



## Hypoglycemic and Hypolipidemic Effects of Pumpkin Seeds Powder and Oil on Alloxan-induced Diabetic in Rats



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THE research was conducted to study the nutritional evaluation of pumpkin seed and the effect of addition of pumpkin seeds powder and oil on blood level of glucose and fat in diabetic rats. Firstly, the nutritional composition and antioxidant activity of pumpkin seeds powder were determined. Secondly, a total of 60 rats were randomized into 6 groups as follows: Group 1: Negative control; Group 2: Positive control; Group 3: Diabetics fed with pumpkin seeds powder (1%); Group 4: Diabetics fed with pumpkin seeds powder (3%); Group 5: Diabetics fed with pumpkin seed oil (1%); Group 6: Diabetics fed with pumpkin seed oil (3%). The rats were injected with alloxan 150 mg / kg BW for induction the diabetes in rats until their blood glucose level reached more than 200 mg / dL. The results showed that pumpkin seeds rich in carbohydrates, protein, crude fiber and crude oil, many unsaturated fatty acids, especially linoleic and oleic acids. It is also a rich source of antioxidants. In addition to that glucose, glycated hemoglobin, cholesterol, triglycerides, LDL, VLDL and lipid peroxidation were significantly increased ( $P < 0.05$ ), while HDL-cholesterol and insulin were decreased in diabetic rats as compared to the negative control group. The use of pumpkin seeds powder and oil resulted in a significant decrease in glucose, glycated hemoglobin, cholesterol, triglycerides, LDL, VLDL and lipid peroxidation compared to positive control. Furthermore, when preparing some products by addition of pumpkin seeds powder and oil, the smell, taste, color, texture and overall acceptability were good. In conclusion, the administration of pumpkin seeds powder and seed oil can lower the side effects of diabetes; improve the insulin levels and health status of diabetic rats. Pumpkin seeds powder and oil can be considered as one of foods that reduced blood glucose level and lipids profile in diabetic rats.

**Keyword:** Pumpkin seeds, Oil pumpkin seeds, Hyperglycemia, Hypolipidemic, Diabetes, antioxidants, Insulin, Rats

### Introduction

Diabetes is caused by insulin deficiency or relative deficiency. This increases blood glucose levels as cells cannot absorb glucose, leading to intracellular glucose deficiency despite abundant extracellular glucose (Savoca et al., 2006). Hyperglycemia is the leading cause of diabetes complications and a major factor in the development of diabetes vascular diseases, especially cardiovascular disease (Soman et al., 2013).

Pumpkins are very popular in many countries. Its seeds were a rich natural source of polyunsaturated fatty acids, proteins, phytosterols, vitamins, trace elements, such as zinc and antioxidant compounds such as tocopherol, phenolic compounds, flavonoids and carotenoids. Pumpkin seed oil offers many health benefits (Xanthopoulou et al., 2009). The pumpkin seeds contain vitamins, oil (37.8-45.4%) especially Omega 6 fatty acids and protein (25.2-37%) (Makni et al., 2011).

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Pumpkin seeds oil mainly contains fatty acids (FAs); oleic, linoleic, and palmitic acids. Also, rich in  $\delta$ -tocopherol,  $\beta$ -sitosterol and syringic acid (Stevenson et al., 2007). A high poly unsaturated FA content and lower free FA content make it highly suitable for use in edible purposes (Habib et al., 2015). D-chiro-Inositol, isolated from pumpkin was considered as an insulin action mediator (insulin sensor) and has been linked to its antidiabetic activity (Yadav et al., 2010).

Pumpkin seeds and oils have drawn the attention of many researchers around the world. It has been shown that pumpkin seeds oil are beneficial as a treatment of benign prostatic hyperplasia, enhancement of immunity, hypolipidemic, antihypertensive, anthelmintic, antidiabetic and anticancer (Patel, 2013).

One of the most health benefits of pumpkin seeds oil is to prevent the growth and reduce the size of the prostate (Tsai et al., 2002 and Gossell-Williams et al., 2006). There is also research showed that pumpkin seeds oil can delay the progress of high blood pressure (Zuhair et al., 2000) and relieve high cholesterol (Zuhair et al., 1997) and arthritis (Fahim et al., 1995). Pumpkin seeds oil also relieves diabetes by promoting the activity of blood sugar (Fu et al., 2006). Pumpkin seeds oil is an important source of vitamin E (tocopherol) in Japanese diets (Imaeda et al., 1999).

This study aims to evaluate the effects of different levels of pumpkin seeds powder and seeds oil on glucose and lipids profile in diabetic rats induced by alloxan.

## **Materials and Methods**

### *Plant materials*

Pumpkin (*Cucurbita maxima*) seeds were obtained from the local market, Alex., Egypt.

### *Animals*

Sixty adult male albino rats Sprague Dawley strain, weighing between (200  $\pm$  10 g) were obtained from the High Institute of Public Health, Alexandria University, Egypt.

### *Materials*

#### *Chemicals*

Casein was acquired from the chemicals and dietary products company Morgan, Cairo, Egypt. Minerals and vitamins mixes, choline and cellulose acquired from the pharmaceutical and chemical company El-gomhoria (Co.) in Cairo, Egypt.

### *Kits*

Kits used for determination of serum analysis were purchased from Gamma Trade Co., Dokki, Egypt.

### *Methods*

#### *Preparation of materials*

The seeds were well washed and cleaned, dried in hot air for 4 hr (60-80°C), roasted in a preheated oven at 120°C for 10-15 min, husking, grinded to make powdered by a domestic grinder (Philips). The oil was hydraulics extracted by pressing with screw presses CA 59 G type (IBG Monforts Oekotec GmbH).

