

## **Ameliorative activity of pumpkin (*Cucurbita Maxima*) fruit and seeds powders on diabetic, oxidative and pancreatic status in rats**

### **Abstract:**

**Background:** Diabetes mellitus is the one of the most common endocrine diseases that is characterized by hyperglycemia, altered metabolism with an increased risk of much complications. Besides drugs classically used for the treatment of diabetes several species of plants have been described as having a hypoglycemic activity with decreased side effects. **Aim of the work:** This work aimed to investigate the possible anti-diabetic effect of oral administration of pumpkin (*Cucurbita maxima*) fruit flesh and seeds powders on Streptozotocin induced diabetic rats via studying blood glucose levels, oxidative biomarkers as well as islets of Langerhans structure changes. **Materials and Methods:** 60 adult albino rats of Sprague-Dawley strains (200±5 gm) were classified into five groups of ten animals each except diabetic control group was composed of twenty rats as follow **Group I:** healthy control ; **Group II:** diabetic control , **Group III** , **IV** and **V** : diabetic rats received 2g pumpkin fruit, seeds, fruit and seeds mixture powders respectively /kg body weight daily by oral intubation . **Results:** The results of present study showed that pumpkin powders caused significant improvements ( $P \leq 0.05$ ) in blood glucose , insulin levels and glycated hemoglobin percent compared to diabetic control group. Also pumpkin powders improved antioxidants activities and healed Langerhans islets by increasing their number and size in comparison with diabetic control group. **Conclusion:** the present study showed that pumpkin powders may normalize the various biochemical and pancreatic tissues abnormalities resulted due to diabetes metabolic disorders and it is a source of potent antidiabetic agent.

### **Keywords**

Pumpkin (*Cucurbita maxima*), Diabetes mellitus; Diabetic status; oxidative biomarkers; Langerhans islets.

## 1. Introduction

Diabetes mellitus, a complex syndrome that is characterized by the imbalance in blood glucose homeostasis leading to hyperglycemia and a series of secondary complications caused by an absolute or relative lack of insulin (Franco *et al.*, 2018). Besides drugs classically used for the treatment of diabetes several species of plants have been described in the scientific literature as having a hypoglycemic activity. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely (Obeagu and Obeagu, 2018). The pumpkin is a vegetable crop belonging to the *cucurbitaceae* family. This family contains chemicals such as saponins, proteins, fibers, polysaccharides as well as minerals (iron, zinc, manganese, copper, etc.). The fruits are a good source of polyphenols, carotenoid and  $\gamma$  - aminobutyric acid. Pumpkin seeds embedded in a bright-yellow fibrous endocarp are large, non-endospermic and usually dark red in colour. Seeds are rich in polyphenolos, vitamins and minerals. Seeds oil typically is a highly unsaturated oil, with predominantly oleic and linoleic acids present (Dar *et al.*, 2017). The present study investigated the effect of oral administration of pumpkin (*Cucurbita maxima*) fruit flesh and seeds powders on blood glucose level, oxidative biomarkers and islets of Langerhans structure changes in Streptozotocin induced diabetic rats.

## 2. Materials and Methods

### 2.1. Materials

**2.1.1. Chemical:** Streptozotocin (STZ) was purchased from sigma-Aldrich (USA).

Other chemicals were obtained from EL-Gomhouria Company for Trading Chemicals, Cairo, Egypt.

**2.1.2. Animals:** Sixty adult male albino rats of Sprague-Dawely strains weighing ( $200\pm 5$ gm), were supplied by Research Center of the Faculty of Medicine, Ain Shams University, Cairo, Egypt.

**2.1.3. Diet:** - Balanced diet according to American Institute of Nutrition AIN-93M and adjusted by *Reeves et al.(1993)* with some modifications (*El-Sheikh and Khalil, 2011*). The composition of the balanced diet (g/100g diet), Cornstarch 62.07, Casein 14.0, Sucrose 10.0, Corn oil 4.0, Fiber 5.0, Mineral mixture 3.5, Vitamin mixture 1.0, L-cystine 0.18 and Choline bitartrate 0.25.

