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Studying the Anti-Diabetic Effect of Gamma-Irradiated Pumpkin Seeds

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ABSTRACT

Natural antioxidants from plant materials have been consumed to replace synthetic ones as supportive therapy in the treatment of diabetes. This research was aimed to explore the influence of gamma (γ)-irradiation on the total phenolic compounds and total flavonoid contents of dried pumpkin seeds as well to determine the hypoglycemic influence of γ-irradiated pumpkin seeds dried powder (GPSDP) on diabetic rats. In this work, the level of total phenolic and total flavonoid contents of raw dried pumpkin seeds was significantly increase under the effect of γ-irradiation (10 kGy). The results showed that glucose and testicular malondialdehyde serum level and the activity of xanthine oxidase were elevated ($P < 0.05$) with obvious reduction in the concentration of insulin, total thyroid hormones triiodothyronin and thyroxine, leutinizing hormone, testosterone and testicular antioxidant parameters (glutathione content , the activity of xanthine dehydrogenase, superoxide dismutase and catalase) in the group of alloxan (150 mg/kg B.WT)-administrated rats as compared to control group. Treatment of diabetic rats with GPSDP (1 g/kg B.Wt/day/ 8 weeks) resulted in significant reduction of glucose level and lipid-peroxidation with amelioration of hormonal disturbance and enhancement of testicular antioxidant status with relative to alloxan-induced diabetic group. Therefore, this study concluded that γ-irradiation can be used as useful technique to improve the antioxidant activity of PSDP without loss in its quality characteristics. Also, the results suggested the effectiveness of GPSDP as a natural antioxidant that can reduce diabetic disorders.

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AMAA and AMM carried out the experiment with support from ANS. AMM and ANS wrote the manuscript with the support from AMAA.

Key words
Natural antioxidants, Diabetes, Gamma-irradiation, Pumpkin seeds, Hormonal disturbance

INTRODUCTION

Diabetes mellitus is the most popular serious metabolic disturbance that is characterized by absolute or relative lack in insulin secretion and/or insulin action associated with chronic hyperglycemia and disorders of carbohydrate, lipid, and protein metabolism (Salah *et al*., 2017). Permanence of high glucose level resulted in metabolic derangements and encourages body damage, including retinopathy, nephropathy, cardiovascular complications and sexual dysfunction (Oliveira *et al*., 2017). Furthermore, oxidative stress originates due to hyperglycemia where the production of reactive oxygen species (ROS) and free radicals (FR) exceeds the defense capacity of the body causing impairing of antioxidant enzymes and disrupting the cellular reduction–oxidation balance (Oliveira *et al*., 2017). Testicular function is also affected by diabetes as a consequence of the lack of insulin and subsequently the impairment of its regulatory action on both leydig and sertoli cells (Zahkok *et al*., 2016). There has been a major concern in finding natural antioxidants from plant materials to substitute synthetic ones to use as remedies to up-regulate insulin deficiency or moderate hormonal imbalance and free-radical damage (Ataman and Osinubi, 2017).

Pumpkin (*Cucurbita pepo* L.), belongs to the family of Cucurbitaceae, is one such plant that has been frequently used as functional food or medicine (AbouSeif, 2014). Pumpkin seeds are rich natural source of fatty acids, phenolic compounds and also antioxidant vitamins (Shaban and Sahu, 2017). So far, pumpkin seeds have been implicated as a natural drug in the improving of fertility and help in preventing arteriosclerosis, high blood pressure and heart diseases (Shaban and Sahu, 2017). Also, pumpkin seeds could be used as anti-oxidant, lipid-lowering, hepatoprotective, anti-carcinogenic, anti-microbial and anti-diabetic agent (Perez-Gutierrez, 2016).

Gamma irradiation has been used as a phytosanitary treatment to improve the hygienic quality of foods and herbal materials by reducing microbial contaminants and disinfestations of insects. Several studies reported that γ-irradiation can be used for enhancing biological activities, increasing yields and improving the color and antioxidant activity of natural products (Ghadi *et al*., 2015; Mounir *et al*., 2019). However, some studies have shown that γ- irradiation reduce the antioxidant properties in plant

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materials (Khalaf *et al.*, 2014).

