in biscuits. The highest total phenolic was found in biscuits fortified with 30% DF (9.10 mg GAE/g) and the increasing was approximately 8-times in comparison to control biscuits (1.10 mg GAE/g). These results perhaps are due to DF has higher amount of TP than wheat flour.

In comparison with control biscuits, flavonoid levels among biscuits fortified with 10–30% DF were significantly increased ($P < 0.05$). The maximum value of TF was noticed in biscuits containing 30% DF (5.31 mg QE/g) compared with control biscuits (0.21 mg QE/g) or other biscuits samples. TF increased in doum biscuits (30% DF) about 25-times in comparison to control biscuits. These findings probably are due to higher level of TF in DF than wheat flour.

With increasing DF content in biscuits DPPH radical scavenging percentage significantly increased ($P < 0.05$) as compared to control biscuits. DPPH radical scavenging percentage for doum biscuits fortified with 10, 20 and 30% DF was 26.70, 32.70 and 38.10%, respectively, while, for control biscuits the percentage was 21.20%. The antioxidant activity of doum biscuits (30% DF) was 79.7% higher than control biscuits. The increase in free radical scavenging percentage in doum biscuits perhaps is due to increase in the proportion of phenolic content in DF than wheat flour (**Abou-Elalla, 2009**). **Seleem, (2015)** obtained that an increase in the amount of total phenolic and antioxidant activity by adding doum flour to cakes.

Table 3. Chemical composition for all types of biscuits.

Parameters	Samples			
	Control	10% DF	20% DF	30% DF
Moisture%	2.71 ± 0.10 ^c	2.89 ± 0.15 ^b	3.09 ± 0.03 ^a	3.25 ± 0.03 ^a
Protein %	7.63 ± 0.16 ^a	7.18 ± 0.12 ^b	6.79 ± 0.15 ^c	6.48 ± 0.11 ^d
Fat%	14.53 ± 0.22 ^a	14.38 ± 0.31 ^{ab}	14.28 ± 0.23 ^c	14.20 ± 0.32 ^d
Fiber%	0.77 ± 0.02 ^d	2.17 ± 0.03 ^c	3.67 ± 0.04 ^b	5.22 ± 0.04 ^a
Ash%	0.36 ± 0.01 ^d	0.96 ± 0.02 ^c	1.51 ± 0.02 ^b	2.00 ± 0.02 ^a
Carbohydrate%	74.00 ± 1.35 ^a	72.42 ± 1.33 ^b	70.66 ± 1.20 ^c	68.85 ± 1.11 ^d
EV Kcal/100g	457.29 ± 2.32 ^a	447.82 ± 2.40 ^b	438.32 ± 2.50 ^c	429.12 ± 2.35 ^d
DPPH %	21.20 ± 1.00 ^d	26.70 ± 1.12 ^c	32.70 ± 1.00 ^b	38.10 ± 1.20 ^a
TP mg GAE/g	1.10 ± 0.05 ^d	3.80 ± 0.14 ^c	6.30 ± 0.15 ^b	9.10 ± 0.13 ^a
TF mg QE/g	0.21 ± 0.01 ^d	1.71 ± 0.11 ^c	3.41 ± 0.10 ^b	5.31 ± 0.10 ^a

Values within the same line followed by various superscripts are significantly different at ($p < 0.05$).

Sensory evaluation for all types of biscuits:

Sensory evaluation results for all types of prepared biscuits are illustrated in Table (4). Findings indicated that odor, taste and overall acceptability scores significantly increased ($P<0.05$) with increasing the percentage of DF in biscuits compared to control biscuits. Addition of DF to biscuits led to improve odor and taste of biscuits this is perhaps is due to the good flavor of doum. Similar observations were reported by **El-Hadidy and El-Dreny, (2020)** who stated that taste and odor scores significantly increased with increasing doum powder in toast bread. Results demonstrated that no significant differences ($P<0.05$) were observed between biscuits fortified with various levels of DF (10, 20 and 30 %) and the control biscuits in color, crispness and appearance. Biscuits fortified with 30% DF recorded the highest scores for all sensory properties.

Table 4. Sensory evaluation of prepared biscuits with various levels of DF.