#### *Nutritional analysis*

The seeds were subjected to proximate analysis (moisture, ash, protein, fat, and fiber, using the method of AOAC (2012). N-free extract content was determined by difference according to Aman and yousef (2000).

#### *Estimation of total phenolic compounds*

The quantity of total polyphenolic compounds in pumpkin seeds was decided calorimetrically by using the Folin-Ciocalteu reagent, according to Francis (1982). Total polyphenol values were expressed in terms of Gallic acid equivalent mg/100g. The test was repeated in triplicate.

#### *Estimation of total flavonoids*

The colorimetric process of aluminum chloride was used to evaluate flavonoids (Aiyegoro and Okoh 2010). One milliliter (1 ml) of seeds (1 mg/ml) was mixed with 0.2 ml of 10% aluminum chloride, 3 ml of methanol, 5.6 ml of distilled water and 0.2 ml of 1 M potassium acetate and remains for 30 min at room temperature. The absorption of the reaction mixture with a UV visible spectrophotometer was measured at 420 nm. The calibration curve was extrapolated to determine the content by preparing a quercetin solution in distilled water. The concentration of flavonoids was expressed in terms of mg/100g.

#### *The free radical scavenging activity of the pumpkin seeds*

The spectrophotometric method according to Sreerama et al. (2010) was used to determine the antioxidant activities of seeds by assessing their 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging ability ( $\mu\text{mol g}^{-1}$ ). 5 g of pumpkin seed with 25 ml of methanol for 2 hr with continuous shaking was then combined with 2.7 mL of DPPH methanol. The samples were analyzed on a spectrophotometer (Thermo Spectronic, Rochester, NY, USA) and the

absorption at 517 nm was measured over a 30 minute period and used to calculate the capacity of seed to scavenge DPPH free radicals ( $\mu\text{mol g}^{-1}$ ).

#### *Gas chromatography (GC) analysis of fatty acid*

Fatty acids were determined in the sample by using the methyl esters boron trifluoride method (A.O.A.C., 2000). Fatty acid methyl esters were identified on an Agilent Technologies 7890A GC equipped with flame ionization detector (PE Auto System XL) with auto sampler and Ezchrom integration system. The carrier gas (He); ca. 25 Psi – air 450 ml/min – Hydrogen 45 ml – split 100 ml/min. Oven temperature 200 °C injector and detector 250 °C.

#### *Experimental animals design*

Six groups of rats (10 rats in each group) weighing between ( $200 \pm 10$  g) were used. All rats were injected with alloxan at (150 mg - kg body weight) for induction of diabetes except for the negative control group.

#### *Grouping of rats and experimental design*

Group (1): “negative control“ fed on basal diet

Group (2): “positive control “ fed on basal diet as diabetic rats without any treatment

Group (3): (diabetic rats) fed on a basal diet containing 1% dried pumpkin seeds powder.

Group (4): (diabetic rats) fed on a basal diet containing 3% dried pumpkin seeds powder.

Group (5): (diabetic rats) fed on a basal diet containing 1% pumpkin seeds oil.

Group (6): (diabetic rats) fed on a basal diet containing 3% pumpkin seeds oil..

#### *Diet planning*

The basal diet contains of protein (13%) , choline (0.2%) , fat (4%) , vitamin mixture (1%) , cellulose (5%), salt mixture (3.5%) and the remainder was starch (Reeves et al., 1993).

#### *Biological assessment*

The biological assessment was conducted by evaluating the food intake (FI) every day throughout the experimental period (4 weeks). Over all body weight gain (BWG) and organs relative weight were determined according to (Chapman et al., 1959) .

#### *Blood sampling*

Rats were starving for 12 hr at the end of the trial duration (4 weeks), then sacrificed under ether

anesthesia. Blood samples were collected from the aortic vein into clean dry centrifuge tubes and stored for 15 min at room temperature, placed in a refrigerator (Toshiba) for 2 hr, then centrifuged to extract serum for 15 min at 3000 rpm. Serum was separated carefully and transferred to dry clean eppendorf tubes using a Pasteur pipette and kept frozen at -20 °C until the test parameters were determined.

#### *The blood serum testing techniques*

##### *Serum glucose determination*

Based on the colorimetric method described by Burrin and price (1985), serum glucose was determined.

##### *Total cholesterol determination*

Serum cholesterol was measured using the enzymatic method of Allain et al. (1974).

##### *Triglycerides determination*

The serum triglycerides is colorimetrically determined by the method of Wahlefeld (1974).

##### *High- density lipoprotein (HDL) cholesterol determination*

According to Albers et al. (1983), the HDL-c was determined.

##### *Low and very low-density lipoprotein (LDL, VLDL) determination*

The VLDL-c and LDL-c concentration was calculated by Friedewald et al. (1972) equation:

$$\text{VLDL-c} = \text{triglycerides} / 5$$

$$\text{LDL-c} = \text{Total cholesterol} - (\text{HDL-c}) - (\text{VLDL-c})$$

##### *Determination of lipid peroxidation*

The levels of lipid peroxides formed was determined by the method of El-Saadani et al. (1989).

##### *Biochemical analysis*

The following reference methods were used to conduct biochemical analysis using Eliza and colorimetric kit test. According to Wayne (1998) & Gonen and Rubenstein (1978), respectively blood glucose levels, insulin levels and glycated hemoglobin percentage are determined.

##### *Preparation of bakery products*

Bakery products were prepared using Saba's (1991) methods.

##### *Preparation of cressina*

The dough was prepared by using the following formula: 720 g wheat flour without additives, 2.5 g sugar, 110 g pumpkin seeds oil, 2.5 g salt, 306 g

warm water and 20 g yeast. The first sample was the control while, the other samples were prepared by replacing 5, 10 and 15% of flour with pumpkin seeds powder /100gm. The dough is prepared by adding all the dry ingredients together and then adding oil and warm water until a smooth dough forms. The dough was placed in a warm place for 30 minutes until it fermented. The dough was cut and formed as round sticks and left to ferment again for 20 min, then baked in the oven (universal) at 200°C for 12-15 min.

