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Evaluation of Anti-Diabetic Activity of Aqueous Extract of Gamma Irradiated Sesame Seeds

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ABSTRACT

This study was conducted for two purposes, the first is to study the effect of gamma irradiation at different doses (0.5, 1.0, 1.5, 2.0 kGy) on the fungal growth, total phenolics, antioxidant activity, oil contents and fatty acid composition of sesame seeds (*Sesamum indicum*). The second objective is to investigate the *in vitro* inhibitory effect of aqueous extract of gamma-irradiated sesame seeds (Irr. SSAE) on α-amylase and α- glucosidase and *in vivo* hypoglycaemic effect in alloxan-diabetic rats. The results observed that gamma-irradiation of sesame seeds at different doses induced significant increase in the total phenolic contents and antioxidant activity, significant decrease in the free fatty acids, fungal growth and colony formation and no significant change in the oil contents and fatty acid composition compared to raw seeds. Also, the inhibitory activity on α-amylase and α- glucosidase of Irr. SSAE (2 kGy) is significantly higher than that of RSSAE. The results revealed that treatment of alloxan-diabetic rats with Irr.SSAE (200 mg/ Kg B.WT/ 8 weeks) significantly reduced hyperglycaemia, the activities of liver enzymes, level of lipid profile contents, malondialdehyde (MDA) and level of inflammatory factors (TNF-α and IL-6) associated with noticeable attenuation of alloxan-induced hypoinsulinaemia and significantly increased level of HDL-C with an enhancement in hepatic total antioxidant capacity (TAC) and level of glutathione content (GSH) relative to the diabetic rats. In conclusion, gamma radiation could be an effective treatment for decontaminating and extending the shelf-life of sesame seeds without destroying its bioactive compounds. In addition, Irr.SSAE is a potent inhibitor of α-amylase and α- glucosidase and has beneficial effects in the treatment of diabetes.

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AAM and AMAA performed animal experiments. ANE-S and MHMAM performed the biological study and collected blood samples. The four authors wrote the manuscript.

Key words
***Sesamum indicum*, α-amylase, α-glucosidase, Gamma irradiation, Diabetes**

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia due to insulin deficiency or insulin resistance or both. DM is associated with metabolic, vascular, nephropathic, and neuropathic disorders. DM metabolic disorders are often diagnosed with hyperglycaemia and lipid abnormalities leading to vascular disorders (Ibrahiem, 2016). Alpha-amylase is the enzyme which is responsible for degradation of complex carbohydrates into disaccharides and oligosaccharides, which, in turn, are converted to monosaccharides by the action of alpha-glucosidase. The final product is absorbed by the intestines and causes an increase in post-prandial glucose levels. Inhibition of these enzymes will help control blood sugar levels by slowing down the breakdown of carbohydrates and its absorption (Amutha and Godavari, 2016). Several studies efforts have focused on the use of natural ingredients that are found in fruits, vegetables, cereal, grains, and legumes towards exploiting their antioxidant potentials and their anti-diabetic efficacy (Talabi *et al*., 2018).

Sesamum indicum Linn. (Sesame) belonging to the family Pedaliaceae is an important oilseed crop, having seed and oil that are highly valued as a traditional health food, and has many medicinal uses. The chemical composition of sesame seed varies with the variety, origin, colour, and size of the seed. Sesame oil is an important component of seeds, and its value varies due to the difference in seed type and the extraction method used.

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Sesame seeds have been reported to contain up to 63% oil with high health-promoting activity (Hassan et al., 2019). Sesame seed has been used to improve health conditions and prevent various illnesses for many decades (Li *et al*., 2020). The high level of mono- and polyunsaturated fatty acids, vitamin E, phytosterols, fiber, and other nutraceutical components as phenolic compounds, bioactive lignans, sesamin, episesamin, sesamol, and sesamolin make the sesame a unique healthy source (Aslam *et al*., 2020).