Samples	Color (10)	Taste (10)	Odor (10)	Crispness (10)	Appearance (10)	Overall Acceptability (10)
Control	9.10 ± 0.22 ^a	7.80 ± 0.23 ^d	7.50 ± 0.22 ^d	8.71 ± 0.20 ^a	9.23 ± 0.31 ^a	8.70 ± 0.26 ^c
10% DF	9.15 ± 0.21 ^a	8.20 ± 0.26 ^c	8.00 ± 0.24 ^c	8.79 ± 0.22 ^a	9.26 ± 0.32 ^a	9.00 ± 0.25 ^b
20% DF	9.20 ± 0.31 ^a	8.70 ± 0.35 ^b	8.60 ± 0.31 ^b	8.88 ± 0.27 ^a	9.30 ± 0.34 ^a	9.20 ± 0.24 ^a
30% DF	9.32 ± 0.36 ^a	9.20 ± 0.33 ^a	9.30 ± 0.33 ^a	8.90 ± 0.31 ^a	9.42 ± 0.41 ^a	9.36 ± 0.30 ^a

Values within the same column followed by various superscripts are significantly different at ($p<0.05$).

Effect of feeding diabetic rats with doum biscuits on BWG, FI and FER:

As shown in Table (5), results indicated that significantly decreased ($P<0.05$) in BWG, FI and FER of diabetic rats (G2) compared to normal control rats (G1) and these results are in agreement with results obtained by **Alsuhaibani and Al-Kuraieef, (2018)**. Current results revealed that significantly increased ($P<0.05$) in food intake among diabetic rats consumed doum biscuits comparing with diabetic rats or diabetic rats fed on control biscuits. Also, results indicated that a significant improve ($P<0.05$) in BWG and FER of diabetic rats consumed doum biscuits as compared with diabetic rats or diabetic rats fed on control biscuits. Findings found that although the food intake in diabetic rats consumed doum biscuits at different levels increased more than normal control group but the body weight gain didn't increase more than control group. These results perhaps are due to DF has higher fiber content

(14.9%) than wheat flour (1.01%). The best improvement was found in diabetic rats consumed doum biscuits (30% DF).

Table 5: Effect of feeding with doum biscuits on (BWG), (FI) and (FER) of diabetic rats.

Groups	Body weight gain (BWG) (g)	Body weight gain (BWG) (%)	Food intake (FI) (g/rat/3months)	Feed efficiency ratio (FER)
G1	110.70 ± 1.10 ^a	85.15 ± 0.60 ^a	1620 ± 2.30 ^c	0.068 ± 0.003 ^a
G2	60.20 ± 0.50 ^l	44.93 ± 0.50 ^l	1458 ± 2.20 ^e	0.041 ± 0.001 ^c
G3	65.00 ± 0.81 ^e	48.15 ± 0.22 ^e	1530 ± 2.10 ^d	0.042 ± 0.001 ^c
G4	73.30 ± 0.67 ^d	53.12 ± 0.23 ^d	1629 ± 2.30 ^c	0.045 ± 0.001 ^{bc}
G5	83.70 ± 0.60 ^c	59.86 ± 0.30 ^c	1665 ± 2.40 ^b	0.050 ± 0.002 ^b
G6	86.20 ± 0.80 ^b	64.81 ± 0.32 ^b	1692 ± 2.30 ^a	0.051 ± 0.001 ^b

Values within the same column followed by various superscripts are significantly different at ($p < 0.05$).

Effect of feeding diabetic rats with doum biscuits on glucose and insulin values:

As presented in Table (6), results obtained from the present study showed that significantly increased ($P < 0.05$) in glucose level as well as this was associated with decrease in insulin value of diabetic rats as compared to healthy rats and these results are in agreement with the observation reported by **Helal et al., (2014)**. Diabetic rats consumed doum biscuits revealed that a significant decrease ($P < 0.05$) in blood glucose values, while, there was significantly increased ($P < 0.05$) in insulin values comparing with diabetic rats or diabetic rats consumed control biscuits and this improvement increased with increasing the level of DF in biscuits. Similar results were reported by **Abdel-moniem et al., (2015)** who stated that *Hyphaene thebaica* extract significantly reduced blood glucose values in diabetic rats. These results perhaps are due to DF has higher level of flavonoid compounds comparing with wheat flour. The best values of glucose and insulin were in diabetic rats treated with biscuits prepared with 30% DF where glucose value was reduced by 54%, whereas, the value of insulin increased by 32.7% compared to diabetic rats. In the best treatment glucose and insulin levels reached to be near that of the healthy control group.