#### *Preparation of patonsalé*

The dough was prepared by using the following formula: 720 g wheat flour without additives, 2 g sugar, 125 g pumpkin seeds oil, 1.5g salt, 15g yeast, 5 g cumin and 250 g water. The first sample was the control, while the other samples were prepared by replacing 5, 10 and 15% of flour with pumpkin seeds powder /100gm. The dough was prepared by adding all the dry ingredients together and then adding oil and warm water until a smooth dough forms. The dough was placed in a warm place for 30 minutes until it fermented. The dough was cut and formed as sticks and left to ferment again for 20 minutes, then baked in the oven (universal) at 200°C for 12-15 min.

#### *Sensory evaluation of the products*

The sensory characteristics were evaluated according to Hooda and Jood (2005) by 40 persons from staff and students of faculty of Specific Education, Alexandria University for a pilot study to select the highest acceptability score in samples. Parameters were taste, color, texture, odor and overall acceptability. The 9-point hedonic scale with a scale ranging from 1 (representing extremely dislike) to 9 (representing extremely like).

#### *Statistical analysis*

Using IBM SPSS software package version 20.0, data were fed to the computer and analyzed. (Armonk, NY: IBM Corp) Kirkpatrick and Feeney (2013). The Kolmogorov Smirnov test has been used to evaluate the normality of variables distribution.

ANOVA was used to compare more than two groups followed by a Post Hoc (LSD) test. The significance of the obtained results was judged at the 5% level Kotz et al. (2006).

## **Results and Discussions**

#### *Nutritional composition of pumpkin seeds*

The results of the chemical composition of the dried pumpkin seeds powder are presented in

Table 1. The dried seeds contained  $6.70 \pm 0.29\%$  of moisture on dry basis. The low moisture content makes these seeds safe for long-term storage without spoiling, as they are less susceptible to attacks by microorganisms (Ajayi et al., 2006). The oil content was found to be  $35.43 \pm 0.07\%$  (Table 1). But this percentage was lower than some Egyptian varieties, *i.e.* 51.0% (El-Adawy and Taha, 2001) and European varieties, *i.e.* 54.9%, (Murkovic et al., 1999), However, they recorded higher values when compared to species in African countries, *i.e.* 21.9-35.0% (Younis et al., 2000). The difference in oil content may be due to different climatic conditions and genetic diversity (Stevenson et al., 2007). The content of pumpkin seeds oil in this study can be compared with other oils such as sunflower (30-35%), olive (12- 50%), cottonseed (22-24%), rapeseed (40-48 %) and soybean (18- 22%) (Nichols and Sanderson, 2003). As a result, pumpkin seeds can be used in many different industries and domestic purposes as a source of vegetable oil.

The protein content  $28.50 \pm 0.33\%$  found in this study (Table 1) agreed with those of Al-Khalifa (1996) for *C. moschata* (24.0%) and *C. pepo* (26.5 %). Protein content may vary from fruit to fruit. These differences are due to species diversity and environmental conditions, this is in good agreement with (Achu et al., 2005). Overall, pumpkin seeds are a rich source of protein. It can meet the daily protein requirements of adults; 23.6 g/100 g as recommended by some authorities (Ajayi et al., 2006). It was found that methionine and tryptophan were the least amino acids, but aspartic, glutamic and arginine were the most abundant amino acids (Devi et al., 2018).

The N- free extract content was calculated to be  $29.31 \pm 0.51\%$  of the dry matter (Table 1). This ratio is close to the N- free extract content of both sesame (26.0%) and cashew nuts (26.2%) (Achu et al., 2005).

Total ash content (4.50 %), which was lower than the value of 4.62% for *Teramnus labialis* seed totally (Viswanathan et al., 1999). Fiber is an important component of many complex carbohydrates. It is mainly found only in plants particularly fruits, vegetables, legumes and nuts. As shown in Table 1, fiber content of the pumpkin seeds was found to be  $(2.26 \pm 0.06\%)$  which was lower than fiber in *cassia hirsute* seed (4.68- 6.92 g %) (Gofur et al., 1993).

These results are consistent with Adebayo et al. (2013) who found that pumpkin seeds contain well carbohydrates, protein, crude fiber and crude oil. Pumpkin seeds oil was found to be highly unsaturated, with preliminary present of linoleic and oleic acids. Both sugars, fixed oils, peptides and proteins are considered active compounds found in pumpkins (Dar et al., 2017).

In recent years, pumpkin seed oil has received great attention due to its high nutritional value and its impact on health. Montesano et al. (2018) also explained that pumpkin seeds oils are rich in PUFA, MUFA, carotenoids and phytosterols, making them important vegetable oils used in cosmetics and also in the preparation of many nutrients that promote human health.

#### *Fatty acids content of pumpkin seeds oil*

The fatty acid content of pumpkin oil is showed in Table 2. The results showed that seven fatty acids in oil were identified, oleic acid was the major fatty acid, its ratio in pumpkin seeds was 44.09 %, while Linoleic acid was 34.70 %; the next important fatty acid in pumpkin seeds

oils was Palmitic acid which make 15.90 %. These values come close to those found by Nyam et al. (2009). Pumpkin seed oil may be oxidized due to its high content of linoleic acid. But on the other hand, these fatty acids have a great nutritional and physiological benefit useful for preventing cancer and coronary heart disease (Oomah et al., 2000). Kulaitienė et al. (2018) showed that polyunsaturated fatty acid ratios were high in pumpkin seed oils and quantities were from 64.29% to 66.71% of total fatty acids, while monounsaturated fatty acids ranged from 16.19% to 18.49%. On the other hand, saturated fatty acids were 15.5% lower to 15.92%. These percentages vary according to category. These results are also identical to Devi et al. (2018). Therefore, pumpkin seeds can be used commercially because they contain a high content of fat and protein, and their composition of amino acids and fatty acids (Alfawaz, 2004). Karanja et al. (2013) found that the fatty acid profile of pumpkin seeds oil, was similar to that of soybean, sunflower and sesame oils which were rich in polyunsaturated fatty acids.