**2.1.4. Plant:** - The fresh fruit of pumpkin, were collected from the local market and authenticated by botanist (Department of Botany, women faculty, Ain shams university). The fruit was washed with water peeled and cut into small pieces. The seeds were cleaned well with water and peeled. They were dried completely in well-ventilated stores under standard conditions away from sunlight, moisture and microbial contamination and ground with an electric grinder into powder (*Sedigheh et al., 2011*).

## **2.2. Methods**

### **2.2.1 Measurement of total polyphenolic compounds**

The amount of total polyphenolic compounds in pumpkin fruit and seeds were determined calorimetrically using the Folin-Ciocalteu reagent, by *Francis (1982)* method. Total polyphenol values were expressed in terms of Gallic acid equivalent (mg/g). The experiment was repeated in triplicate.

### **2.2.2 Nutritional analysis**

The sample of the fruit and seeds was subjected to proximate analysis (moisture, ash, crude protein, crude fat, and crude fiber, using the method of *AOAC (2012)*. Carbohydrate content was determined by difference.

## **2.3. Experimental Design**

Animals were randomly classified into five groups of ten animals each except diabetic control group which was composed of twenty rats. Diabetes was induced in fifty rats with Streptozotocin (STZ). STZ was dissolved in cold citrate buffer (0.1M, pH 4.5) and then injected subcutaneously in a dose of 40mg/kg body weight after an overnight

fasting. The other 10 rats (control healthy rats) were injected subcutaneously with citrate buffer (*Volpato et al., 2011*). After injection of rats with STZ, rats were given a sucrose solution 5% for 24h instead of drinking water to overcome STZ-induced hypoglycemia (*Ibrahim and Rizk, 2008*) and was treated as follows: **Group I:** healthy control group, rats received a placebo 2gm of physiological saline daily by oral intubation ; **Group II:** diabetic control group, rats received a placebo 2gm of physiological saline daily by oral intubation.; **Group III :** diabetic rats received 2gm pumpkin fruit powder/kg body weight daily by oral intubation .; **Group IV:** diabetic rats received 2gm pumpkin seeds powder/kg body weight daily by oral intubation. **Group V :** diabetic rats received a mixture of 2gm pumpkin fruit and seeds powder mixture(1:1) per kg body weight daily by oral intubation.

### 2.3.1. Handling of blood and pancreas samples

At the end of the experiment (30 days) all rats were fasted overnight and sacrificed under ether anesthesia. Blood samples were collected from the hepatic portal vein to separate serum for biochemical analyses. Pancreas samples were immediately removed to be examined microscopically.

### 2.4. Biochemical analysis:

Blood glucose concentration, insulin level and glycated hemoglobin (HbA1c) percentage were determined by glucometer and Enzyme Immunoassay Test kit according to **Wayne (1998)** and **Gonen and Rubenstein (1978)**, respectively. nitric oxide (NO), malondialdehyde (MDA), advanced oxidation protein products (AOPPs) and reduced glutathione (GSH) levels were determined according to **Montgomery and Dymock (1961)**, **Uchiyama and Mihara (1978)**, modification of Witko's method (**Witko et al ., 1992**) and **Beutler et al. (1963)**, respectively . Catalase (CAT) and superoxide dismutase ( SOD ) activities according to *Aebi (1984)* and *Nishikimi et al.( 1972 )*.

**2.5. Microscopical examination:** At the end of the experiment the rats were euthanized and the pancreas was removed. The specimens were washed with normal saline , fixed in 10% formalin, then dehydrated and cut into 5 micron sections and were stained with hematoxylin and eosin. Prepared slides were examined for mean diameter and number of Langerhans islets under the light microscope (*Asgary et al., 2008*).

**2.6. Statistical analysis:** - Results were analyzed using the SPSS software (version 16). (ANOVA; F-test) and least significant difference (L.S.D) were calculated according to *Levesque (2007)*.

### **3. Results:**

#### **3.1. Nutritional composition of pumpkin fruit and seeds**

**Table (1): Nutritional composition of pumpkin fruit and seeds**

Parameter	Fruit	Seeds
Ash( g%)	15.5	4.8
Moisture(mg%)	0.532	5.7
Fat ( g%)	2.3	39.43
Fiber( g%)	11.54	1.26
Protein( g%)	3.16	26.5
Carbohydrates ( g%)	66.96	22.31

Table (1) showed that pumpkin fruit is a rich source of carbohydrates and fiber while the seeds are rich in fat , protein and carbohydrates.

