

Effect of high protein food containing pumpkin bread on diabetic rats

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ABSTRACT:

The aim of this study was to investigate the effects of a high protein diet alone or a high protein diet containing bread fortified with a pumpkin on daily feed intake (FI), body weight gain% (BWG%), serum glucose, lipid profile, kidney functions and liver enzymes in diabetic rats, in addition to, the determination of the chemical composition of (pumpkin and the bread which fortified with pumpkin), also sensory evaluation for the bread fortified with 10%, 15%, and 20% pumpkin. A total of 48 adult male albino rats of Sprague-Dawley Strain weighing 150 ± 10 g were used in this study. The rats were divided into two main groups: The first main group: (12 rats) divided into (2 subgroups), as a following: Subgroup (1): fed on normal protein diet (14% protein) containing 300 g unfortified bread with pumpkin. Subgroup (2): was fed on high protein diet (20% protein) containing 300 g unfortified bread with pumpkin, these subgroups used (as control negative groups). The second main group (36) rats were injected with alloxan (150 mg/kg b.w.) to induce, hyperglycemia. The rats in the second main group were divided into (six subgroups) as a following. The first subgroup (6 rats) was fed on a normal protein diet (14% protein) containing 300 g unfortified bread (as a control positive group). The second and third subgroups (12 rats) were fed on a normal protein diet (14% protein) containing the best two fortified bread which were identified by the sensory evaluation (300 g fortified bread with 10% and 15% pumpkin powdered / 1000g diets), respectively. The fourth subgroup (6 rats) was fed on a high protein diet (20% protein) containing 300 g unfortified bread (as a control positive group). The fifth and sixth subgroups (12 rats) were fed on a high protein diet (20% protein) containing (300 g fortified bread with 10% and 15% pumpkin powdered / 1000g diets), respectively. At the end of the experiment, rats were sacrificed. Blood samples were collected, left to clot, the serum was separated. The obtained results revealed that, feeding diabetic rats on normal or high protein diets containing (300 g fortified

bread with 10 & 15% pumpkin powdered) induced significantly decreased body weight gain%, serum glucose, cholesterol, triglyceride, LDL-c, VLDL-c, uric acid, urea nitrogen, creatinine, AST, ALT, and ALP, while HDL-c increased significantly, as compared to the positive control groups. The best results recorded for diabetic group which was fed on high protein diet containing 300 g fortified bread with 15% pumpkin. Conclusion: According to the results of this study, a high protein diet containing bread fortified with pumpkin powder decreased blood glucose and the complications of diabetes. Bread fortified with pumpkin powder can be used as functional food for diabetics.

Keywords: Diabetes, Pumpkin, Fortified Bread, Rats.

INTRODUCTION:

Diabetes mellitus is affecting millions of people worldwide, the prevalence of this disease is increasing annually and the number of diabetics is projected to rise above 300 million before 2025 (Maiti et al., 2004).

Cultivars of pumpkin include *Cucurbita pepo*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita moschata* and they are classified according to the texture and shape of their stems (Rakcejeva et al., 2011). Pumpkin is a seasonal crop, and since fresh pumpkins are very sensitive to microbial spoilage, even in refrigerated conditions, they must be frozen or dried (Doymaz, 2007).

Pumpkin (*Cucurbita pepo*, *C. moschata*, *C. maxima*, and *C. mixta*) is one of the vegetables used in healthy diets as well as in traditional medicine in many countries. Since ancient times, it has been essential in the diet of rural communities. Nowadays, it is cultivated both, for fruit and seeds that are used in a variety of ways. Fruit is regarded by consumers due to its sweet and mild taste as well as high nutritive value (Sharma and Rao, 2013). In addition, (Rakcejeva et al., 2011) reported that pumpkin flesh is widely used as a component in a variety of products for children and adults.

(Noor et al., 2011) reported that Pumpkins can be processed into flour, which has a longer shelf life. This flour can be used for its flavor, sweetness, deep yellow-orange color, and a considerable amount of dietary fiber. It can be also used to supplement cereal flours in bakery products, soups, sauces, instant noodles, and as a natural coloring supplement for food.

The major contributory factors of the nutritional and medicinal value of pumpkin fruit are high total content of carotenoids with >80%

of β -carotene (Azevedo and Rodriguez, 2007), as well as the presence of pectin and non-pectin polysaccharides, minerals (potassium, phosphorus, magnesium, iron, and selenium), vitamins (C, E, K, thiamine (B1) and riboflavin (B2), pyridoxine (B6)), dietary fiber, phenolic and polyphenol components (flavonoids, phenolic acids) and other substances beneficial to human health (Nawirska, 2014)

Pumpkins are traditionally used in many countries due to their wide range of properties, which include anti-diabetic, antihypertension, immunomodulation, anti-bacterial, anti-hypercholesterolemia, antitumor, antioxidant activity, intestinal antiparasitic, anti-inflammation and antalgic (Adams et al., 2011). On the other hand, (Jin et al., 2013) showed that a pumpkin-rich diet could reduce blood glucose. A similar result was obtained by (Zhao et al., 2014) who showed increased levels of serum insulin, reduced blood glucose levels, and improvement of glucose tolerance in mice by pumpkin polysaccharides. Also, hypocholesterolemic, antibacterial, anti-inflammatory, and antitumor activities were reported (Fu et al., 2006)

AIM OF STUDY :

The study aims to investigate the effects of a high protein diet alone or a high protein diet containing bread supplemented with a pumpkin on diabetic rats.