Sesame and other oleaginous seeds are good food sources, widely used in the pharmaceutical and cosmetics industry because of their perceived antigenicity, but they can be contaminated during the cultivation and storage with a wide variety of contaminants and microorganisms result in food deterioration (Rizki *et al*., 2019). There is a need for using simple preservation techniques to increase storage life of seeds to avoid contamination and achieving the food security. Gamma rays have been used as an excellent tool for sterilization and preservation of food and can safely and effectively eradicate pathogenic bacteria from food, disinfests seeds, extend the shelf life of many products. Also, gamma rays help maintain the quality of processed food compared to other techniques (Rizki *et al*., 2019).

Therefore, the present study was conducted to investigate the decontaminating effect of gamma irradiation on the fungal growth and the impact of this treatment on the total phenolics, and antioxidant activity of sesame seeds. Additionally, this study was aimed to investigate the in-vitro inhibitory effect of aqueous extract of gamma-irradiated sesame seeds on α- amylase and α- glucosidase and its in-vivo hypoglycaemic effect in alloxan-diabetic rats.

MATERIALS AND METHODS

All experiments were carried out during 2021 at the Egyptian Atomic Energy Authority, Food Irradiation Department.

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

Gamma irradiation of seeds and preparation of aqueous and organic solvent extract

Sesame seeds were purchased from local store of spices, grains, and oils (Cairo, Egypt). After cleaning the seeds were transferred into polyethylene bags and treated with gamma rays at the doses of 0.5, 1.0, 1.5, and 2.0 kGy, using Indian Gamma Cell (Ge 4000 A)^{60}Co source at a dose rate of 0.8053 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Either raw or gamma-irradiated sesame seeds were ground into fine powder by an electrical blender. The aqueous extracts of the of treated and untreated seeds were prepared by soaking 50 g of the ground samples in 500 mL of distilled water for 48 h according to (Ojo *et al*., 2013). The mixture was later filtered through Whatman filter paper, and the extract was kept for further analysis.

For extraction of oils about 150 g of either raw or gamma-irradiated crushed sesame seeds were placed in dark flasks (1L) and homogenized with 750 ml hexane (1:5, g: v). The mixture was maintained under agitation over night. The extracts were subjected to evaporation using a rotary evaporator at 40°C and then the obtained oil was analysed (Hassan, 2012).

Determination of free fatty acids (FFA) and fatty acid composition

The analysis of the FFA content in raw and gamma-irradiated sesame seeds oil was done according to AOAC official method (AOAC, 1995).

The fatty acid composition was determined by using gas chromatography after derivatization to fatty acid methyl esters with 0.5 M Na-methylate in methanol. Hewlett Packard Gas Chromatograph used in this analysis was equipped with a Series 11 5890 GC, electronic pressure control (EEP), capillary split/splitless injection system with a Focus Liner™ (Glass liner, 4 mm id), for optimum needle tip wiping and sample transfer and flame ionization detector (FID), and A HP7673 controller with an automatic liquid sample. The injector and detector temperature were set to 250 °C and 275°C. The initial oven temperature was 120 °C (hold 4 min), and final temperature was 230°C at a rate of 5°C /min with final time of 10 min. Fatty acids were expressed as a percentage of the total fatty acids. The identification and quantification of fatty acid methyl ester was calculated by comparing retention times of the peaks with those of fatty acid standards.

Fungal growth and colony formation

The fungal growth of the treated and raw seeds was measured following the agar method according to ISTA (2006). About 25 disinfested seeds were placed on PDA (Potato Dextrose Agar) in each petri dish. The plates were incubated at 20-25 °C for 5 days. The total number of infected seeds with the pathogen were counted and calculated into a percentage of infection using the following formula:

$$\text{Fungal growth (\%)} = \frac{\text{Number of seeds with fungal growth}}{\text{Total number of the seeds}} \times 100$$

The fungal colony formation unit per gram (CFU/g) of treated and untreated samples was determined according to standard methods (AOAC, 1995). One ml of selected dilution (10-3) of each sample was poured and platted (in triplicate) onto petri dishes containing sterile PDA and then

incubated at 25±2°C for 5 days. Colony formation was observed and identified according to spore morphology under a stereoscopic microscope, and the colonies on the reverse side of the petri-dishes, as well as the fruiting body, were observed under a compound microscope.