**TABLE 1. Nutritional composition of pumpkin seeds (n = 3)**

Parameter	Amount
Moisture Content (%)	6.70±0.29
Ash Content (%)	4.50 ±0.06
Oil Content (%)	35.43±0.07
Fiber Content (%)	2.26±0.06
Protein Content (%)	28.50±0.33
N-free extract Content (%)	29.31±0.51

Data was expressed using Mean ±SE. of three replicate

**TABLE 2. Levels of fatty acids (%) in pumpkin seed oil (n = 3)**

Fatty acid	Amount (%)
Palmitic acid (C16:0)	15.90±0.07
Stearic acid (C18:0)	4.60±0.01
Oleic acid (C18:1 n-7)	44.09±0.17
Linoleic acid (C18:2 n-6)	34.70±0.45
Arachidic acid (C20:0)	0.39±0.04
Linolenic (C18:3)	Tr.
Palmitoleic (C16:1)	Tr.

Data was expressed using Mean ±SE. of three replicate

*Total phenolic, total flavonoids and antioxidant activity in pumpkin seeds*

The total phenolic contents of pumpkin seeds were  $32.58 \pm 0.46$  mg gallic acid equivalent/100g, While the total flavonoids of pumpkin seeds were ( $19.50 \pm 0.37$  mg quercetin equivalent/100g respectively). These results were consistent with Zdunić et al. (2016) who reported that polyphenolic compounds are rich in pumpkin fruit and seeds. In general Polyphenols, such as flavonoids, are found largely in food products derived from plant sources and are characterized by their high antioxidant content (Van Acker et al., 1996). Studies have also shown that the higher level of flavonoids in food, lower the incidence of many human diseases (Hertog et al., 1993). Sopian et al. (2014) propose the presence of phenolic compounds in the pumpkin powder. Such phenolic compounds have many groups of hydroxyls including o-groups of hydroxy with very high antioxidant potential.

Antioxidant activity of pumpkin seeds are presented in Table 3. Results showed that pumpkin seeds have highly values for DPPH% ( $36.22 \pm 0.35$ ). Sopian et al. (2014) reported that the uses of pumpkin powder extracts led to improvement in the absorption of DPPH radical after the reaction between the antioxidant molecules and the extracts, resulting in the scavenging of the radical by hydrogen donation. Kalantzakis et al. (2006) observed a difference in antioxidant activity in many vegetable oils such as soybeans cottonseed, olive oil, sunflower and commercial oils. The cause may be different content of phenolic compounds and tocopherol which directly affect oxidative stress. The seed is one of the residues resulting from the circulation of fruits and vegetables, but it plays an important role in human nutrition as it can be used regularly without any harm to human health (Maheshwari et al., 2015).

*Effect of pumpkin seeds powder oil on food consumption and food efficiency ratio*

Data in Table 4 show the impact of powder for pumpkin seeds and oil on food consumption

and food efficiency ratio (FER) in diabetic rats. It was found from the results that all diabetic groups treated with different concentrations of dried pumpkin seeds powder and pumpkin seeds oil by 1 and 3% results in a significant decrease in food intake compared with negative control group. From the same data, It was also observed that all diabetic groups treated with different concentrations of dried pumpkin seeds powder and pumpkin seeds oil by 1&3% had non – significant increase in food efficiency ratio F.E.R. comparing to positive control groups. While, The results showed significant increase in body weight gain compared to positive control group when treated groups with different levels of dried pumpkin seed powder and pumpkin seed oil by 1 and 3%.

The results were agreed with Al-Okbi et al. (2014) where it was found that the groups treated with pumpkin seeds oil at different doses had a significantly lower intake than the control group and hypercholesterolemia. It was also observed that there were no significant differences in the body weight gain, final body weight and the ratio of food efficiency among different groups. Also, after treatment with *Cucurbita pepo*. L. extracted oil Bardaa et al. (2016) showed a slight increase in rat weight. However, no significant differences in mean body weight were observed between the different groups studied at the end of the experiment and it can be concluded that the groups are homogeneous and the rats grow normally.

*Effect of pumpkin seeds powder and oil on organs/ body weight ratio in diabetic rats*

From the data in Table 5, there were no significant differences between the ratio of the liver, spleen and kidney to body weight between all diabetic groups, were treated at different concentrations of dried pumpkin seed powder and pumpkin seeds oil by 1 and 3% when compared to the positive and negative control.