The present work was undertaken to examine the impact of γ-rays on the total phenolic compounds and total flavonoid contents of dried pumpkin seeds. As well, determine the hypoglycemic influence of irradiated pumpkin seeds dried powder (GPSDP) on diabetic rats.

MATERIALS AND METHODS

Chemicals and reagents were purchased from alphabetic character Chemical Co. (St. Louis, MO, USA).

Plant material

Pumpkin seeds were purchased from native market (Cairo, Egypt). The outer core of the pumpkin seed was removed manually. They were dried in air then in an oven at 50°C till completely dried. Finally, the dried seeds were crushed into powder form:

Gamma irradiation treatment

The powder of dried pumpkin seeds was packed in plastic bags and irradiated with gamma rays at dose level (10 kGy), using Indian Gamma Cell (Ge 4000 A) 60Co source at a rate of 1.667 kGy/h at the National Centre for Radiation analysis and Technology (NCRRT), Egypt.

Determination of total phenolic compounds

The amounts of phenolic compounds of raw and gamma-irradiated pumpkin seeds were determined with Folin-Ciocalteu chemical agent using the tactic of (Spanos and Wrolstad, 1990). 2.5 ml of 100% Folin-Ciocalteureagent and 2 ml of Na2CO3 (2% w/v) was intercalary to resulting.5 ml of every sample of plant extract answer (1 mg/ml). The resulting mixture was incubated at 45°C with shaking for fifteen min. The absorbance of the samples was measured at 765 nm using UV/visible light. Results were expressed as milligrams of gallic acid (mg GAE) dissolved in distilled water. The experiment was repeated in triplicate.

Estimation of total flavonoids

Aluminum chloride technique method was used for determination of flavonoid contents of raw and γ-irradiated pumpkin seeds according to Aiyegoro and Okoh (2010). One milliliter (1 ml) of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with UV visible spectrophotometer. The content was determined from extrapolation of calibration curve which was made by preparing quercetin (QC) solution in distilled water. The concentration of flavonoids was expressed in terms of QC mg/100g DW. The experiment was repeated in triplicate.

Animals

Male albino rats (170-220g body weight (B.WT)) were purchased from the Egyptian company for Biological product and Vaccines (Cairo, Egypt) and used for the many investigations allotted during this study. Rodents were acclimated to controlled laboratory conditions for 2 weeks. Rats were preserved on stock rodent diet and water that were allowed *ad libitum*.

Induction of diabetes

Male albino rats were made diabetic by injecting them with alloxan hydrate dissolved in saline intraperitoneally with medication dose of one hundred fifty mg/kg B.WT (Pari and Venkateswaran, 2002). Alloxan could induce fatal hypoglycemia because of the massive pancreatic insulin release; consequently, rats were treated with 30% glucose solution orally at different time intervals after 6 h alloxan induction, and 5% glucose solution was kept in bottles in their cages for the next 24 h. After one week, blood was extracted from the tail vein for glucose analysis by the method of Trinder (1969). Tested animals exhibited fasting glucose levels within the range of 200 to 250 mg/dl.

Grouping of animals

Animals were randomly divided into four groups, each of seven rats: Group C has rats fed on normal diet and served as control. Group D was diabetic group. Group Diab. and RPSDP has diabetic rats treated with raw pumpkin seeds dried powder (RPSDP) (1 g/kg B. Wt/day) (Sedigheh *et al*., 2011) for eight weeks. Group Diab. and GPSDP has diabetic rats treated with γ-irradiated pumpkin seeds (GPSDP) (1 g/kg B. Wt/day) (Sedigheh *et al*., 2011) for eight weeks.

At the end of the experiment, animals from every group were sacrificed 24 h post the last dose of treatment. Blood samples were taken by cardiac puncture once slight anathesation of rodents using diethyl ether and ready for biochemical analysis.