Materials and Methods

Materials:

- Casein, alloxan, vitamins, minerals, cellulose, choline chloride were purchased from EL-Gomhoria company, Cairo, Egypt.
- Soy oil and pumpkin were obtained from in Agricultural research center, Giza, Egypt.
- Starch, wheat flour (80% extraction) and yeast were obtained from local market, Cairo, Egypt.
- Forty-eight male albino rat Sprague Dawley strain weighting 150 ± 10 g were purchased from Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.
- Kits for biochemical analysis were obtained from Alkan for pharmaceutical and chemical Dokki, Egypt.

Methods:

Preparation of dried pumpkin:

pumpkin was washed thoroughly with tap water following with cutting into similar pieces and dehydrated into air circulated oven at 40-50°C for 48 hrs. The dried samples were finely powdered by using a coffee grinder and stored in polyethylene bags at - 20°C until used .

Preparation of bread: normal bread consists of wheat flour (90g), yeasts (5gm), salt (2.5gm), sugar (2.5gm)

Fortified of bread with dried pumpkin: in this study, white flour fortified with dried pumpkin by partially replacing the wheat flour by (5, 10, 15 and 20g) pumpkin.

Sensory evaluation of all samples was used to identify the best two samples from pumpkin bread.

Sensory evaluation:

The organoleptic properties of produced bread were measured by twenty personal trained judges. The judges asked to give a score from zero to 100 for color, odor, taste, texture and general acceptance as reported by, (Abd El-latif, 1990 and Amerine et al.,1965) .

Chemical analysis :

Moisture content, total protein, fat, fiber and ash were determined in unfortified bread and also the best two fortified bread with (pumpkin), according to the methods outlined in (A.O.A.C 1990), while the carbohydrates content were calculated by difference.

Experimental Design:

A total of 48 adult male albino rats of Sprague-Dauley Strain weighing 150 ± 10 g were used in this study. The rats were divided into two main groups: The first main group: (12 rats). This group was divided into (2 subgroups), as a following: Subgroup (1): fed on normal protein diet containing (14% protein) containing 300 g unfortified bread. Subgroup (2): was fed on high protein diet containing (20% protein) containing 300 g unfortified bread, these groups used (as control negative groups). The second main group (36) rats were injected with alloxan (150 mg/kg b.w.) to induce, hyperglycemia, and fed on a basal diet for four days (Buko et al., 1996). After this period, serum glucose was determined in each rat in the first and second main group, to ensure from the induction. The rats in the second main group was divided into (six subgroups) as a following. The first subgroup (6 rats) was fed on a normal protein diet (14% protein) containing 300 g unfortified bread (as a control positive

group). The second and third subgroups (12 rats) were fed on normal protein diet (14% protein) containing the best two levels which were identified by the sensory evaluation (300 g fortified bread with 10% and 15% pumpkin powdered / 1000g diets), respectively. The fourth subgroup (6 rats) was fed on a high protein diet (20% protein) containing 300 g unfortified bread (as a control positive group). The fifth and sixth subgroups (12 rats) were fed on a high protein diet (20% protein) containing the best two levels which were identified by the sensory evaluation (300 g fortified bread with 10% and 15% pumpkin powdered / 1000g diets), respectively.

At the end of the experiment (4 weeks), rats were sacrificed, liver and kidney removed and weighed. Blood samples were collected, left to clot, the serum was separated and stored at - 20 C° until analysis. Biological evaluation of the different diets was carried out by determination of food intake, organs weight/body weight% and body weight gain % (BWG %) according to (Chapman et al, 1959). Determination of serum glucose according to (Trinder, 1959). Total cholesterol according to (Allain and Poon.,1974). Triglycerides (Fossati and Principle, 1982). High density lipoprotein cholesterol (HDL-C) (Burstein, 1970). Low and very low density lipoprotein cholesterol (LDL-c and VLDL-c) (Friedewald et al., 1972). Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) (Reitman and Frankel, 1957). Alkaline phosphates (ALP) (Bergmeyer and Brent, 1974). Uric acid (Fossati et al, 1980). Urea nitrogen (Patton and Crouch, 1977) and creatinine according to (Bohmer, 1971). Results of biochemical analysis and biological evaluation of each group were statistically analyzed, mean, standard deviation, and one-way ANOVA test using SAS package with the level of significant $p < 0.05$ (SAS,2004) .

Results and Discussion:

Chemical composition of pumpkin (% dry weight basis)

Data in Table (1) presented proximate chemical composition of pumpkin powder (fresh & dry weight basis). Moisture, total protein, fat, ash, crude fiber and carbohydrate were (85.7, 2.48, 1.51, 0.32, 0.24 and 9.75 g / 100 g fresh weight, respectively) and (7.16, 17.4, 10.59, 2.23, 1.70 and 60.92 g/100g dry weight, respectively). From these data it was revealed that pumpkin powder is rich source of carbohydrates followed by total protein and fat.