Table 6: Effect of feeding with doum biscuits on glucose and insulin values of diabetic rats.

Groups	Glucose mg/dl	Insulin μ IU/ml
G1	117.00 \pm 1.00 ^e	4.25 \pm 0.11 ^a
G2	260.50 \pm 2.11 ^a	2.60 \pm 0.02 ^e
G3	259.30 \pm 2.10 ^a	2.63 \pm 0.04 ^e
G4	208.00 \pm 1.13 ^b	2.90 \pm 0.03 ^d
G5	161.70 \pm 1.12 ^c	3.15 \pm 0.03 ^c
G6	120.00 \pm 1.10 ^d	3.45 \pm 0.04 ^b

Values within the same column followed by various superscripts are significantly different at ($p < 0.05$).

Effect of feeding diabetic rats with doum biscuits on lipid profile:

Lipid profile for diabetic rats fed on biscuits fortified with DF are clarified in Table (7). Values of TG, TC, LDL-C and VLDL-C were significantly higher ($P < 0.05$) in diabetic rats than normal control group. Level of HDL-C in positive control group was significantly lower ($P < 0.05$) than negative control group. While, diabetic rats consumed biscuits containing DF demonstrated that a significant improve ($P < 0.05$) in all lipid's parameters compared to positive control group or diabetic rats consumed control biscuits and this improvement increased with increasing the level of DF in biscuits. These results perhaps are due to that DF has higher fiber and flavonoids than wheat flour. Current study found that diabetic rats fed on biscuits fortified with 30% DF recorded the best result for all measurements of lipid profile and values of parameters reached to nearly normal values. In the best treatment levels of TG, TC, LDL-C and VLDL-C decreased by 52.8, 47.6, 68.6 and 52.8%, respectively, and values of HDL-C increased by 60.1% compared to diabetic rats. Similar results were obtained by **Abdulazeez *et al.*, (2019)** who found that treatment of obese rats with flavonoid-rich fraction of *H. thebaica* led to a significant decrease in TC, TG and LDL-C, while, the levels of HDL-C significantly increased.

Table 7: Effect of feeding with doum biscuits on lipid profile of diabetic rats.

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
G1	73.30 \pm 1.00 ^e	108.50 \pm 1.10 ^e	49.90 \pm 0.40 ^a	43.94 \pm 0.50 ^f	14.66 \pm 0.32 ^d
G2	160.00 \pm 2.12 ^a	210.00 \pm 2.10 ^a	30.30 \pm 0.30 ^e	147.70 \pm 1.30 ^a	32.00 \pm 0.40 ^a
G3	159.00 \pm 2.21 ^a	209.30 \pm 2.20 ^a	31.00 \pm 0.20 ^e	146.50 \pm 1.12 ^b	31.80 \pm 0.32 ^a
G4	131.84 \pm 1.22 ^b	176.82 \pm 1.24 ^b	36.40 \pm 0.20 ^d	114.05 \pm 1.11 ^c	26.37 \pm 0.26 ^b
G5	104.28 \pm 1.13 ^c	142.80 \pm 1.13 ^c	42.72 \pm 0.30 ^c	79.22 \pm 1.10 ^d	20.86 \pm 0.21 ^c
G6	75.52 \pm 1.00 ^d	110.04 \pm 1.10 ^d	48.50 \pm 0.22 ^b	46.44 \pm 0.60 ^e	15.10 \pm 0.20 ^d

Values within the same column followed by various superscripts are significantly different at ($p < 0.05$).