#### **3.2. Total polyphenol content of pumpkin fruit and seeds**

**Table (2): total polyphenol content of pumpkin fruit and seeds**

Parameter	Fruit	Seeds
Total polyphenols (mg GAG/g)	0.925	0.415

Table (2) showed that pumpkin fruit contains (0.925 mg GAG/g) while seeds contain (0.415mg GAG/g).

#### **3.3 Impact of pumpkin fruit and seed powders on the progression of diabetic status in rats**

**Table (3):Impact of pumpkin fruit and seed powders on the progression of diabetic status in rats**

Parameter Group	blood glucose (mg/dl)	Insulin ( $\mu$ IU/ml)	Glycated hemoglobin (%)
Healthy	77 $\pm$ 0.936 <sup>e</sup>	4.756 $\pm$ 0.156 <sup>a</sup>	7.834 $\pm$ 0.132 <sup>e</sup>
Diabetic	272.18 $\pm$ 1.631 <sup>a</sup>	2.15 $\pm$ 0.339 <sup>e</sup>	29.14 $\pm$ 0.642 <sup>a</sup>
Diabetic supplemented with pumpkin fruit powder	104.1 $\pm$ 1.53 <sup>d</sup>	3.914 $\pm$ 0.149 <sup>b</sup>	8.82 $\pm$ 1.047 <sup>d</sup>
Diabetic supplemented with pumpkin seed powder	149.14 $\pm$ 1.73 <sup>b</sup>	3.05 $\pm$ 0.161 <sup>d</sup>	13.22 $\pm$ 0.526 <sup>b</sup>
Diabetic supplemented with pumpkin fruit and seed mixture powder	121.96 $\pm$ 1.38 <sup>c</sup>	3.382 $\pm$ 0.134 <sup>c</sup>	10.20 $\pm$ 0.624 <sup>c</sup>

- Values are expressed as means  $\pm$ S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts letter in the same column. (p $\leq$ 0.05).

Table (3) clarified that STZ-diabetic rats elucidated a significant rise in blood glucose level and HbA1c percent but a low insulin level as compared to the healthy control group .On the other hand, the STZ diabetic rats treated with pumpkin fruit ,seeds and mixture of both powders showed a significant decrease in blood glucose levels and HbA1c percents also there were a significant increase in insulin levels, especially the rats that were administered with the pumpkin fruit powder, which exhibited the highest improvement for blood glucose, HbA1c and insulin levels.

### 3.4. Effect of pumpkin fruit and seed powders on some oxidative markers

**Table (4):Effect of pumpkin fruit and seed powders on some oxidative markers**

parameter Group	Nitric oxide ( $\mu$ mol/L)	MDA ( $\mu$ mol/L)	Advanced oxidation protein products ( $\mu$ mol/L)
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<b>Healthy</b>	18.42±0.396 <sup>e</sup>	2.5±0.291 <sup>e</sup>	84.48±0.349 <sup>e</sup>
<b>Diabetic</b>	41.32 ±0.605 <sup>a</sup>	8.12±1.389 <sup>a</sup>	181.82±1.304 <sup>a</sup>
<b>Diabetic supplemented with pumpkin fruit powder</b>	22.42±1.084 <sup>d</sup>	3.21±1.51 <sup>d</sup>	101.62±1.087 <sup>d</sup>
<b>Diabetic supplemented with pumpkin seed powder</b>	32.28±1.279 <sup>b</sup>	5.78±1.92 <sup>b</sup>	138.6±0.65 <sup>b</sup>
<b>Diabetic supplemented with pumpkin fruit and seed mixture powder</b>	27.78±0.881 <sup>c</sup>	4.48±0.283 <sup>c</sup>	120.6±0.809 <sup>c</sup>

- Values are expressed as means ±S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts letter in the same column. (p≤0.05).

Table (4) displayed that the levels of nitric oxide, MDA and AOPPs significantly increased in diabetic rats as compared to healthy control rats. While treatment with pumpkin powders caused a significant reduction in oxidative stress markers NO, MDA and AOPPs values.