Table (1): Chemical composition of pumpkin (% fresh and dry weight basis).

Components (g)	Ingredients	
	Fresh	Dry
Moisture	85.7	7.16
Total Protein	2.48	17.40
Fat	1.51	10.59
Ash	0.32	2.23
Crude fiber	0.24	1.70
Carbohydrate*	9.75	60.92

Each value represents the mean of two determinations .

*Total carbohydrates were calculated by differences

Pumpkin have high composition of carbohydrates, salts and minerals, dietary fiber, vitamins, fatty acids and amino acid gives a unique value in human nutrition. Moreover, pumpkin is playing a significant role in neutralization of free radical and finally suppress the various types of diseases development and progression (Jun et al. 2006).

Sensory evaluation of bread fortified with pumpkin powder.

Bread is one of the bakery products that is consumed daily. Forasmuch, nutrition plays an important role in many diseases such as diabetes, Whole-wheat bread diet leads to improving the postprandial blood glucose, as compared to a refined grains diet like White bread. On the other hand, making bread with pumpkin powder is a good step in upgrading the nutritive value of the bread. Table (2) showed the sensory characteristics of prepared bread fortified with pumpkin powder. The results revealed that all sensory characteristics including (color, taste, odor, texture, and general acceptability) in the bread fortified with 10% showed non-significant changes, as compared to the control sample.

Bread fortified with 15% pumpkin powder recorded a significant decrease ($p \leq 0.05$) in (taste, odor, and general acceptability), as compared to the bread fortified with 10% and un-fortified bread (control). On the other hand, the results in this table showed that fortified bread with 20% pumpkin powder decreased all sensory characteristics significantly ($p \leq 0.05$), as the other samples of bread.

The highest score of sensory evaluation recorded for the control sample, followed by bread fortified with 10%, 15%, and 20%, respectively. Therefore, the biological part of this study was done to Knowing the effect of fortified bread with 10% and 15% pumpkin powder on diabetic rats.

In this respect, (Malkanathi and Umadevi, 2018) revealed that pumpkin mix blended biscuits had enhanced nutrient composition, textural and sensory properties. Therefore, it can be supplemented successfully into food products to increase the nutrient content and this will be benefited in a wide variety of food applications such as functional and therapeutic food products.

Table (2): Sensory evaluation of bread fortified with pumpkin powder.

Sensory Characteristics	Control	Bread with pumpkin %		
		10%	15%	20%
Color (20)	19.30±.329 _a	19.00±.415 _a	18.92 ±.485 ^a	12.95±.570 ^b
Taste (20)	19.29±.275 _a	18.89±.292 _a	18.00 ±.728 ^b	14.50 ±.400 ^c
Odor (20)	19.14±.210 _a	18.90 ±.475 ^a	17.41 ±.424 ^b	14.35±.530 ^c
Texture (20)	19.45±.388 _a	19.25 ±.382 ^a	19.21 ±.321 ^a	17.12±.36 ^b
General Acceptance (20)	19.65±.295 _a	19.61±.366 _a	18.10 ±.566 ^b	14.11±.630 ^c
Total Score (100)	96.83±.372 ^a	95.65±.478 ^b	91.64±1.390 ^c	73.03±1.223 ^d

Mean values in the same row which are not followed by the same letter are significantly different ($p \leq 0.05$).

Chemical composition of bread fortified with 10% and 15% pumpkin powder (g/100g)

Data in Table (3) presented proximate chemical composition of unfortified and fortified bread with (10 & 15% pumpkin powder). The data in this table revealed that, moisture, total protein, fat, ash, crude fiber and carbohydrate of unfortified bread were (37, 9.6, 2.37, 1.52, 3.5 and 46.01 g / 100 g fresh weight, respectively) and (6.05, 15.25, 3.76, 2.41, 5.55 and 66.98 g/100g dry weight, respectively.)

On the other hand, fortified bread with 10% pumpkin powder showed changed in moisture, total protein, fat, ash, crude fiber and carbohydrate, which recorded (37.8, 10.493, 1.685, 1.355, 2.510 and 46.157 g/100 g fresh weight, respectively) and (6.40, 16.87, 2.71, 2.18, 4.053 and 67.805 g/100g dry weight, respectively). While these nutrients in fortified bread with 15% pumpkin powder recorded (38.40, 10.958, 2.21, 1.632, 2.566 and 44.234 g/100 fresh weight basis, respectively) and (6.43, 17.79, 3.59, 2.65, 4.125 and 65.415 g/100g dry weight basis, respectively)

Effect of high protein diets containing pumpkin bread on Feed intake and body weight gain % of diabetic rats.

Data in Table (4) illustrate the effect of high protein diets containing bread fortified with (10% & 15% pumpkin powder) on feed intake (g/day/each rat) and body weight gain % of diabetic rats. The mean value \pm SD of feed intake (FI) of healthy rats fed on normal protein diet containing 300 g unfortified bread/kg diet was (16.600 \pm 1.140 g/day/each rat), while the mean value of FI of healthy rats fed on high protein diet containing 300 g unfortified bread/ kg diet was (15.600 \pm 1.140 g/day/each rat). A non-significant change was observed between these groups in the mean values of FI .