Blood serum analysis

Serum samples were analyzed for glucose (Trinder, 1969) and insulin hormone determined by radioimmunoassay kit provided by Diasari, Italy. Total thyroid hormones triiodothyronin (T_3) and tetra iodothyronine (T_4) levels were measured by in-vitro nuclear diagnostic radioimmunoassay technique as represented by Burtis *et al*. (1994). Analysis of testosterone hormone was performed based on the method of Wilson and Foster (1992) and leutinizing hormone (LH) according to Garrett (1989). Testes homogenates were obtained employing a tissue homogenizer. The homogenates (1:10 w/v) were ready using a employing a buffer (pH 7) containing EDTA 0.3 mM. All homogenates were centrifuged at 20× g for 20 minutes at 4°C, and also the supernatants were used to estimate the amount of malondialdehyde (MDA) (Yoshioka *et al*., 1979), the activity of xanthine oxidase (XO) and xanthine dehydrogenase (XDH) (Kaminski and Jewezska, 1979), glutathione content (GSH) (Gross *et al*., 1967) and the activity of superoxide dismutase (SOD) (Minami and Yoshikawa, 1979) and catalase (CAT) (Aebi, 1984).

Statistical analysis

Results were subjected to one-way analysis of variance and Duncan's multiple range tests by computer program SPSS (SPSS, 1998) and represented as mean ± SE (n = 7) (P <0.05).

RESULTS

The data in Table I indicated that γ-rays processing considerably increase the total phenolic and total flavonoid contents of raw pumpkin seeds by percent change 4.2% and 6.5%, severally.

Diabetic rats showed a significant increase in the level of serum glucose with significant reduction in insulin concentration compared to control rats. Whereas, treatment of diabetic rats with RPSDP or GPSDP considerably reduced the glucose level and increased the level of insulin relative to alloxan-induced diabetic rats (Table II).

Table I. Total phenols and total flavonoid contents of raw and γ-irradiated pumpkin seeds.

Parameters	Chard leaves		% Change
	Raw	Irradiated	
Total phenolic content (mg GAE/100g DW±S.D)	33.15 ± 0.42	34.61± 0.46	4.2%
Total flavonoids (QCmg/100g DW±S.D)	19.62± 0.36	20.90± 0.39	6.5%

Values are means of three replicates (± SD).

As a result of alloxan injection a significant reduction was induced in the level of serum total thyroid hormones triiodothyronin (T3) and thyroid hormone (T4), LH and testosterone (T) compared to control rats. Administration of RPSDP or GPSDP to diabetic rats resulted in significant elevation in the level of these hormones (Table II).

The results indicate that alloxan administration provoked a significant rise in MDA level and also the activity of testicular XO accompanied by a significant decrease in XDH, SOD and CAT activities and GSH content in testes tissues relative to the corresponding values of control rats. On the other wise, a remarkable reduction in MDA level and the activity of XO and significant elevations in XDH, SOD and CAT activities and GSH content were observed in the testes of diabetic rats treated with either RPSDP or GPSDP relative to the diabetic group (Table II).

Table II. Effect of treatment with RPSDP or GPSDP on the serum level of glucose, insulin, total thyroid hormones, testosterone, LHand oxidants and antioxidant status in tests of alloxan-induced diabetic rats.