Effect of feeding diabetic rats with doum biscuits on liver and kidney function parameters:

Kidney and liver function assessments for healthy control rats, diabetic rats and diabetic rats consumed biscuits fortified with different levels of DF are showed in Table (8). Levels of alanine and aspartate aminotransferase (ALT and AST) as well as alkaline phosphatase (ALP) were significantly increased ($P<0.05$) in diabetic rats (G2) comparing with healthy control group (G1). Similar findings reported by **Tohamy *et al.*, (2013b)**. Findings indicated that no significant change recorded ($P<0.05$) in alanine and aspartate aminotransferase (ALT and AST) as well as alkaline phosphatase (ALP) among diabetic rats consumed control biscuits as compared to diabetic rats. Data showed that significantly decreased ($P<0.05$) in values of alanine and aspartate aminotransferase (ALT and AST) as well as alkaline phosphatase (ALP) of diabetic rats consumed biscuits fortified with DF with increasing the doum flour level in biscuits as compared to diabetic rats. These results perhaps are due to DF has higher amount of phenolic and flavonoid compounds than wheat flour. The best improvement for liver functions found in diabetic rats fed on biscuits fortified with 30% DF where serum values of alanine and aspartate aminotransferase (ALT and AST) & alkaline phosphatase (ALP) were significantly decreased ($P<0.05$) from 40.30 to 26.15(U/L), 60.0 to 45.0 (U/L) and 110.0 to 87.0 (U/L), respectively. Current findings are in line with the observation of **Salib *et al.*, (2013)** who demonstrated that there was improved in liver parameters of rats with diabetes treated with flavonoid compounds from *Hyphaene thebaica*.

Urea and creatinine are parameters used to evaluate kidney functions. In the present study, both urea and creatinine values were significantly increased ($P<0.05$) in diabetic rats comparison with control rats and these results are in agreement with findings obtained by **Abdel-moniem *et al.*, (2015)**. Furthermore, results revealed that urea and creatinine were significantly decreased ($P<0.05$) in diabetic rats feeding with biscuits containing DF at different levels as compared to diabetic rats or diabetic rats consumed control biscuits. This improvement in kidney functions increased with increasing the level of DF in biscuits. These findings are attributed to DF has higher flavonoids content than wheat flour. The highest improve in kidney functions was found in diabetic rats treated with biscuits fortified with 30% DF where values of creatinine and urea decreased from 1.70 to 0.94 (mg/dl) and 60.80 to 46.30 (mg/dl), respectively. **Shehata and Abd El-Ghffar, (2017)** stated that

administration of *H. thebaica* extract led to a significant improve in creatinine and urea in mercuric chloride treated animals.

Table 8: Effect of feeding with doum biscuits on kidney and liver functions of diabetic rats.

Groups	Liver functions (U/L)			Kidney functions (mg/dl)	
	ALT	AST	ALP	Urea	Creatinine
G1	25.20 ± 0.30 ^e	36.40 ± 0.40 ^e	84.00 ± 1.10 ^e	39.80 ± 1.10 ^e	0.86 ± 0.02 ^d
G2	40.30 ± 0.50 ^a	60.00 ± 0.5 ^a	110.00 ± 1.30 ^a	60.80 ± 1.10 ^a	1.70 ± 0.03 ^a
G3	40.00 ± 0.32 ^a	59.40 ± 0.3 ^a	109.10 ± 1.20 ^a	60.00 ± 1.1 ^a	1.68 ± 0.02 ^a
G4	35.58 ± 0.24 ^b	55.00 ± 0.22 ^b	102.30 ± 1.20 ^b	55.90 ± 1.11 ^b	1.44 ± 0.03 ^b
G5	31.20 ± 0.13 ^c	50.40 ± 0.20 ^c	94.00 ± 1.30 ^c	51.40 ± 1.10 ^c	1.21 ± 0.03 ^c
G6	26.15 ± 0.20 ^d	45.00 ± 0.35 ^d	87.00 ± 1.22 ^d	46.30 ± 1.00 ^d	0.94 ± 0.03 ^d

Values within the same column followed by various superscripts are significantly different at ($p < 0.05$).