Table (3): Chemical composition of bread fortified with 10% and 15% pumpkin powder (% fresh weight & dry weight basis).

Components (g)	Unfortified Bread		Fortified Bread with			
			10% Pumpkin		15% Pumpkin	
	FW	DW	FW	DW	FW	DW
Moisture	37	6.05	37.8	6.40	38.4	6.43
Total Protein	9.6	15.25	10.493	16.87	10.958	17.79
Fat	2.37	3.76	1.685	2.71	2.21	3.59
Ash	1.52	2.41	1.355	2.18	1.632	2.65
Crude fiber	3.5	5.55	2.510	4.035	2.566	4.125
Carbohydrate*	46.01	66.98	46.157	67.805	44.234	65.415

*Each value represents the mean of two determinations. * Total carbohydrates were calculated by differences FW: Fresh weight DW: dry weight

Injected rats which were fed on (normal and high protein diets containing 300 g unfortified bread/ kg diet) with alloxan (control +ve groups) led to a significant decrease $p \leq 0.05$ in the mean values of FI, as compared to the negative control group fed on normal protein diet containing 300 g unfortified bread/kg diet.

The results revealed that non-significant deference's in the mean values of FI between all treated diabetic groups. On the other hand, all treated diabetic groups which were fed on normal and high protein diets containing 300 g fortified bread with 10% and 15% pumpkin powder showed non-significant changes, as compared to the negative and positive control groups .

Table (4): Effect of high protein diets containing pumpkin bread on feed intake and body weight gain % of diabetic rats.

Parameters		Feed intake (g/day/each rat)	Body weight gain %
Groups			
NPD containing unfortified bread Control (-ve)		16.600 ± 1.140 ^a	29.600 ± 1.673 ^a
HPD containing unfortified bread Control (-ve)		15.600 ± 1.140 ^{a b}	26.600 ± 1.816 ^b
Diabetic rats fed on	NPD containing unfortified bread Control (+ve)	15.000 ± 1.224 ^b	21.800 ± 1.923 ^c
	HPD containing unfortified bread Control (+ve)	14.400 ± 0.547 ^b	18.000 ± 1.224 ^d
	NPD containing fortified bread with 10% pumpkin	15.520 ± 1.066 ^{a b}	18.800 ± 1.303 ^d
	NPD containing fortified bread with 15% pumpkin	15.680 ± 0.661 ^{a b}	17.800 ± 1.643 ^d
	HPD containing fortified bread with 10% pumpkin	15.200 ± 1.083 ^{a b}	14.200 ± 1.650 ^e
	HPD containing fortified bread with 15% pumpkin	15.460 ± 0.753 ^{a b}	14.200 ± 0.836 ^e

NPD: Normal protein diet HPD: High protein diet Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Feeding normal rats on a high protein diet containing a 300 g unfortified bread/kg diet showed a significant decrease $P \leq 0.05$ in BWG%, as compared to rats fed on a normal protein diet containing 300 g unfortified bread/ kg diet (26.600 ± 1.816 vs. 29.600 ± 1.673), respectively. The same trend was observed in the diabetic groups. Feeding diabetic group on normal protein diet containing 300 g fortified bread with (10% pumpkin and 15% pumpkin) powder recorded significant decrease $p \leq 0.05$, as compared to the positive control group fed on normal protein diet containing 300g unfortified bread. Data in this Table showed that feeding diabetic groups on a high protein diet containing 300 g fortified bread/kg diet with 10% & 15% pumpkin powder recorded also a significant decrease of $P \leq 0.05$ in BWG%, as compared to the positive control group fed on high protein diet containing 300g unfortified bread/kg diet. The highest decrease in body weight gain% recorded for diabetic groups fed on high protein diet containing fortified bread with (10% and 15% pumpkin powdered), followed by the other treated groups .

A high protein diet decreased body weight gain% in diabetic rats. In this respect, Astrup, (2005) reported that protein is more satiating than the isoenergetic ingestion of carbohydrates or fat. Also (Pesta and Samuel 2014) reported that high protein diets are increasingly popularized in lay media as a promising strategy for weight loss by providing the twin benefits of improving satiety and decreasing fat mass. Also (Halton and Hu 2004) reported that diets high in protein have been shown to be a potential tool for weight loss .

Ali, (2015) reported that feeding rats that suffer from hypercholesterolemia on diets containing cake fortified with pumpkin (5%, 10%, and 15%) induced a significant decrease in body weight gain, feed intake, and feed efficiency ratio, the author reported also that these results may be due to the contents of fiber in these cake. Abete et al., (2008) cleared that high protein diets reduce energy intake, increase weight loss, enhance body composition, and help to maintain a reduced body weight following food restriction in humans.

Effect of high protein diets containing pumpkin bread on serum glucose of diabetic rats.

The effect of normal and high protein diets containing 300 g bread fortified with (10% and 15% pumpkin powdered) / kg diet on serum glucose (mg/dl) of diabetic rats presented in Table (5.)