**TABLE 3. Total phenolic, total flavonoids and antioxidant activity in pumpkin seeds (n = 3)**

Sample	Total phenolic (mg/100g)	Flavonoids (mg/100g)	DPPH %
Pumpkin	$32.58 \pm 0.46$	$19.50 \pm 0.37$	$36.22 \pm 0.35$

Data was expressed using Mean  $\pm$ SE. of three replicate

**TABLE 4. Effect of pumpkin seeds powder and oil on food consumption and food efficiency ratio (FER) in diabetic rats**

Parameter	Food consumption (gm / day)	Body Weight Gain (%)	Food Efficiency Ratio
<b>Treatment</b>			
Negative control	13.47 ± 0.165 <sup>a</sup>	35.16 ± 4.97 <sup>a</sup>	0.059 ± 0.016 <sup>a</sup>
Positive control	12.57 ± 0.172 <sup>b</sup>	14.80 ± 3.26 <sup>b</sup>	0.041 ± 0.017 <sup>a</sup>
Dried pumpkin seeds powder 1%	12.20 ± 0.161 <sup>b</sup>	32.64 ± 4.37 <sup>a</sup>	0.068 ± 0.016 <sup>a</sup>
Dried pumpkin seeds powder 3%	9.90 ± 0.172 <sup>c</sup>	34.02 ± 7.50 <sup>a</sup>	0.084 ± 0.017 <sup>a</sup>
Pumpkin seeds oil 1%	11.83 ± 0.186 <sup>b</sup>	31.21 ± 1.78 <sup>a</sup>	0.072 ± 0.018 <sup>a</sup>
Pumpkin seeds oil 3%	10.90 ± 0.21 <sup>c</sup>	32.84 ± 6.37 <sup>a</sup>	0.078 ± 0.012 <sup>a</sup>

\* Values with the same letters indicate insignificant difference (P<0.05) and vice versa.

Values are expressed as means ±SE

**TABLE 5. Effect of pumpkin seeds powder and oil on organs/ body weight ratio in diabetic rats**

Parameter	Liver / BW ratio	Kidney/ BW ratio	Spleen / BW ratio
<b>Treatment</b>			
Negative control	3.22 ± 0.23 <sup>ab</sup>	0.96 ± 0.044 <sup>a</sup>	0.43 ± 0.035 <sup>a</sup>
Positive control	2.94 ± 0.24 <sup>ab</sup>	0.78 ± 0.046 <sup>b</sup>	0.39 ± 0.036 <sup>a</sup>
Dried pumpkin seeds powder 1%	2.68 ± 0.23 <sup>b</sup>	0.75 ± 0.043 <sup>b</sup>	0.39 ± 0.034 <sup>a</sup>
Dried pumpkin seeds powder 3%	3.46 ± 0.24 <sup>a</sup>	0.89 ± 0.046 <sup>ab</sup>	0.40 ± 0.036 <sup>a</sup>
Pumpkin seeds oil 1%	3.65 ± 0.26 <sup>a</sup>	0.83 ± 0.050 <sup>ab</sup>	0.50 ± 0.039 <sup>a</sup>
Pumpkin seeds oil 3%	3.33 ± 0.22 <sup>a</sup>	0.79 ± 0.053 <sup>ab</sup>	0.49 ± 0.037 <sup>a</sup>

\* Values with the same letters indicate insignificant difference (P<0.05) and vice versa.

Values are expressed as means ±SE

#### *Effect of pumpkin seeds powder and oil on the progression of diabetic status in rats*

Table 6 shows that glucose and glycated hemoglobin increased significantly in diabetic rats, while insulin levels decreased compared to the negative control group. On the other hand, treatment with pumpkin seeds powder and oil in different proportions in diabetic rats resulted in a significant reduction in blood glucose and glycated hemoglobin levels compared with positive control group. But there was a significant increase in insulin levels, especially rats that were administered with pumpkin oil, which showed the highest improvement in blood glucose levels, glycated hemoglobin and insulin. The diabetic group showed a significant increase in plasma glucose compared with the the negative control group. Alloxan generates active oxygen molecules that damage a large number of  $\beta$ -cells, leading to a decrease in the level of insulin. This process takes a very short time for the rats to become diabetic (Martinez and Milagro, 2000). On the other hand, pumpkin seeds groups displayed lower blood glucose levels which are in line with Sedigheh et al. (2011) who reported lower blood glucose levels of pumpkin seeds.

Anti-diabetic plants promote insulin impact either by releasing insulin bound or by increasing the insulin secretion of the pancreas (Pari and Amarnath 2004) inhibition of the production of liver glucose (Eddouks et al., 2003) inhibition of intestinal glucose absorption, (Youn et al., 2004) or insulin-resistance correction (Hu et al., 2003). These mechanisms mentioned above suggested that plant seeds can be used in diabetes prevention or treatment. Pumpkin seeds contain many active compounds such as phenol and flavonoids. Quercetin is an essential flavonoid known to increase the production of hepatic glucokinase, possibly by raising the release of insulin from pancreatic islets (Vessal et al., 2003). Fatty acids affect the secretion of insulin depending on the degree of saturation and the length of the chain (Poitout and Robertson, 2008). As reported by Feng et al. (2006) linoleic acid the main fatty acid in pumpkin fats may be involved in modulating pancreatic  $\beta$ -cell function. The active chemical components of pumpkin play a very important role in lowering blood sugar. This function is performed by the polysaccharides from the fruit pulp, protein in germinated seed and oils from un-germinated seeds (Zhang et al., 2002).

**TABLE 6. Effect of pumpkin seeds powder and oil on the progression of diabetic status in rats.**

Parameter Treatment	Glucose (gm/dl)	Insulin ( $\mu$ IU/ml)	Glycated hemoglobin(%)
Negative control	63.0 $\pm$ 0.48 <sup>f</sup>	4.76 $\pm$ 0.06 <sup>a</sup>	7.83 $\pm$ 0.42 <sup>e</sup>
Positive control	159.03 $\pm$ 0.37 <sup>a</sup>	2.15 $\pm$ 0.51 <sup>d</sup>	29.14 $\pm$ 0.36 <sup>a</sup>
Dried pumpkin seeds powder 1%	126.93 $\pm$ 0.34 <sup>b</sup>	3.00 $\pm$ 0.26 <sup>cd</sup>	13.22 $\pm$ 0.08 <sup>b</sup>
Dried pumpkin seeds powder 3%	112.40 $\pm$ 0.04 <sup>d</sup>	3.05 $\pm$ 0.29 <sup>bc</sup>	12.20 $\pm$ 0.41 <sup>c</sup>
Pumpkin seeds oil 1%	117.60 $\pm$ 0.35 <sup>c</sup>	3.38 $\pm$ 0.29 <sup>bc</sup>	11.82 $\pm$ 0.12 <sup>c</sup>
Pumpkin seeds oil 3%	101.60 $\pm$ 0.02 <sup>e</sup>	3.91 $\pm$ 0.03 <sup>ab</sup>	10.85 $\pm$ 0.18 <sup>d</sup>
F (p)	9859.05(<0.001*)	9.361(0.001*)	648.204(<0.001*)