Parameters	C	Diab.	Diab. and RPSDP	Diab. and GPSDP
Glucose (mg/dl)	117.94±3.57^{c}	276.25±5.21^{a}	173.65±4.81^{b}	165.35±4.37^{b}
Insulin (µU/ml)	31.82 ±2.93^{a}	19.55±2.38^{c}	25.45±2.27^{b}	26.35±2.72^{b}
T_3 (ng/dl)	93.56±2.05^{a}	49.96±1.68^{c}	74.06±1.92^{b}	77.65±1.77^{b}
T_4 (µg/dl)	7.80 ±0.46^{a}	5.34 ±0.38^{c}	6.63±0.43^{b}	6.92 ±0.51^{b}
LH (mIu/ml)	1.33±0.25^{a}	0.61±0.12^{c}	1.11±0.16^{b}	1.17±0.18^{b}
Testosterone (ng/dl)	216.72±5.32^{a}	144.52±4.68^{c}	183.76±3.66^{b}	186.64±3.50^{b}
MDA (n mol/ml)	197.27±3.72^{c}	393.28±4.53^{a}	251.78±4.15^{b}	247.29±4.37^{b}
XO (mU/mgprotein)	2.52±0.08^{c}	3.90±0.11^{a}	2.96±0.08^{b}	2.92±0.09^{b}
XDH (mU/mg protein)	3.25±0.22^{a}	1.45±0.14^{c}	2.76± 0.17^{b}	2.84± 0.15^{b}
GSH (mg/g tissue)	28.28±0.96^{a}	16.93± 0.75^{c}	24.97±0.73^{b}	25.26±0.68^{b}
SOD (U/mg protein)	44.86±1.23^{a}	28.96±1.11^{c}	38.92±1.20^{b}	39.58±1.17^{b}
CAT (U/g protein)	3.58±0.04^{a}	1.54± 0.03^{c}	2.78±0.04^{b}	2.85± 0.04^{b}

C, control; Diab., diabetic; RPSDP, raw pumpkin seeds dried powder; GPSDP, γ-irradiated pumpkin seeds. Means in the same row with different superscripts are significantly different at (P<0.05); Values are expressed as mean ± S.E. (n=7).

DISCUSSION

The data of this work obtained that pumpkin seeds possess high amounts of total phenolic compound and flavonoid contents in agreement with the results of Farid *et al*. (2015). Also, the results discovered that γ-irradiation of raw pumpkin seeds induced significant increase in the amounts of total phenolic compound and flavonoid contents suggesting that γ-irradiation has positive role on the inhibitor activity and quality of pumpkin seeds. Variyar *et al*. (1998) reported that the increase in phenolic compound contents as a result of the irradiation treatment was related to the degradation of tannins and changes within the conformation of the molecules. Moreover, the result of γ- irradiation on the total phenolic compound contents is also because of increasing the activity of essential amino acid ammonia-lyase that is responsible for the synthesis of polyphenolic acids (Oufedjikh *et al*., 2000). Harrison and Were (2007) suggested that the rise in total phenols and total flavonoids is because of the release of phenolic compound bounded with glycosidic elements and also the breakdown of chemical bonds in larger phenolic compounds to produce smaller ones when exposed to γ- rays.

Results of this study indicated that alloxan administration induced significant elevation in the level of glucose and reduced the insulin concentration. Sarfraz *et al*. (2017) discovered that alloxan causes time and concentration dependent degradation lesions of the pancreatic beta cells leading to hyperglycemia. Also, Salah *et al*. (2017) reported that alloxan acts as diabetogenic agent due to its destructive effect of pancreatic ß-cells, inflicting a vast reduction in insulin release which leads to an increase in blood glucose. On the other hand, treatment of diabetic rats by either RPSDP or GPSDP resulted in vital reduction in glucose level and elevation of insulin concentration relative to alloxan-induced diabetic rats. These results are in line with Jin *et al*. (2013) who proposed that pumpkin polysaccharides might play a crucial role to restore the islets of Langerhans, repairing impaired islets or act as an insulin sensitizer to boost insulin action by rising the insulin sensitivity of target tissues like the liver, muscle and adipose tissue. Also, GPSDP may has exerted its anti-diabetic effect by rising the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin, inhibition of hepatic glucose production inhibition of intestinal glucose absorption, or correction of internal secretion resistance (Farid *et al*., 2015).