The mean value \pm SD of serum glucose of normal rats fed on a high protein diet containing 300 g un-fortified bread/kg diet decreased significantly ($p \leq 0.05$), as compared to normal rats fed on normal protein diet containing the same amount and type of this bread. Feeding rats that were injected with (150 mg alloxan /kg body weight to induce diabetes) on a high protein diet containing 300 g un-fortified bread induced a significant decrease $p \leq 0.05$ in serum glucose, as compared to diabetic rats fed on a normal protein diet containing 300 g unfortified bread. A high protein diet decreased the mean value of serum glucose in diabetic rats by about 8.8% than that of diabetic rats fed on a normal protein diet.

Treating diabetic rats with normal protein or high protein diets containing 300 g fortified bread with (10% and 15% pumpkin powdered) / kg diet caused a significant decrease $p \leq 0.05$ in serum glucose, as compared to the positive control groups. The highest decrease in the mean value of serum glucose recorded for diabetic group fed on high protein diet containing 300 g fortified bread with 15% pumpkin powdered / kg diet, followed by diabetic group fed on normal protein diet containing the same amount of fortified bread with 10% pumpkin powdered, these treatments decreased the mean values of serum glucose by about 27.152% and 15.099%, than that of the positive control group fed on normal protein diet containing 300 g un-fortified bread. In this respect, a high-protein diet decreases blood glucose postprandially in patients with type 2 diabetes and enhances overall glucose control. On the other hand, higher protein intake increases satiety and enhances the leptin concentrations in central nervous system CNS as well as elevates leptin sensitivity which tends to be weight maintenance (Weigle et al., 2005)

Zhang & Bai (2004) reported that, pumpkin powder showed a significant decrease in glucose level and a rise in blood insulin and protected the diabetic nephropathy. Also the result was obtained by Zhao et al. (2014) showed raised levels of serum insulin, decreased blood glucose and improvement of glucose tolerance in mice by pumpkin polysaccharides. Alenaz et al., (2017) showed that pumpkin extract has positive effects on glycemic control and pancreatic β cells; however, most of the studies were done on animals and human study are needed in this field.

Table (5): Effect of high protein diets containing pumpkin bread on serum glucose of diabetic rats.

Parameters		Glucose (mg/dl)
Groups		
NPD containing unfortified bread Control (-ve)		103.400 ± 8.203 ^f
HPD containing unfortified bread Control (-ve)		92.800 ± 2.167 ^g
Diabetic rats fed on	NPD containing unfortified bread Control (+ve)	151.000 ± 8.573 ^a
	HPD containing unfortified bread Control (+ve)	136.200 ± 1.788 ^{b c}
	NPD containing fortified bread with 10% pumpkin	141.600 ± 2.073 ^b
	NPD containing fortified bread with 15% pumpkin	133.400 ± 5.079 ^{c d}
	HPD containing fortified bread with 10% pumpkin	128.200 ± 2.280 ^d
	HPD containing fortified bread with 15% pumpkin	110.400 ± 6.024 ^e

NPD: Normal protein diet HPD: High protein diet

Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Effect of high protein diets containing pumpkin bread on lipid profile of diabetic rats.

The effect of normal and high protein diets containing 300 g bread fortified with (10% and 15% pumpkin powdered) /kg diet on serum lipid profile including (cholesterol, triglycerides, high-density lipoprotein-cholesterol HDL-c, low and very low-density lipoprotein-cholesterol LDL-c & VLDL-c) of diabetic rats presented in Table (6).

Treating diabetic rats on high protein diet HPD containing unfortified bread control (+ve) induced a significant decrease $p \leq 0.05$ in serum cholesterol and LDL-c, while the other lipid profile (triglycerides, HDL-c and VLDL-c) showed non-significant changes, as compared to diabetic rats on normal protein diet NPD containing unfortified bread control (+ve).

Table (6): Effect of high protein diets containing pumpkin bread on lipid profile of diabetic rats.

Parameters Groups		Cholesterol	Triglycerides	HDL-c	LDL-c	VLDL-c
		mg/dl				
NPD containing unfortified bread Control (-ve)		73.400 ± 3.646 ^{ef}	61.000 ± 2.449 ^d	48.80 ± 5.02 ^a	12.40 ± 2.62 ^g	12.20 ± 0.49 ^d
HPD containing unfortified bread Control (-ve)		70.800 ± 2.387 ^f	59.200 ± 1.788 ^d	47.40 ± 2.61 ^{ab}	11.56 ± 1.17 ^g	11.84 ± 0.36 ^d
Diabetic rats fed on	NPD containing unfortified bread Control (+ve)	121.800 ± 2.774 ^a	81.600 ± 1.673 ^a	36.80 ± 2.77 ^e	68.28 ± 0.769 ^a	16.32 ± 0.33 ^a
	HPD containing unfortified bread Control (+ve)	113.800 ± 2.949 ^b	79.400 ± 4.098 ^a	37.20 ± 1.79 ^e	60.72 ± 0.77 ^b	15.88 ± 0.82 ^a
	NPD containing fortified bread with 10% pumpkin	93.200 ± 3.193 ^c	75.200 ± 2.863 ^b	40.80 ± 2.17 ^d	37.36 ± 3.42 ^c	15.04 ± 0.57 ^b
	NPD containing fortified bread with 15% pumpkin	85.200 ± 4.549 ^d	67.200 ± 3.271 ^c	45.60 ± 2.07 ^{ab}	26.76 ± 3.04 ^e	13.44 ± 0.65 ^c
	HPD containing fortified bread with 10% pumpkin	88.000 ± 2.738 ^d	73.600 ± 3.847 ^b	41.20 ± 1.09 ^{cd}	32.08 ± 2.03 ^d	14.72 ± 0.76 ^b
	HPD containing fortified bread with 15% pumpkin	76.800 ± 2.949 ^e	66.800 ± 3.420 ^c	44.40 ± 0.55 ^{bc}	19.04 ± 2.33 ^f	13.36 ± 0.68 ^c