Data was expressed using Mean  $\pm$ SE. F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (LSD). p: p value for comparing between the studied groups

\* Values with the same letters indicate non-significant difference ( $P < 0.05$ ) and vice versa.

#### *Effect of pumpkin seeds powder and oil on the Lipid profile of Diabetic Rats.*

Table 7 shows that all diabetic rats treated with different concentrations of pumpkin seeds powder and oil by 1 and 3% had a significant decrease in LDL-C, VLDL-C, total cholesterol and triglycerides compared with the positive control group. While the use of pumpkin seeds powder and oil resulted in an improvement in HDL-C level compared to positive control group. Such results are consistent with previous Farid et al. (2015) research, who observed that glucose, cholesterol, triglycerides, LDL were significantly increased, compared to the normal control group in diabetic rats. Also, the results agree with the previous study of Aboelnaga (2015), who suggested that pumpkin seeds revealed signs of improvement in obese-diabetic rats which might be a good approach to be applied in human suffering from diabetes complications. Next, alloxan administration was found to have increased levels of triglycerides, cholesterol and LDL-cholesterol (Table 7). However, when diabetes is caused by alloxan, high blood glucose results in increased triglycerides and cholesterol in the plasma (Pari and Saravanan, 2002). In case of diabetes, insulin deficiency causes the non-activation of lipoprotein lipase, resulting in high blood lipids. Therefore, the level of sugar in the blood is responsible for the regulation of blood lipid concentrations (Laakso, 1995). In addition to that, pumpkin oil groups showed lower levels of lipid profile parameters, which are in line with Ramadan et al. (2011) who found that pumpkin and apricot oils supplementation in diets decreased triglyceride, total cholesterol, and LDL levels.

Many human trials have proven fatty acids to lower cholesterol especially linoleic acid and oleic acid (Mensink et al., 2003). They are the main fatty acids in pumpkin seeds oil as shown in Table

2. Pumpkin seeds are rich sources of phytosterol and phenolic compounds. Phytosterol has been shown to inhibit the absorption of cholesterol leading to low cholesterol (Ostlund et al., 2002). Phenols also improve plasma lipid profile (Covas et al., 2006). The lipid-lowering effect of pumpkin seeds may be due to the presence of fiber in a large proportion. Hannan et al. (2003) showed that active soluble dietary fiber can lower blood fat by retarding carbohydrates and absorbing fat. The increment in HDL levels observed in the present study may be due to the stimulation of pre- $\beta$  HDL and reverse cholesterol transport. This is consistent with the results of (Gupta et al., 1993).

#### *Effect of pumpkin seeds powder and oil on serum lipid peroxidation and atherogenic index*

Data in Table 8 showed the effects of pumpkin seeds powder and oil on the serum lipid peroxidation and atherogenic index of male rats. Results indicated that using alloxan alone significantly ( $P < 0.05$ ) increased lipid peroxidation and atherogenic index compared to the negative control group. On the other hand, pumpkin seeds powder and pumpkin seeds oil by 1&3% had a significant decrease in serum lipid peroxidation and atherogenic index compared with positive control group.

One of the most important factors for antiatherogenic agents is the rise in the HDL-c. Pumpkin seeds found in this study may have an antiatherogenic effect due to the presence of  $\beta$ -carotene, PUFAs, tocopherols, and phytosterols (Vijaimohan et al., 2006). The main cause of diabetes is the formation of free radicals that form lipid peroxides. Thus, inhibition of free radical generation by antioxidants is important in protecting against diabetic hepatopathy (Castro



**TABLE 7. Effect of pumpkin seeds powder and oil on the Lipid profile in diabetic rats.**

Parameter	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
<b>Treatment</b>					
Negative control	54.48± 0.21 <sup>a</sup>	68.64± 0.12 <sup>f</sup>	24.60± 0.13 <sup>d</sup>	147.72± 0.18 <sup>f</sup>	123.0± 0.05 <sup>f</sup>
Positive control	36.21± 0.31 <sup>f</sup>	130.69± 0.38 <sup>a</sup>	31.86± 0.11 <sup>a</sup>	198.76± 0.08 <sup>a</sup>	159.30± 0.31 <sup>a</sup>
Dried pumpkin seeds powder 1%	40.31± 0.23 <sup>e</sup>	111.79± 0.38 <sup>b</sup>	29.83± 0.08 <sup>b</sup>	181.93± 0.07 <sup>b</sup>	149.13± 0.48 <sup>b</sup>
Dried pumpkin seeds powder 3%	45.87± 0.15 <sup>d</sup>	93.57± 0.51 <sup>c</sup>	28.23± 0.38 <sup>c</sup>	168.67± 0.08 <sup>c</sup>	146.15± 0.47 <sup>c</sup>
Pumpkin seeds oil 1%	48.06± 0.50 <sup>c</sup>	84.42± 0.07 <sup>d</sup>	27.87± 0.26 <sup>c</sup>	160.35± 0.49 <sup>d</sup>	138.87± 0.02 <sup>d</sup>
Pumpkin seeds oil 3%	52.24± 0.10 <sup>b</sup>	79.02± 0.50 <sup>c</sup>	25.09± 0.24 <sup>d</sup>	156.11± 0.15 <sup>c</sup>	131.0± 0.49 <sup>c</sup>
F (p)	612.517 (<0.001*)	3862.988 (<0.001*)	150.686 (<0.001*)	6609.839 (<0.001*)	1284.119 (<0.001*)

Data was expressed using Mean ± SE.