In-vitro *antioxidant determination*

For determination of the antioxidant activity of sesame extracts, the stable, 1 diphenyl-2-picryl hydrazyl (DPPH) radical was used (Sun and Ho,2005). The amount of total phenolic compounds was determined by using Folin-Ciocalteu reagent (Taga *et al*., 1984).

Enzymes assays

The α-amylase inhibitory activity was determined according to the protocol described by Shai *et al*. (2010). The α-glucosidase inhibitory activity was assessed in line with the protocol described by Ademiluyi and Oboh (2013), with little modifications.

Animals and induction of diabetes

Male rats (Sprague Dawley) (170 to 220g body weight (B.WT)) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were available *ad libitum*.

Male albino rats were made diabetic by injecting alloxan monohydrate intraperitoneally at 150mg/kg B.WT (Pari and Venkateswaran, 2002). Rats were treated with 30% glucose solution orally at different time intervals after 6 h of 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 (Trinder, 1969). Experimental animals exhibited fasting blood glucose levels in the range of 200 to 250 mg/dl.

Experimental plan

The animals were randomly divided into 4 groups, each of 7 rats: (i) Group C: rats fed on balanced diet and served as control, (ii) Group D: diabetic group, (iii) Group D + RSSAE (diabetic rats received raw sesame seeds aqueous extract orally at dose 200 mg/Kg B.WT/ day for 8 weeks (Prasad *et al*., 2018) and (iv) Group D + Irr. SSAE (diabetic rats treated with oral γ-irradiated sesame seeds aqueous extract at 200 mg/Kg B.WT for 8 weeks). At the end of the experiment, animals from each group were sacrificed 24 h after the last dose of treatment. Blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether and allowed to coagulate and centrifuged to get serum for biochemical analysis.

Biochemical analysis

Serum samples were analyzed for glucose (Trinder, 1969) and insulin hormone was determined by radioimmunoassay kit supplied by Diasari, Italy. Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain *et al*. (1974), Fossati and Prencipe (1982) and Demacker *et al*. (1980), respectively. Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C)were evaluated according to Friedwald *et al*. (1972) by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957) and serum γ-glutamyl transferase (GGT) was assessed according to Rosalki (1975). Detection of serum tumour necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) was performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions.

Hepatic tissues (100 mg tissue/ml buffer) were homogenized in 50 mM phosphate buffer (pH 7.2; St. Louis, MO, USA); the homogenates were then centrifuged at 1,200 x g for 15 min and the supernatant was used for determination of malondialdehyde (MDA) concentration (Yoshioka *et al*., 1979), total antioxidant capacity (TAC) (Mahfouz *et al*., 2009) and glutathione (GSH) content (Beutler *et al*., 1963).

Statistical analysis

Results were presented as mean±SE (n= 6). Experimental data were analysed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Data were statistically analysed by the aid of Statistical Package of the Social Sciences, SPSS version 25 (copyrighted by IBM SPSS software, USA). Differences between means were considered significant at $P < 0.05$.

RESULTS

Table I shows the percentage of fungal growth in raw sesame seeds is 98.7% and is significantly reduced to 76.0% at 0.5 kGy reaching the lowest value (9.3%) in the sample treated at 2 kGy. In addition, the impact of gamma radiation processing in reducing fungi counts was observed. There are too many colony forming units were noticed in raw sesame seeds so that the colony formation was impossible to count. However, the colony formation was significantly decreased as the irradiation dose increased, reaching its minimum at 2 kGy to 1.0×102 cfu/g.

Table I. Effect of gamma irradiation on fungal growth (%) and colony formation (cfu/g) in sesame seeds.