NPD: Normal protein diet HPD: High protein diet

Means in the same column with different letters are significantly different at ($p \leq 0.05$).

All treated diabetic groups with normal and high protein diets containing 300 g fortified bread with (10 and 15%) pumpkin powder showed a significant decrease in serum (cholesterol, triglycerides, LDL-c, and VLDL-c), while HDL-c increased significantly, as compared to the positive control groups. On the other hand, the highest improvement was recorded for the diabetic group which was treated with a high protein diet HPD containing fortified bread with 15% pumpkin.

In this respect, (Appel et al., 2005) reported that, dietary protein content affects body weight and lipid profiles. While differences in dietary protein content appear to have similar effects on body weight, diets higher in protein may produce more favorable changes in lipid profiles. For example, replacing carbohydrates with protein was found to significantly reduce TG levels and LDL cholesterol. Several experimental studies carried out in normal as well as diabetic animals have shown hypo-cholesterolemic effect by pumpkin. (Adams et al., 2011 and Caili et al., 2006) reported that, pumpkins are usually used in many countries due to their varied range of properties, which include anti-diabetic, antihypertension, immunomodulation, anti-bacterial, anti-hypercholesterolemia, antitumor, antioxidant activity, intestinal antiparasitic, anti-inflammation, and antalgic. Danilchenko et al., (2000) reported that, furthermore the high nutritional value of pumpkin, pumpkin possesses numerous medicinal properties. It is a rich source of biologically active compounds and is recommended for atherosclerosis and helps to decrease the cholesterol in people suffering from obesity.

Rubi and Sadewa (2016) reported that, treating rats which were fed on high fat diet and fructose with pumpkin decreased total cholesterol, triglyceride, and increased HDL levels. The authors reported also the changes were positively correlated with the amount of pumpkin intake. The reduce of cholesterol levels was positively correlated with glucagon-like peptide-1 GLP-1 level, and negatively associated with homeostatic model assessment beta-cell function HOMA- β . Silva et al. (2013) suggested that β carotene which presents in pumpkin can decrease serum cholesterol and increase cholesterol excretion in rats that received a cholesterol-enriched diet.

Effect of high protein diets containing pumpkin bread on kidney functions of diabetic rats.

The effect of normal and high protein diets containing 300 g bread fortified with (10% and 15% pumpkin powdered) /kg diet on kidney functions including (uric acid, urea nitrogen and creatinine) mg/dl of diabetic rats presented in Table (7). Feeding normal or diabetic rats on high protein diet containing 300g un-fortified bread / kg diet resulted in significant increase in serum uric acid, urea nitrogen and creatinine, as compared to the normal and diabetic rats fed on normal protein diet .

All treated diabetic groups with normal and high protein diets containing 300 g fortified bread with (10 and 15%) pumpkin powder showed significant decrease in serum uric acid, urea nitrogen and creatinine, as compared to the positive control groups. On the other hand, the highest decrease in these parameters recorded for the diabetic group which treated with normal protein diet NPD containing fortified bread with 15% pumpkin, followed by the diabetic group treated with normal protein diet NPD containing fortified bread with 10% pumpkin. In this respect, (Juraschek et al., 2013) reported that, consumption of a diet high in protein can cause glomerular hyperfiltration, a potentially maladaptive response, which may accelerate the progression of kidney disease. (Ayoade et al., 2009) reported that, the significant increase in serum urea and creatinine of a group of rats treated with alloxan might be due to damage of pancreatic cells and not as a result of kidney damage

Table (7): Effect of high protein diets containing pumpkin bread on kidney functions of diabetic rats.