It was found that serum levels of T_3, T_4, LH and testosterone were significantly lower in diabetic rats than those in controls. The results are consistent with the results of Baydas *et al*. (2002) who showed a decrease in serum T3 and T4 and an increase in serum glucose level in diabetic rats. Sluszkiewicz (1986) reported that impairments of glucose utilization in diabetes mellitus cause inhibition in the activities of peripheric 5′-deiodinase enzyme, leading to a decrease in peripheral deiodination of T_4 to T_3. Furthermore, reduction of thyroid hormones could be due to reduced iodide pump activity resulting in the suppression of tyrosine iodination or owing to the decrease in the synthesis of thyroxine-binding globulin, the major serum thyroid hormone-binding protein (Concannon *et al*., 1999). The significant harmful effects of alloxan on the serum levels of sex hormones (LH and testosterone) may be due to suppression of Leydig cell activity by alloxan, accumulation of lipids in Leydig cells (Murray *et al*., 1983) or diminution of their number (Ballester *et al*., 2004). Also, the reduction in insulin levels led to decline in serum testosterone levels in diabetic rats because insulin augments testicular androgen production by inhibiting sex hormone binding globulin (SHBG) concentration (Saha *et al*., 2015). Administration of RPSDP or GPSDP to diabetic rats caused an obvious increase in T_3, T_4, LH and testosterone hormones relative to diabetic rats. Pumpkin seed is natural supplement rich in phenolic compounds and antioxidant activity which is combined with alpha-glucosidase and antigiotensin converting enzyme inhibitory activities and has the potential to reduce hyperglycemia-induced pathogenesis and also complication linked to diabetes (Kwon *et al*., 2007). The action mechanism of pumpkin seed is well known by its inhibition on 5-α-reductase which converts testosterone into dihedrontestosterone (Tsai *et al*., 2006). Also, the thyroid hormones responded to antioxidants content of pumpkin seeds indicating the significance of antioxidants for the prevention of toxic effect of alloxan in thyroid gland by protecting biological system against potentially harmful influence of free radicals that can cause oxidative stress.

It was observed that oxidative damage by alloxan administration are exhibited by a significant elevation in MDA level and the activity of testicular XO with significant diminish in XDH, SOD and CAT activities and GSH content in testes tissues compared to control rats. Alloxan induces oxidative stress in the testis by increasing the formation of free radicals and suppress endogenous antioxidant enzymes, subsequently leading to oxidative stress causes mitochondrial injury and lipid peroxidation in both, germ and Leydig cells and, subsequently, a defect in sperm production (Aybek *et al*., 2008). Hypoinsulinemia induced by alloxan stimulates the activity of fatty acyl coenzymes A oxidase, which oxidizes fatty acids and therefore results in lipid peroxidation and leads to decrease membrane fluidity and disturbs the interaction between the membrane-bound enzymes and receptors, ultimately resulting in cell damage (Saravanan and Pari, 2005). The reduction of GSH probably due to the higher levels of superoxides and free radicals, GSH converts more to oxidized glutathione (GSSG) or higher rate of conversion of GSH to hydrogen-sulfide (H_2S) liver during diabetes process (Derouiche *et al*., 2017). The results indicate that RPSDP and GPSDP exhibited antioxidant effect against alloxan evidenced by a remarkable reduction in MDA level and the activity of testicular XO with significant elevations in XDH, SOD and CAT activities and GSH content. The antioxidant and antiradical effects of pumpkin seeds could be attributed to its composition such as vitamin E and phenolic compound (Andjelkov *et al*., 2010) that can bind to free radicals and so inhibit the cell membrane oxidation and occurrence of lipid peroxidation. Also, pumpkin seeds are rich in Zinc that acts as an anti-oxidant by neutralizing free radical generation and by engrossing the iron or copper binding sites of lipids, proteins, and DNA (Amara *et al*., 2008).

CONCLUSION

In general, this study concluded that γ-radiation (10 kGy) can improve the quality of pumpkin seeds by significant increase of the total phenolic and total flavonoid contents of raw samples supporting that γ-irradiation can be used as a phytosanitary treatment for pumpkin seeds with enhancement of its antioxidant activity. Additionally, the results showed that the γ-irradiated pumpkin seeds afforded substantial treatment of hyperglycemic disorders by improving the endocrine disturbance induced by alloxan with enhancing the testicular antioxidant status and reducing the lipid-peroxidation.

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Statement of conflict of interest

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