Radiation dose (kGy)	Fungal incidence	
	Fungal growth (%)	Colony formation (cfu/g)
0.0	$95.6^{a} \pm 2.220$	Uncountable
0.5	$72.5^{b} \pm 1.927$	$2.5 \times 10^{5\,a} \pm 1.033$
1.0	$18.5^{c} \pm 1.725$	$1.9 \times 10^{3\,b} \pm 0.976$
1.5	$12.7^{d} \pm 1.635$	$4.5 \times 10^{2\,c} \pm 1.050$
2.0	$8.8^{e} \pm 1.576$	$1.1 \times 10^{2\,d} \pm 1.035$

Values are represented as the mean ±SE of mean of triplicate experiments (n = 3). Values in the same column with different superscript are significantly different at P<0.05.

The results revealed that the total phenolic contents and antioxidant activity in sesame seeds were significantly increased as the radiation dose elevated. The highest phenolic content (75 mg GAE/g) and the highest antioxidant activity (47 %) was observed in the sesame seeds irradiated at 2 kGy (Table II). The data revealed that the oil contents of the raw sesame seeds was found to be 56.79% and gamma-irradiation has no effects on oil content of sesame seeds. In contrast, the free fatty acids (FFA) in raw seeds (3.90 mg/g) were significantly reduced under the effect of gamma-radiation. Gamma-irradiated sesame seeds at 0.5 kGy had significant higher FFA contents than those of irradiated seeds at 1.0, 1.5 and 2.0 kGy. Whereas there is no significant different in FFA contents of irradiated seeds at 1.0, 1.5 and 2.0 kGy.

Table III shows the main fatty acids identified in both irradiated and non-irradiated sesame seeds samples. The most abundant fatty acid in sesame seed oil was oleic acid while eicosenoic (C20:1) was the least. Also, the results showed there in no significant difference was observed between the main fatty acids identified in raw and irradiated sesame seeds.

Table II. Total phenolic compounds, antioxidant activity and Free fatty acids of raw and γ-irradiated sesame seeds.

Radiation dose (kGy)	Total phenolic (mg GAE/g)	Antioxidative activity (%)	Oil content %	Free fatty acids (mg/g)
0.0	$47^{e} \pm 0.14$	$32^{e} \pm 1.20$	56.79	$3.90^{a} \pm 0.09$
0.5	$52^{d} \pm 0.12$	$36^{d} \pm 1.26$	56.59	$2.50^{b} \pm 0.07$
1.0	$60^{c} \pm 0.15$	$39^{c} \pm 1.32$	56.63	$2.15^{c} \pm 0.07$
1.5	$66^{b} \pm 0.22$	$42^{b} \pm 1.15$	56.66	$2.11^{c} \pm 0.06$
2.0	$75^{a} \pm 0.25$	$47^{a} \pm 1.09$	56.69	$2.09^{c} \pm 0.08$

Values are represented as the mean ±SE of mean of triplicate experiments (n= 3). Values in the same column with different superscript are significantly different at P<0.05.

The effect of treating sesame seeds with gamma-radiation has revealed that the treatment of sesame seeds with 2.0 kGy had the most noticeable effect in terms of increasing the total phenolic contents, increasing the antioxidant activity, and decreasing the free fatty acids content. Therefore, gamma-irradiated sesame seeds at a dose of 2.0 kGy were used to conduct the biological experiment and study its antidiabetic effect.

Table III. Fatty acid composition (%) of sesame oil extracted from raw and γ-irradiated seeds.