Histological examination:

Histological examination of the healthy rat's pancreas showed normal islets cells with prominent beta and alpha cells beside normal pancreatic acini (Photo. 1). While, diabetic rat's pancreas displayed focal apoptotic and necrotic change of islets. In addition, reduction in the islet cellular density and a severe decrease in the number of cells in the islets. Moreover, congested pancreatic blood vessels were also seen (Photo. 2). These findings are in accordance with results reported by **Mohamed *et al.*, (2019)**. Pancreas of diabetic rats fed on control biscuits showed congested pancreatic blood vessels. Some of the islets cells showed reduction of cellular population which represented by some apoptotic and necrotic islets cells. Furthermore, some inflammatory cells were infiltrated periductal and perivascular (Photo. 3). Pancreas of diabetic rats fed on biscuits fortified with 10% DF revealed some normal islets of Langerhans and pancreatic acini. In addition, few apoptotic islets cells were also detected (Photo. 4). Most of pancreatic islets of diabetic rats fed on biscuits fortified with 20% DF restore their sizes, number and cell population. Moreover, normal exocrine parts were also observed (Photo. 5). Majority of pancreatic islets of diabetic rats consumed doum biscuits (30% DF) were large in size or normal sizes with apparently normal active cellular contents. Moreover, the exocrine pancreas displayed clearly normal pancreatic acini (Photo. 6). These findings probably are due to doum has antioxidant activity causes increase the number of β -cells by increasing the repair and regeneration of β -cells and protect cell membranes from oxidative damage. **Tohamy *et al.*, (2013a)** reported that

diabetic rats fed on *Hyphaene thebaica* suspensions displayed normal pancreatic tissues.

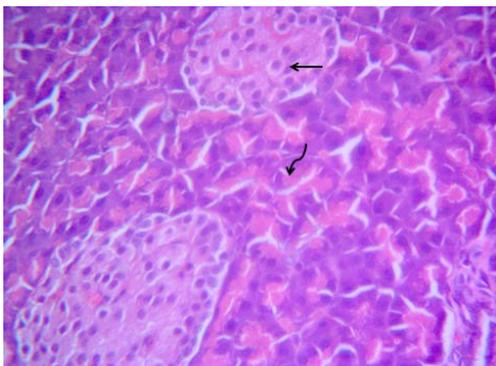


Photo. 1 (G1): Photomicrograph of pancreas showing normal islets cells (arrow) and pancreatic acini (curved arrow). H&E X400.

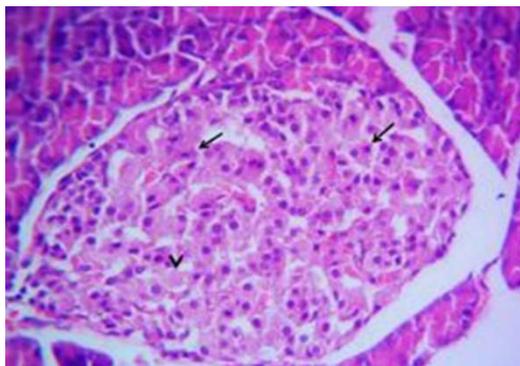


Photo. 2 (G2): Photomicrograph of pancreas showing apoptotic (arrows) and necrotic change (arrow head) of islets. H&E X400.

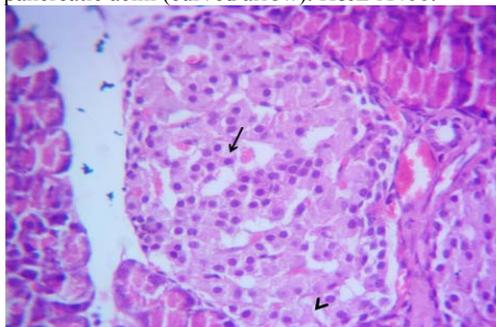


Photo. 3 (G3): Photomicrograph of pancreas showing some apoptotic (arrow) and necrotic islets cells (arrow head). H&E X400.

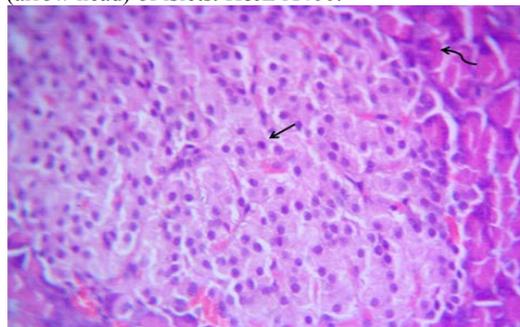


Photo. 4 (G4): Photomicrograph of pancreas showing apparently normal pancreatic acini (curved arrow) with few apoptotic islets cells (arrow). H&E X400.