F: F for ANOVA test, Pair wise comparison bet. each 2 groups was done using Post Hoc Test (LSD)

p: p value for comparing between the studied groups

\* Values with the same letters indicate insignificant difference ( $P < 0.05$ ) and vice versa.

**TABLE 8. Effect of pumpkin seeds powder and oil on serum lipid peroxidation and atherogenic index .**

Parameter	Lipid peroxidation (mmol/l)	Atherogenic Index (TC – HDL-c / HDL-c)
<b>Treatment</b>		
Negative control	3.35 ± 0.45 <sup>bc</sup>	1.71±0.37 <sup>d</sup>
Positive control	6.29 ± 0.37 <sup>a</sup>	4.49± 0.02 <sup>a</sup>
Dried pumpkin seeds powder 1%	4.25 ± 0.26 <sup>b</sup>	3.51± 0.06 <sup>b</sup>
Dried pumpkin seeds powder 3%	3.40 ± 0.16 <sup>bc</sup>	2.68± 0.47 <sup>bc</sup>
Pumpkin seeds oil 1%	4.27 ± 0.37 <sup>b</sup>	2.34± 0.38 <sup>cd</sup>
Pumpkin seeds oil 3%	3.27 ± 0.19 <sup>c</sup>	1.99± 0.03 <sup>cd</sup>
F (p)	13.125 (<0.001*)	12.766 (<0.001*)

Data was expressed using Mean ± SE.

F: F for ANOVA test, Pairwise comparison bet. every 2 groups were done using Post Hoc Test (LSD)

p: p value for comparing between the studied groups

\* Values with the same letters indicate insignificant difference ( $P < 0.05$ ) and vice versa.

et al., 1974). High lipid peroxide is observed in plasma diabetic rats. This rise is an indicator of damage to membranes and changes in the structure and function of cellular membranes. This results in tissue damage and the failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (Amresh et al., 2007). Supplements of pumpkin seeds powder and oil improve these changes significantly. Thus, it is possible to be a protective mechanism because of its antioxidant activity. The results are agreement with Bora (2018) who concluded that the seeds of pumpkin are highly popular as an edible delicacy in different countries of the world and possess a high antioxidant value along

with anti-diabetic, anti-carcinogenic and anti-inflammatory properties. The oil from this seed is also documented to contain high phenolic content which translates to its high antioxidant capability. The pumpkin seeds oil has been envisaged as a preservative and functional ingredient in many foods, cosmetics, and nutraceutical.

#### *Sensory evaluation of some bakery products*

##### *Sensory evaluation of patonsalé*

Sensory scores of patonsalé samples with pumpkin seeds powder and oil are shown in Table 9. It is noticeable when comparing the results obtained from the addition of pumpkin seeds oil (125g) and powder by 5-10-15% to the patonsalé

that taste, color, smell, texture, and acceptability were significantly affected ( $P \leq 0.05$ ) compared to the control. Besides, the highest mean value of overall acceptability of patonsalé was ( $8.10 \pm 1.07$ ) for control followed by ( $7.60 \pm 1.59$ ) for 5%, ( $6.70 \pm 0.86$ ) for 10%, and ( $5.95 \pm 0.94$ ) for 15%. Data indicate that the patonsalé with 5% pumpkin seed powder exhibited the highest score for color ( $7.10 \pm 1.59$ ) and texture ( $6.95 \pm 1.47$ ) compared to 10 and 15 %. However, 10% ( $7.25 \pm 1.33$ ) was higher than 5% ( $7.15 \pm 1.39$ ) and 15% ( $5.0 \pm 1.21$ ) when evaluating odor. It was obvious that adding pumpkin powder by 15% led to a reduction in score of taste, color, smell, texture and overall acceptability.

#### *Sensory evaluation of cressina*

Sensory score of cressina products with pumpkin seeds oil (110g) and powder by 5-10-15% are shown in Table 10. It can be observed that the highest mean value of overall acceptability of cressina was ( $8.25 \pm 1.02$ ) for control followed by 5% ( $7.90 \pm 1.37$ ), 10% ( $6.75 \pm$

2.10) then 15% ( $4.80 \pm 0.70$ ) respectively. Taste, color, odor, texture and overall acceptability of cressina were significantly affected ( $p \leq 0.05$ ) by adding different amount of pumpkin seed powder to cressina products compared to the control.

From Table 10 results indicated that adding 15% of pumpkin seed powder led to a decrease in the level scores of taste, color, odor, texture and overall acceptability in cressina compared to other concentrations. The results showed that there were no significant differences between the products prepared by adding 5 and 10% pumpkin seeds powder in both color, taste, odor and texture.

#### **Conclusion**

This study showed that the use of both pumpkin seeds and oil resulted in maintaining the function of the pancreas, improving glucose level and lowering the level of fats in diabetic rats. More research is needed, which explains how these nutrients are used and how they are included in many diets.