Fatty acids	Radiation dose (kGy)				
	0.0	0.5	1.0	1.5	2.0
Palmitic (C 16-0)	12.70 ± 0.08	12.82 ± 0.06	12.75 ± 0.08	12.72± 0.07	12.68 ± 0.09
Palmitoleic (C16:1)	0.24 ± 0.03	0.25 ± 0.02	0.23 ± 0.01	0.24 ± 0.04	0.22 ± 0.02
Stearic (C18:0)	5.75 ± 0.05	5.80 ± 0.03	5.77 ± 0.04	5.74 ± 0.02	5.75 ± 0.05
Oleic (C18:1)	41.76 ± 0.58	41.74 ± 0.53	41.71 ± 0.48	41.68 ± 0.42	41.65 ± 0.41
Linoleic (C18:2)	38.31 ± 0.22	37.89 ± 0.18	37.80 ± 0.16	37.72 ± 0.14	37.68 ± 0.13
Linolenic (C18:3)	0.49 ± 0.03	0.52 ± 0.03	0.55 ± 0.02	0.57 ± 0.03	0.58 ± 0.04
Arachidic (C20:0)	0.57 ± 0.02	0.59 ± 0.02	0.63 ± 0.03	0.65 ± 0.04	0.67 ± 0.03
Eicosenoic (C20:1)	0.16 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.18 ± 0.03	0.19 ± 0.02
SAFA	19.02 ± 0.17	19.21 ± 0.05	18.97 ± 0.05	19.11 ± 0.05	19.10 ± 0.05
MUFA	42.16 ± 0.67	42.16 ± 0.35	42.11 ± 0.35	42.10 ± 0.35	42.06 ± 0.35
PUFA	38.80 ± 0.30	38.41 ± 0.14	38.35 ± 0.14	38.29 ± 0.14	38.26 ± 0.14

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values are represented as the mean ±SE of mean of triplicate experiments and are not significantly different ($P \geq 0.05$).

Table IV. Inhibitory activities of aqueous extracts from raw and and γ-irradiated sesame seeds against α-amylase and α-glucosidase.

		α-amylase inhibition (%)	α- glucosidase inhibition (%)
20 µg/mL	RSSAE	2.47	5.65
	Irr. SSAE	3.11	6.80
40 µg/mL	RSSAE	7.82	8.17
	Irr. SSAE	8.25	9.33
60 µg/mL	RSSAE	8.91	10.26
	Irr. SSAE	10.05	11.97
80 µg/mL	RSSAE	12.71	15.69
	Irr. SSAE	14.12	17.75
100 µg/mL	RSSAE	16.55	19.40
	Irr. SSAE	18.16	21.38

Values are represented as the mean ±SE of mean of triplicate experiments (n = 3). RSSAE: raw sesame seeds aqueous extract; Irr. SSAE: γ-irradiated sesame seeds aqueous extract

Table IV shows that there is a significant increase in the inhibitory activities of aqueous extracts of raw and gamma-irradiated sesame seeds against α-amylase and α-glucosidase at different concentrations ranging from 20 to 100 µg/mL. However, the inhibitory activity of Irr. SSAE is significantly higher than that of RSSAE. The inhibitory activity of RSSAE and Irr.SSAE against α-amylase and α-glucosidase at the low centration 20 µg/mL was 2.47 and 5.65%, respectively and 4.19 and 7.15%, respectively. The inhibitory activity concentration 100 µg/mL against α-amylase for both RSSAE and Irr. SSAE was16.55 and 18.21%, respectively and against α-glucosidase it was 19.40 and 22.16%, respectively.

As obtained in this study, induction of diabetes by using alloxan resulted in significant elevation in the serum level of glucose, TC, TG, LDL-C and vLDL-C, serum activities of AST, ALT and GGT, concentration of hepatic MDA and serum level of TNF-α and IL-6 with an obvious reduction in the serum level of insulin, level of HDL-C, and the level of hepatic a TAC and GSH content when compared to control group (Table V).

Table V. Effect of raw and γ-irradiated sesame seeds aqueous extract on serum glucose, insulin, lipid profile, liver enzymes activity, hepatic antioxidants status, MDA and TNF-α and IL-6 levels in alloxan-induced diabetic rats.