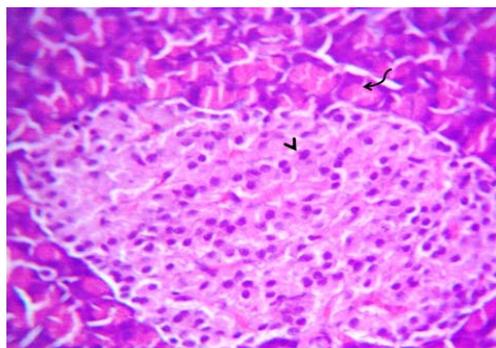


Photo. 5 (G5): Photomicrograph of pancreas showing clearly active cellular contents of pancreatic islets cells (arrow head) and normal pancreatic acini (curved arrow). H&E X400.

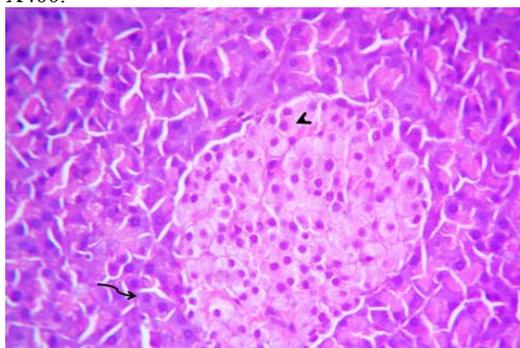


Photo. 6 (G6): Photomicrograph of pancreas showing normal pancreatic acini (curved arrow) and islets of Langerhans (arrow head). H&E X400.

As shown in Table (9) current study summarized that the best finding of histological examination was found in the pancreatic tissue of diabetic rats consumed biscuits fortified with 30% DF comparing with diabetic rats. This result is in agreement with results of glucose and insulin values in the present study. Also, this finding is in line with previous results for lipid profile as well as kidney and liver functions in the current study.

Table 9: Histological changes severity in the pancreatic tissue of diabetic rats consumed biscuits fortified with different levels of DF.

Criteria	G1	G2	G3	G4	G5	G6
Degenerative changes of Islets cells	-	+++	+++	++	+	-
Islets cell death (apoptosis)	-	+++	+++	++	-	-
Inflammatory cells infiltration	-	+++	+++	+	-	-
Regenerative attempts	-	-	-	-	+	++

-: No alterations.

+: Mild (25-39% alterations).

++: Moderate (40-65% alterations).

+++: Severe (up to 65% alterations).

Conclusion:

The present study revealed that inclusion DF in biscuits formula led to increase its nutritional functions. Organoleptic properties showed that biscuits fortified with DF up to 30 % was acceptable. TP, TF and antioxidant activity in doum biscuits were higher than control biscuits in all substitution levels. Thus, addition of DF improved TP and TF levels in biscuits, hence led to higher antioxidant activity. Furthermore, doum biscuits have potent activity against hyperglycemia experimentally induced by alloxan due to its antioxidant properties. It can be advised to use doum biscuits as a protective and treatment of diabetes. Also, doum biscuits might be useful for people suffering from obesity and hyperlipidemia.

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الملخص العربي

تأثير تدعيم البسكويت بدقيق الدوم على سمات الجودة والقياسات البيوكيميائية والفحص النسيجي للبنكرياس الفئران المصابة بمرض السكري

مع الاستخدام الواسع النطاق للأغذية الوظيفية، يبحث العالم عن منتجات غذائية صحية جديدة بنسب مناسبة من المكونات النشطة بيولوجياً مثل الألياف والمركبات الفينولية والفلافونويدات. ويُعتبر الدوم مصدر غني بالألياف ومضادات الأكسدة. ويهدف هذا البحث إلى استخدام دقيق الدوم في تحضير البسكويت لزيادة قيمة الغذائية، حيث تم استبدال دقيق القمح بثلاث مستويات من دقيق الدوم ١٠٪ و ٢٠٪ و ٣٠٪ لتحضير البسكويت. وتمت دراسة الخواص الكيميائية والحسية للبسكويت المحضر. كما تم تقييم تأثير تغذية الفئران المصابة بمرض السكري ببسكويت الدوم على الاختبارات البيوكيميائية والتغيرات النسيجية للبنكرياس. وقد أظهرت النتائج أن زيادة نسبة دقيق الدوم في البسكويت أدى إلى زيادة معنوية ($P < 0.05$) في قيم الألياف والرماد في البسكويت المحضر مقارنة بالكنترول. وسجلت النتائج زيادة معنوية بشكل تدريجي ($P < 0.05$) في مستويات المركبات الفينولية والفلافونويدات