### 3.5. Effect of pumpkin fruit and seed powders on some antioxidants

**Table (5):Effect of pumpkin fruit and seed powders on some antioxidants biomarkers**

<b>Parameter Group</b>	<b>Reduced glutathione (mg/dL)</b>	<b>Superoxide dismutase (U/mL)</b>	<b>Catalase (U/L)</b>
<b>Healthy</b>	80.82±0.319 <sup>a</sup>	310.72±0.84 <sup>a</sup>	97.88±0.975 <sup>a</sup>
<b>Diabetic</b>	50.30 ±0.886 <sup>c</sup>	197.86±0.578 <sup>c</sup>	51.76±0.626 <sup>c</sup>
<b>Diabetic supplemented with pumpkin fruit powder</b>	74.09±610 <sup>b</sup>	298.52±0.623 <sup>b</sup>	84.12±1.23 <sup>b</sup>
<b>Diabetic supplemented</b>	58.38±0.890 <sup>d</sup>	238.82±1.63 <sup>d</sup>	63.10±1.106 <sup>d</sup>

<b>with pumpkin seed powder</b>			
<b>Diabetic supplemented with pumpkin fruit and seed mixture powder</b>	66.90±0.483 <sup>c</sup>	262.26±1.39 <sup>c</sup>	78.14±0.618 <sup>c</sup>

- Values are expressed as means ±S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts letter in the same column. ( $p \leq 0.05$ ).

Table (5) illustrated that diabetic rats showed a significant decrement in GSH content, SOD and Catalase activities as compared to healthy control rats, while treatment with pumpkin powders caused a significant increase in antioxidants like GSH content, SOD and catalase activities.

### 3.6. Impact of pumpkin fruit and seed powders on the number and size of langerhans islets

**Table (6): Impact of pumpkin fruit and seed powders on the number and size of langerhans islets**

<b>parameter Group</b>	<b>No of islets</b>	<b>Size (micron)</b>
<b>Healthy</b>	7.3±0.81 <sup>a</sup>	1.45±0.32 <sup>a</sup>
<b>Diabetic</b>	2.9±0.12 <sup>e</sup>	0.67±0.09 <sup>e</sup>
<b>Diabetic supplemented with pumpkin fruit powder</b>	5.88±0.26 <sup>b</sup>	1.09±0.16 <sup>b</sup>
<b>Diabetic supplemented with pumpkin seed powder</b>	3.8±0.19 <sup>d</sup>	0.81±0.07 <sup>d</sup>
<b>Diabetic supplemented with pumpkin fruit and seed mixture powder</b>	4.2±0.35 <sup>c</sup>	0.96±0.05 <sup>c</sup>

- Values are expressed as means ±S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts letter in the same column. ( $p \leq 0.05$ ).



Table (6) cleared that the size and the number of langerhans islets cells decreased in diabetic rats as a result of oxidative stress and tissue degeneration caused by STZ while supplementation with pumpkin powders caused significant improvement that may be because active constituents like polyphenols, proteins , fat and polysaccharides that prevent oxidative stress and promote tissues building.

#### 4. Discussion

pumpkin, pulp is a good supplement of protein, carbohydrate and fat with low anti nutrients. Our results go hand in hand with (**Karanja et al., 2013 and Adebayo et al., 2013**) who stated that the pumpkin seeds were well endowed in crude oil, protein, carbohydrates and crude fiber. Pumpkin seed oil typically is a highly unsaturated oil, with predominantly oleic and linoleic acids present. Pumpkin fruit contains biologically active compounds like polysaccharides, fixed oils, proteins and peptides (**Dar et al., 2017**). Our results were in agreement with (**Zdunić et al., 2016**) who stated that pumpkin fruit and seeds are rich in polyphenolic compounds.

Streptozotocin (STZ), an analogue (2-deoxy-2-(3-methyl-3-nitrosourea)-D-glucopyranose), is a strong diabetogenic agent and widely used for inducing diabetes in a variety of animals by the selective degeneration and necrosis of pancreatic cells. Streptozotocin selectively

destroys pancreatic insulin secreting  $\beta$ -cells causing diabetes close to type-2 diabetes of humans (**Sharma et al., 2013**). STZ has a structure like glucose (Glu) as well as N-acetyl glucosamine (GlcNAc). STZ is taken by pancreatic  $\beta$ -cells via the GLUT 2 transporter where it leads to  $\beta$ -cell death by DNA fragmentation due to the nitroso-urea moiety. (**Ventura-Sobrevilla et al., 2011**).