Parameters	C	D	D+ RSSAE	D + Irr. SSAE
Glucose(mg/dl)	125.23±7.2^{d}	270.23±8.5^{a}	165.34±6.9^{b}	142.38±5.8^{c}
Insulin(µU/ml)	33.26±3.19^{a}	18.68±3.61^{d}	27.37±2.39^{c}	29.89±2.47^{b}
Lipid profile				
TC (mg/dl)	164.15± 4.55^{d}	233.46±4.72^{a}	185.61±4.37^{b}	177.29±4.95^{c}
TG (mg/dl)	116.79±2.71^{d}	185.65±2.81^{a}	140.51±2.61^{b}	132.33±2.45^{c}
HDL-C (mg/dl)	49.38±1.62^{a}	38.63±1.46^{d}	42.71±1.39^{c}	45.55 ±1.75^{b}
LDL-C (mg/dl)	91.41±2.96^{d}	157.70±3.56^{a}	114.80±3.23^{b}	105.18±2.68^{c}
vLDL-C (mg/dl)	23.36±1.82^{d}	37.13±1.75^{a}	28.10 ±1.67^{b}	26.47±1.63^{c}
Liver enzymes				
AST (U/ml)	33.52±1.59^{d}	56.24±2.71^{a}	45.58±1.63^{b}	39.42±1.58^{c}
ALT (U/ml)	30.65±1.42^{d}	51.52±1.76^{a}	39.65±0.93^{b}	36.47±0.92^{c}
γGT (U/ml)	5.43±0.38^{d}	13.67±0.53^{a}	8.31±0.59^{b}	6.87±0.47^{c}
Hepatic antioxidants and MDA				
MDA (n mol/g tissue)	175.18±4.31^{d}	310.64±6.38^{a}	205.36±4.73^{b}	190.68±4.57^{c}
TAC (U/mg protein)	0.74±0.05^{a}	0.51±0.04^{d}	0.60±0.03^{c}	0.66±0.05^{b}
GSH (mg/g tissue)	28.63 ±2.39^{a}	18.53±1.48^{d}	23.92±0.87^{c}	25.71±0.85^{b}
Inflammatory factors				
TNF-α (pg/mL)	630.34 ± 26.31^{d}	917.23 ± 41.25^{a}	764.37 ± 31.72^{b}	705.43 ± 32.51^{c}
IL-6 (pg/mL)	326.31 ± 22.41^{d}	495.28± 26.97^{a}	410.35± 21.13^{b}	379.28± 23.16 ^{c}

Values are expressed as means ± S.E. (n=7). Values in the same row with different superscript are significantly different at P<0.05. D, diabetic; RSSAE, raw sesame seeds aqueous extract; Irr. SSAE, γ-irradiated sesame seeds aqueous extract.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GSH, glutathione; γGT, gamma glutamyl transferase; HDL-C, high-density lipoprotein; IL-6, interleukin 6; LDL-C, low-density lipoprotein; MDA, malondialdehyde; TC, total cholesterol; TG, triglycerides; TAC, total antioxidant capacity; TNF-α, tumour necrotic factor-alpha; vLDL-C, very-low-density lipoprotein.

However, treatment of diabetic rats with either RSSAE or Irr. SSAE resulted in significant reduction in serum glucose level, the activities of liver enzymes, lipid profile contents, lipid peroxidation (MDA) and level of inflammatory factors (TNF-α and IL-6) associated with noticeable elevation in the insulin level, level of HDL-C with an enhancement in hepatic TAC and level of GSH relative to the diabetic rats (Table V).

DISCUSSION

Gamma-irradiation food processing is considered is an effective technique for controlling food spoilage and protecting seeds from damaging by different types of microorganisms (Rizki *et al*., 2019). In this study, it has been observed that gamma-irradiation significantly decreased the fungi growth and colony formation in sesame seeds in a dose-dependent fashion when compared to raw samples. The decontamination of sesame seeds under the effect of different doses of gamma rays in this study could be effective in elongation of the shelf-life and preservation of the quality of sesame seeds during post-harvest processing (Hassan *et al*., 2019). McNamara *et al*. (2003) concluded that the inhibitory effect of gamma-irradiation processing on fungal growth and colony formation could be due to damaging of fungal membrane under the effect of gamma-rays that can lead the loss of nucleic acid proteins, as well as to osmatic balance, thereby causing death of fungal cells.