Groups		Parameters	Uric acid	Urea nitrogen	Creatinine
			mg/dl		
NPD containing unfortified bread Control (-ve)			1.48 ± 0.13 _e	28.16 ± 2.30 _f	0.58 ± 0.07 _f
HPD containing unfortified bread Control (-ve)			1.62 ± 0.08 _d	41.76 ± 1.41 _c	0.77 ± 0.08 _e
Diabetic rats fed on	NPD containing unfortified bread Control (+ve)		1.91 ± 0.07 _b	46.58 ± 1.80 _b	1.33 ± 0.06 _b
	HPD containing unfortified bread Control (+ve)		2.04 ± 0.11 _a	52.46 ± 0.94 _a	1.52 ± 0.11 _a
	NPD containing fortified bread with 10% pumpkin		1.67 ± 0.07 _{cd}	36.94 ± 2.27 _d	1.17 ± 0.06 _c
	NPD containing fortified bread with 15% pumpkin		1.57 ± 0.04 _{de}	34.60 ± 2.30 _e	0.93 ± 0.10 _d
	HPD containing fortified bread with 10% pumpkin		1.88 ± 0.08 _b	43.50 ± 1.18 _c	1.35 ± 0.04 _b
	HPD containing fortified bread with 15% pumpkin		1.76 ± 0.05 _c	38.56 ± 1.64 _d	1.14 ± 0.05 _c

NPD: Normal protein diet

HPD: High protein diet

Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Each pumpkin parts contained a significant amounts of antioxidants, tocopherols, and carotenoids with values of 171.9 to 461.9 $\mu\text{g.g}^{-1}$ (Rahman et al., 2019). Therefore, pumpkin potentially has antioxidant activity, which might be important for pre-diabetics, diabetics, and patients with heart diseases, (Yadav et al., 2010). Administering pumpkin extract significantly increased antioxidant enzymes including (superoxide dismutase and glutathione peroxidase activities) in the liver, Chang et al., (2004). Pumpkin is an edible food; pumpkin gives many health benefits to enhance our overall health. Pumpkin has many effects beneficial to health such as anti-diabetic, anti-carcinogenic, and antioxidant. There are other many health beneficial effects of pumpkin also reported such as inhibition of kidney stone formation, and hypotensive, anti-inflammatory, and blood-coagulator effects (Yadav et al., 2010). On the other hand, (Browne et al., 2019) reported that, significant associations between the carotenoids and glomerular filtration rate and improves kidney functions. The author reported also, further investigations are required to confirm these findings.

Effect of high protein diets containing pumpkin bread on liver enzymes of diabetic rats.

The effect of normal and high protein diets containing 300 g bread fortified with (10% and 15% pumpkin powdered) /kg diet on liver enzymes including (Aspartate Aminotransferase AST and Alanine Aminotransferase ALT and Alkaline phosphates ALP) U/l of diabetic rats presented in Table (8).

Table (8): Effect of high protein diets containing pumpkin bread on liver enzymes of diabetic rats.

Groups		Parameters	AST	ALT	ALP
			U/l		
NPD containing unfortified bread Control (-ve)			17.40 \pm 1.14 ^e	12.20 \pm 0.84 ^e	95.80 \pm 9.93 ^f
HPD containing unfortified bread Control (-ve)			21.80 \pm 1.79 ^c	17.40 \pm 1.14 ^c	95.04 \pm 8.01 ^f
Diabetic rats fed on	NPD containing unfortified bread Control (+ve)		31.20 \pm 2.59 ^b	24.20 \pm 2.49 ^b	209.40 \pm 7.27 ^b
	HPD containing unfortified bread Control (+ve)		37.80 \pm 2.17 ^a	27.60 \pm 2.89 ^a	245.40 \pm 11.10 ^a
	NPD containing fortified bread with 10% pumpkin		19.80 \pm 1.92 ^e	17.40 \pm 0.55 ^c	137.40 \pm 4.28 ^d
	NPD containing fortified bread with 15% pumpkin		17.60 \pm 1.14 ^u	15.60 \pm 0.89 ^{cd}	124.00 \pm 4.24 ^e
	HPD containing fortified bread with 10% pumpkin		21.60 \pm 0.89 ^c	15.80 \pm 0.84 ^{cd}	150.40 \pm 4.39 ^c
	HPD containing fortified bread with 15% pumpkin		18.40 \pm 1.14 ^u	13.80 \pm 1.64 ^{de}	120.00 \pm 6.96 ^e

NPD: Normal protein diet HPD: High protein diet

Means in the same column with different letters are significantly different at ($p \leq 0.05$)

Treating normal and diabetic rats on high protein diet HPD containing 300g unfortified bread (control -ve and +ve groups) induced significant increase in serum liver enzymes, as compared to normal and diabetic rats fed on normal protein diet. All treated diabetic groups with normal and high protein diets containing 300 g fortified bread with (10 and 15%) pumpkin powder showed significant decrease in serum (AST, ALT and ALP) enzymes, as compared to the positive control groups. On the other hand, the highest decrease in these parameters recorded for the diabetic group which treated with high protein diet HPD containing fortified bread with 15% pumpkin.