**TABLE 9. Sensory scores of patonsalé containing 5, 10, 15% of pumpkin seed powder**

Patonsalé'	Color	odor	Taste	Texture	Acceptability
Control	8.40± 0.75 <sup>a</sup>	8.10± 0.85 <sup>a</sup>	8.30± 0.73 <sup>a</sup>	7.85± 1.09 <sup>a</sup>	8.10± 1.07 <sup>a</sup>
5%	7.10± 1.59 <sup>b</sup>	7.15± 1.39 <sup>b</sup>	6.90± 1.02 <sup>b</sup>	6.95± 1.47 <sup>b</sup>	7.60± 1.59 <sup>b</sup>
10%	7.05± 1.67 <sup>b</sup>	7.25± 1.33 <sup>b</sup>	6.90± 2.13 <sup>b</sup>	6.80± 1.61 <sup>b</sup>	6.70± 0.86 <sup>b</sup>
15 %	2.0± 0.65 <sup>c</sup>	5.0± 1.21 <sup>c</sup>	4.30± 0.86 <sup>c</sup>	5.80± 1.15 <sup>c</sup>	5.95± 0.94 <sup>c</sup>
F(p)	101.702* (<0.001*)	23.650* (<0.001*)	32.583* (<0.001*)	7.771* (<0.001*)	12.106* (<0.001*)

F: F test (ANOVA)

\*: Statistically significant at  $p \leq 0.05$

Different superscripts are statistically significant

Data was expressed by using mean  $\pm$  SD.

**TABLE 10. Sensory scores of cressina containing 5, 10, 15% of pumpkin seed powder**

Cressina	Color	Taste	Odor	Texture	Acceptability
Control	8.15± 1.46 <sup>a</sup>	7.95± 1.39 <sup>a</sup>	7.90± 1.48 <sup>a</sup>	7.95± 1.39 <sup>a</sup>	8.25± 1.02 <sup>ab</sup>
5%	7.20± 1.44 <sup>b</sup>	8.05± 1.90 <sup>b</sup>	7.35± 1.57 <sup>a</sup>	7.25± 1.83 <sup>a</sup>	7.90± 1.37 <sup>a</sup>
10%	6.75± 1.16 <sup>b</sup>	7.15± 1.04 <sup>ab</sup>	7.60± 1.57 <sup>a</sup>	7.0± 1.75 <sup>a</sup>	6.75± 2.10 <sup>b</sup>
15 %	3.15± 0.81	4.15± 1.18 <sup>c</sup>	4.75± 1.21 <sup>b</sup>	4.95± 1.05 <sup>b</sup>	4.80± 0.70 <sup>c</sup>
F(p)	61.619* (<0.001*)	27.577* (<0.001*)	19.641* (<0.001*)	14.064* (<0.001*)	18.262* (<0.001*)

F: F test (ANOVA)

\*: Statistically significant at  $p \leq 0.05$

Different superscripts are statistically significant

Data was expressed by using mean  $\pm$  SD.

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## التأثير الخافض للجلوكوز و دهون الدم لمسحوق بذور زيت اليقطين في الفئران المصابة بالسكر بواسطة الألوكسان

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أجري البحث لدراسة التقييم الغذائي لمسحوق بذور اليقطين وتأثير إضافة مسحوق و زيت بذور اليقطين على مستوى الدم من الجلوكوز والدهون في الفئران المصابة بالسكري. أولاً، تم تقدير التركيب الكيميائي والنشاط المضاد للأكسدة لبذور اليقطين. ثانياً، تم تقسيم عدد 60 من الفئران إلى 6 مجموعات على النحو التالي: المجموعة 1: المجموعة الضابطة السالبة، المجموعة 2: المجموعة الضابطة الموجبة، المجموعة 3: مصابة بالسكري و تأخذ مسحوق بذور القرع بنسبة (1%)، المجموعة 4: مصابة بالسكري و تأخذ مسحوق بذور القرع بنسبة (3%)، المجموعة 5: مصابة بالسكري و تأخذ زيت بذور اليقطين بنسبة (1%)، المجموعة 6: مصابة بالسكري و تأخذ زيت بذور اليقطين بنسبة (3%). تم حقن الفئران بواسطة الألوكسان (150 ملجم / كجم من وزن الجسم) لإصابتها بالسكري واستمر العلاج حتى وصل مستوى السكر في الدم إلى أكثر من 200 ملجم / ديسيلتر. أظهرت النتائج أن بذور اليقطين غنية بالكربوهيدرات، البروتين، الألياف الخام والدهون الخام والعديد من الأحماض الدهنية غير المشبعة، و خاصة حمض اللينوليك و حمض الأوليك. كما أنها مصدر غني بمضادات الأكسدة. بالإضافة إلى ذلك زادت نسبة الجلوكوز، السكر التراكمي، الكوليسترول، الجلسريدات الثلاثية، الدهون منخفضة الكثافة، الدهون شديدة الانخفاض في الكثافة و مستوى البيروكسيدات الدهنية بشكل كبير. بينما انخفضت نسبة الدهون مرتفعة الكثافة و الانسولين في الفئران المصابة بالسكر مقارنة □ بالمجموعة الضابطة السالبة. عند استخدام مسحوق بذور اليقطين والزيت انخفض مستوى الجلوكوز، السكر التراكمي، الكوليسترول، الجلسريدات الثلاثية، الدهون منخفضة الكثافة، الدهون شديدة الانخفاض في الكثافة و مستوى البيروكسيدات الدهنية مقارنة بالمجموعة الضابطة الموجبة. علاوة على ذلك، عند إعداد بعض المنتجات المحضرة بإضافة مسحوق و زيت بذور اليقطين، كانت الرائحة والتذوق واللون والملمس والتقبل العام جيد. في الختام، استخدام مسحوق بذور اليقطين وزيت بذور اليقطين يمكن أن يقلل من الآثار الجانبية لمرض السكري، يحسن مستويات الأنسولين والحالة الصحية للفئران المصابة بالسكري. و لذلك يمكن اعتبار مسحوق بذور اليقطين والزيت أحد الأغذية التي تقلل من مستوى الجلوكوز و الدهون في الدم.