Treatment of sesame seeds in this work by different doses of gamma-rays resulted in significant increase in the total phenolic contents and the DPPH scavenging activity and the highest increase was observed at dose level 2 kGy. The same results were observed by Hassan *et al*. (2019) and Ghadi *et al*. (2015). The elevation of total phenolic contents by gamma-irradiation could be due to release of phenolic compounds from glycosidic components of the seeds and degradation of larger phenolic compounds into smaller ones, thereby increasing their solubility and extractability in the seeds (Hassan *et al*., 2019). Formation of more free phenolic compounds due to radiation-induced breakdown of glycosides might be resulted in an increase in antioxidant activity of sesame seeds (Variyar *et al*., 2004; Apaydin *et al*., 2017). The elevation of total phenolic contents and the antioxidant activity following gamma-irradiation processing could increase the effectiveness and health potentials of sesame seeds (Hassan *et al*., 2019).

The results indicated that sesame seeds can be considered as a good source of vegetable oil for domestic and industrial purposes as it had higher oil content (56.87%) than that of some common edible oils such as cotton seeds (22-24%), sunflower (30-35%), soybean (18-22%), rapeseed (40- 48%), pumpkin seeds (31.2-51.1%) and olive (12-50%) (Rizki *et al*., 2019). Also, the results revealed that gamma irradiation has no effects on oil content of sesame seeds, similar studies reported that gamma irradiation had no real effect on the moisture content of oilseeds (Rizki *et al*., 2019).

It can be observed from the results in this study that the FFA found in raw seeds is significantly higher than that of irradiated seeds which in accordance with the results that reported by Mahmoud *et al*. (2016) and Pankaj *et al*. (2013). The significant reduction in the FFA by gamma-irradiation at low doses (≤ 2.0 kGy) could be due to deactivation of lipases by gamma rays and preventing the release of more FFA into the irradiated oil (Hassan *et al*., 2019). Hassan *et al*. (2019) showed that the decrease in FFA contents of sesame seed oil as gamma radiation doses increase is desirable as the FFA is an indicator of rancidity which helps in the development of off-flavor and off-odor in the oil during storage. Therefore, from the results of this study it might be revealed that low gamma irradiation doses (≤ 2.0 kGy) can be considered as an effective technique for stabilizing and prolonging the storage life of sesame seed.

The results in this study revealed that there is no significant difference between the fatty acids composition of raw and gamma-irradiated seeds. Iqbal *et al*. (2016) concluded that gamma-irradiation processing (2-10 kGy) does not cause any significant change in the fatty acids composition of oil extracted from walnuts. From the results it can be observed that there are a lot of prominent fatty acids in both raw and non-irradiated sesame seeds such as arachidic acid, palmitic acid, stearic acid, linoleic acid, α-linoleic acid and oleic acid. The presence of high levels of oleic and linoleic acid in sesame seed oil is an indication that sesame seed oil is nutritious and can be an indicator of oil stability as oil stability depends on the level of oleic and linoleic acid present in the seeds (Hassan *et al*., 2019).

Pancreatic α-amylase is involved in the breakdown of starch into disaccharides and oligosaccharides before intestinal α-glucosidase causes the breakdown of disaccharides to release glucose later absorbed into the blood circulation. Inhibition of these enzymes can reduce the deterioration of starch in the intestinal tract, thereby reducing postprandial hyperglycemia (Kwon *et al*., 2007; Ojo *et al*., 2016). *In-vitro* results have shown that Irr. SSAE and RSSAE inhibit α-amylase and α-glucosidase activity in a concentration-dependent manner. However, the results indicated that Irr. SSAE (2 kGy) has inhibitory α-amylase and α-glucosidase activity higher than that of RSSAE. This suggests that gamma-irradiated Sesame indicum L. is more effective and can be considered a good treatment for diabetes complications. Talabi *et al*.

(2018) suggested that the antioxidant activity and radical scavenging potential of the *Sesame indicum* seeds could be beneficial in the inhibition of α-amylase and α-glucosidase and management of type 2 diabetes.