A previous study was conducted by (Bolter & Critz, 1974) shown that feeding for two weeks on diet containing a high amount of protein (casein) caused a slight increase in serum (ALT) and (AST) activity levels in healthy rats. On the other hand, (Oarada et al., 2012) reported that the feeding healthy animal on high-protein diet after fasting led to acute hepatocellular injury, and proposes that awareness should be given when using such a diet.)Sharma et al., 2013) reported that, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase increased in diabetic rats, as compared to control rats. (Rahayu et al., 2020) showed that the pumpkin flour had the hypoglycemic effect of lowering blood glucose level at 4 g/kg bwt doses; pumpkin flour had antioxidant activity by decreasing the activity of AST and ALT enzymes ($p < 0.05$)

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تأثير الغذاء عالي البروتين والمحتوى علي خبز القرع العسلي علي الفئران المصابة بالسكر

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المستخلص العربي:

تهدف هذه الدراسة الي التحقق من تأثير الغذاء عالي البروتين منفردا أو الغذاء عالي البروتين والمحتوى علي خبز مدعم بالقرع العسلي علي المأخوذ اليومي من الغذاء، النسبة المثوية للزيادة في الوزن، مستوى الجلوكوز، صورة الدهون، وظائف الكلي، وانزيمات الكبد في الفئران المصابة بالسكر. هذا بالاضافة الي تقدير التركيب الكيميائي (للقرع العسلي و الخبز المعزز بالقرع العسلي)، أيضا التقييم الحسي للخبز المعزز القرع العسلي بمستويات (١٠%، ١٥% و ٢٠%). استخدم في هذه الدراسة عدد ٤٨ فأر من نوع الالبينو (فصيلة اسبراجداولي) أوزانهم 150 ± 10 جرام. تم تقسيم الفئران الي مجموعتين رئيسيتين: المجموعة الرئيسية الأولى (١٢ فأر) تم تقسيمهم الي (مجموعتين فرعيتين)، كالتالي: المجموعة الفرعية (١): تم تغذيتها علي غذاء قياسي (١٤% بروتين) يحتوي علي ٣٠٠ جرام خبز غير معزز بالقرع العسلي. المجموعة الفرعية (٢): تم تغذيتها علي عالي البروتين (٢٠% بروتين) يحتوي علي ٣٠٠ جرام خبز غير معزز بالقرع العسلي، هذه المجموعات الفرعية تم استخدامها (كمجموعات ضابطة سالبة). المجموعة الرئيسية الثانية (٣٦ فأر) تم حقنهم بمادة الأولوكسان (١٥٠ ملجم / كجم وزن) لإحداث إرتفاع مستوى سكر الدم. تم تقسيم فئران المجموعة الثانية الرئيسية الي (٦ مجموعات فرعية) كالتالي، المجموعة الفرعية الأولى (٦ فئران) تم تغذيتها علي غذاء قياسي (١٤% بروتين) يحتوي علي ٣٠٠ جرام خبز غير مدعم بالقرع العسلي (وتم استخدامها كمجموعة ضابطة مصابة). المجموعة الفرعية الثانية والثالثة (١٢ فأر) تم تغذيتهم علي غذاء قياسي (١٤% بروتين) تحتوي على أفضل نوعين من الخبز المدعم والتي تم تحديدها من خلال التقييم الحسي (٣٠٠ جرام خبز مدعم بنسب ١٠% و ١٥% مسحوق القرع العسلي / ١٠٠٠ جم غذاء)، علي التوالي. المجموعة الرابعة

الفرعية (٦ فئران) تم تغذيتها علي غذاء عالي البروتين (٢٠% بروتين) يحتوى علي ٣٠٠ جرام خبز غير مدعم (واستخدمت كمجموعة ضابطة ايجابية). المجموعات الخامسة والسادسة الفرعية (١٢ فأرا) تم تغذيتهم علي غذاء عالي البروتين (٢٠% بروتين) تحتوى علي (٣٠٠ جرام خبز مدعم بنسب ١٠% و ١٥% مسحوق القرع العسلي/ ١٠٠٠ جرام غذاء)، علي التوالي. في نهاية فترة التجربة، تم ذبح الفئران. تم تجميع عينات الدم، وتم تركة لإحداث التجلط، تم فصل مصل الدم. أشارت النتائج المتحصل عليها بأن، تغذية الفئران المصابة بالسكر علي غذاء قياسي أو عالي البروتين والمحتوى علي (٣٠٠ جرام خبز مدعم بنسب ١٠% و ١٥% مسحوق القرع العسلي) أحدثت إنخفاضا في النسبة المئوية للزيادة في الوزن و الجلوكوز والكولسترول و الجلسريدات الثلاثية و كولسترول الليبوبروتينات منخفضة الكثافة والمنخفضة الكثافة جدا، وحامض اليوريك، ونيروجين اليوريا، والكرياتينين، وانزيمات الكبد (AST, ALT and ALP)، في حين احدثت ارتفاعا في مستوى كولسترول الليبوبروتينات عالية الكثافة، مقارنة بالمجموعات الضابطة المصابة. أفضل النتائج سجلت للمجموعة المصابة بإرتفاع مستوى الجلوكوز والتي تم تغذيتها على نظام غذائي عالي البروتين يحتوي على ٣٠٠ جرام خبز مدعم بنسبة ١٥٪ مسحوق القرع العسلي. الخلاصة: وفقا لنتائج هذه الدراسة ، فإن اتباع نظام غذائي عالي البروتين ويحتوي على خبز مدعم بمسحوق القرع العسلي يقلل نسبة الجلوكوز في الدم ومضاعفات مرض السكري. يمكن استخدام الخبز المدعم بمسحوق القرع العسلي كغذاء وظيفي لمرضي السكري.

الكلمات المفتاحية: السكري - القرع العسلي - تعزيز الخبز - فئران