These results are linked with that of the glucose and insulin levels as previously illustrated. It may be explained according to the high ability of glucose in case of hyperglycemia to bind with Hb forming glycated Hb. (**Ventura-Sobrevilla et al., 2011**)

The possible mechanism by which pumpkin powders bring about their antidiabetic action may be by potentiating the insulin effect of plasma by stimulating insulin release from the remnant pancreatic  $\beta$ -cells or its release from the bound form. Also, it might involve an extra-pancreatic action in these diabetic rats, which might include the stimulation of

peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis. The antihyperglycemic activity of pumpkin powders may also be due to the presence of polysaccharides, fiber, proteins, healthy fat as well as polyphenols.

Our results are similar to *Sharma et al.(2013)* who stated that oral administration of pumpkin seed extracts produces significant antidiabetic effect in controlling the blood glucose level and have a protective role on complications associated with diabetes. which may be due to presence of flavanoids, polyphenols and saponins.

Current evidence has demonstrated that oxidative stress plays an important role in the pathogenesis of chronic diseases such as DM and may diminish the antioxidative defense system of the body, increasing the oxidative load (*Ceriello et al.,2016*). Oxidative stress is increased in diabetes because of multiple factors. Among these factors, glucose autooxidation which is a dominant factor leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances

and reduction in antioxidant defenses including decreased cellular antioxidant levels like GSH content and a reduction in the activity of antioxidant enzymes as, CAT and SOD activities that dispose of free radicals. Increase in the levels of oxygen and nitrogen free radicals is closely related to lipid peroxidation, non-enzymatic glycation of proteins and oxidation of glucose which contributes toward diabetes mellitus. (*Jebur et al.,2016*).

Plant polyphenols are among the most abundant phytochemicals present in the human diet. Supplementation with certain phytochemicals may be effective in improving human glucose disorder. Therefore, antioxidant vitamins and phytonutrients could be used as a potential natural therapy to reduce oxidative stress and alleviates diabetic complications (*Jebur et al.,2016 and Singh et al., 2015*).

Pumpkin is one of the well-known edible plants and has substantial medicinal properties due to the presence of unique natural edible substances. It contains several phyto-constituents belonging to the categories of alkaloids, flavonoids, and palmitic, oleic as well as linoleic acids. (*Dar et al., 2017*).

Streptozotocin makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in about 2-4 days. Induction of diabetes with Streptozotocin decreases Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes. ( *Ramakrishnan et al., 2017* )

*Aboulthana et al., (2018)* reported that STZ caused inflammatory changes in pancreatic islets of diabetic rats. Consequently, this leads to atrophy in islands of the Langerhans cells associated with vacuolation of islet cells. This might be due to the destruction of  $\beta$ -cells and hence decrease in a number of the pancreatic islets.

An effective therapeutic strategy is in demand to prevent or delay the progression of pancreatic  $\beta$ - cells dysfunction or death .  $\beta$ -cell is particularly sensitive to damage by free radicals because of their low level of free radical scavenging enzymes that leads to hyperglycemic condition. Oral hypoglycemic agents and insulin, currently used have serious side effects, so there is a need to find

another more safe anti diabetic traditional medicine (*Hossen et al., 2017*).

Numerous studies have shown that administration of antioxidants to diabetic rats significantly increases the number of  $\beta$  cells (*Asgary et al., 2008*). Therefore, the pancreas protective effect of pumpkin and its hypoglycemic properties should be attributable, in part, to antioxidant activity of this fruit.

*Sedigheh et al., (2011)* stated that histological analysis showed significant difference in the mean diameter and the number of Langerhans islets between the diabetic control group and the normal control group. The mean diameter of islets in the diabetic group was significantly decreased as compared to that of the normal control group, which was consistent with other studies (*Asgary et al., 2008 and Mohajeri et al., 2009*). It is evident from the results that the number and mean diameter of pancreatic islets increased in the group fed with pumpkin as compared to the diabetic group which illustrates the effects of pumpkin powder on repair and restoration of pancreatic tissue. Antioxidant compounds also increase the number of  $\beta$  pancreatic cells by enhancing the

repair and restoration of these cells.

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