وكذلك النشاط المضاد للأكسدة بزيادة مستوى دقيق الدوم في البسكويت مقارنة بالكنترول. وقد أظهر التقييم الحسي أن البسكويت المدعم بدقيق الدوم حتى 30% مقبول من قبل المحكمين مقارنة بالبسكويت الكنترول. وقد لوحظ انخفاضاً معنوياً ($P < 0.05$) في مستويات الجلوكوز في الدم والكوليسترول والدهون الثلاثية والليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جداً بعد تغذية الفئران المصابة بمرض السكري على بسكويت الدوم المحتوى على مستويات مختلفة من دقيق الدوم، بينما لوحظ أن هناك زيادة معنوية ($P < 0.05$) في مستويات الأنسولين والليبوبروتينات مرتفعة الكثافة مقارنة مع الفئران المصابة بمرض السكري. وقد أظهرت النتائج أن هناك انخفاضاً معنوياً ($P < 0.05$) في مستويات AST وALT وكذلك ALP لدى الفئران المصابة بمرض السكري التي تمت معاملتها بالبسكويت المحتوى على دقيق الدوم بالمقارنة مع الفئران المصابة بمرض السكري. أيضاً، تحسنت وظائف الكلى معنوياً ($P < 0.05$) في الفئران المصابة بمرض السكري التي تغذت على بسكويت الدوم، حيث انخفضت قيم كلاً من الكرياتينين واليوريا معنوياً ($P < 0.05$) في المصل مقارنة بالفئران المصابة بمرض السكري. إضافة إلى ذلك، تغذية الفئران المصابة بمرض السكري على بسكويت الدوم أدى إلى تحسناً ملحوظاً في أنسجة البنكرياس وتتفق هذه النتائج مع النتائج التي تم الحصول عليها أعلاه. وأكدت النتائج أن أفضل تحسن لجميع القياسات وُجد في مجموعة الفئران المصابة بمرض السكري التي تم تغذيتها على بسكويت الدوم (30% دقيق دوم) بالمقارنة مع الفئران المصابة بمرض السكري حيث وصلت القياسات إلى قيم قريبة من مجموعة الكنترول الصحية. علاوة على ذلك، يحتوي بسكويت الدوم (30% دقيق دوم) على أعلى قيم للألياف والرماد بالإضافة إلى المركبات الفينولية والفلافونويدات وفي المقابل أقل قيمة للطاقة مقارنة بالبسكويت الكنترول. وبناءً على ذلك، يمكن الاستنتاج أن بسكويت الدوم الذي تم إنتاجه بتكلفة منخفضة له فوائد محتملة في الوقاية من مرض السكري وعلاجه ويلعب دوراً في إدارته وكذلك يُقلل من خطر مضاعفات مرض السكري. أيضاً، يُمكن أن يكون بسكويت الدوم مفيداً للأشخاص الذين يُعانون من السمنة وارتفاع دهون الدم. وبالتالي سوف يساعد ذلك في الترويج لاستهلاك بسكويت الدوم ومعالجة المشاكل المتعلقة بالتغذية في مصر. لذلك، توصي الدراسة الحالية بزيادة استهلاك المخبوزات المدعمة بدقيق الدوم وزيادة وعي المستهلكين بفوائد استهلاك المنتجات الغذائية المصنعة من الدوم لأهميتها الكبيرة في الوقاية والعلاج من مرض السكري وبعض الأمراض الأخرى عبر وسائل الاعلام المسموعة والمرئية والمراكز الصحية.

الكلمات المفتاحية: الدوم - البسكويت - التدعيم - النشاط المضاد للاكسدة - الالوكزان - التقييم الحسي - مرض السكري.