The results of biological study revealed that DM is induced in the group of rats injected by alloxan which evidenced by significant low level of insulin, level of HDL-C, and the level of hepatic a TAC and GSH associated with significant high level of glucose, level of TC, TG, LDL-C and vLDL-C, serum activities of AST, ALT and GGT, concentration of hepatic MDA and serum level of TNF-α and IL-6 when compared to control group. Alloxan-induced diabetes by producing hydrogen peroxide and other free radicals, including $O_2^{\bullet}$ and $^{\bullet}OH$ that causing oxidative damage and severe necrosis of pancreatic β-cells leading to reduction of insulin release following by induction of hyperglycemia (Lenzen and Munday, 1991). Sakurai and Tsuchiya (1988) reported that alloxan induced sustained hyperglycemia aggravates the oxidative stress status by auto-oxidation of glucose and its primary and secondary adducts. Over production of free radicals impaired antioxidant defenses and increase the level of TNF-α and IL-6 (Ceretta *et al*., 2012). The elevation in the activities of serum AST, ALT and GGT in diabetic rats indicates that alloxan might have been induced liver tissue damage and might have resulted to leakage of these enzymes from the cytosol of hepatic cells into the blood stream (Ohaeri, 2001).

However, treatment of diabetic rats with either Irr. SSAE or RSSAE resulted in significant hypoglycemic effect compared to non-treated diabetic group. The results showed that the anti-diabetic effect of Irr.SSAE is significantly greater than that of RSSAE and that is probably due to the potent effect of gamma-irradiation on increasing the antioxidants activity and increasing the total phenolic content in sesam seeds.

Significant reduction in serum glucose degree and insulin levels might be due to the presence of flavonoids, saponins, alkaloids, tannins and MUFA in sesame seeds (Akanya *et al*., 2015). Haidari *et al*. (2016) have suggested that multiple MUFA dietary supplements may control glycemic responses through suppression of β-cellular destruction and increased insulin sensitivity. Also, sesamin can improve glycogen production, inhibit blood glucose uptake, and may play a role in carbohydrate metabolism, as well as insulin signaling mechanisms by regulating genetic expression in type 2 diabetic rats (Bigoniya *et al*., 2012).

A significant decrease in the level of some lipid profile found within the diabetic groups received Irr.SSAE or RSSAE may be due to the presence of saponins that can form cholesterol-containing and bile acids complexes and prevent them from being absorbed through the small intestine hence lowers the cholesterol level in the blood and liver (Akanya *et al*., 2015). In addition, Irr.SSAE and RSSAE had been observed to maintain good cholesterol (HDL) and reduces bad cholesterol (LDL) which may be initiated by reducing insulin deficiency that might be linked to a elevate level of LDL receptor thereby reducing LDL particles resulting in the decrease in LDL-cholesterol levels (Shih *et al*., 1997).

Furthermore, sesamin has been known to protect the liver from oxidative damage by scavenging reactive oxygen species (ROS) and reduce the activity of liver enzymes (AST, ALT and GGT) and prevent the leakage of liver enzymes into blood stream (Akanya *et al*., 2015). Additionally, sesamin has the ability to decrease messenger RNA (mRNA) levels of pro-inflammatory cytokine IL-6 (Collins *et al*., 2008) .The antioxidant effects of Irr.SSAE or RSSAE could be due to presences of lignans such as the sesamol that can increase vitamin E level, increase TAC and GSH level, suppress the over production of ROS and thereby reduce MDA level (Sankar *et al*., 2006).

CONCLUSION

The results concluded that, gamma-irradiation caused a significant increase in the total phenolic composition and DPPH activity of sesame seed, while decreasing the fungal growth and colony formation in the seeds and free fatty acid formation in the oil associated with no significant effect on the oil content and individual fatty acids in the oil extracted from treated seeds. Also, this work has shown that Irr.SSAE exhibited inhibitory activity on key enzymes (α-amylase, α-glucosidase) and show potential as functional food in the management of diabetes mellitus and other related diseases.

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IRB approval and ethical statement

All animals procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH guidelines).

Statement of conflict of interest

The authors have declared no conflict of interest.

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