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The potential hypoglycemic effect of aqueous extract of gamma-irradiated olive leaves in alloxan-induced diabetic rats

M.H.M. Abd el Megid[1*], A.M. Abdul Azeem[2], A.N. El-Shahat[2], A.M. Mounir[2]

[1]Natural Products Department, [2]Food Irradiation Research Department, National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt

► ***Original article***

Corresponding author:
Dr. Mohamed H.M. Abd el Megid,
E-mail:
mohamed.h.m.abd.elmegid@gmail.com

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ABSTRACT

Background: The present study aimed to evaluate the effect of gamma-irradiation (5kGy) on total phenolic and total flavonoid content, antioxidant capacity, and concentration of phenolic compounds of olive leaves, and to assess the potential hypoglycemic effect of gamma-irradiated olive leaf extract (GOLE) in alloxan-induced diabetic rats. ***Results:*** The results indicated that gamma-irradiation caused a significant increase in the total phenol content, total flavonoids, and antioxidant activity in olive leaves compared to raw leaves. High-Performance Liquid Chromatography (HPLC) analysis showed that gamma-irradiation increased the values of phenolic compounds in olive leaves such as hydroxytyrosol, hydroxytyrosol-1-glucoside, rutinhydrate, and tyrosol. The results of the biological study showed that the co-administration of alloxan (150 mg/kg body weight) together with either raw (ROLE) or gamma-irradiated olive leaf extract (GOLE) (1ml/100 gm /body weight/ day/ 8 weeks) to rats caused hypoglycemia, hyperinsulinemia, decreased level of the homeostatic index of insulin resistance (HOMA-IR), α-amylase and α-glucosidase activity, and a significant elevation in hepatic glycogen storage and the activity of hepatic hexokinase, glucose-6-phosphate dehydrogenase, pyruvate kinase and glycogen synthase associated with a significant reduction in hepatic glucose 6-phosphatase, fructose 1,6-bisphosphatase, glycogen phosphorylase, the activity of liver marker enzymes and decreased level of serum creatinine, uric acid, and urea compared to the diabetic group. ***Conclusion:*** The results also showed that the anti-diabetic effect of GOLE was more significant than that observed with ROLE because of the effectiveness of gamma-irradiation (5 kGy) in improving the antioxidant activity and increasing the concentration of biological components of olive Leaf.

INTRODUCTION

Diabetes mellitus (DM) is the most widespread metabolic disorder in the world affecting about 2.8% of the population globally [1]. It is a prevalent metabolic disease that leads to neuropathy, cardiomyopathy, and nephropathy, among other consequences. It encompasses both type 1 (T1DM) and type 2 (T2DM) versions of the disease; T1DM is distinguished by insulin insufficiency, whereas T2DM is marked by insulin resistance (IR) [2]. Hyperglycemia, or a high level of glucose in the blood, is a result of both insulin insufficiency and insulin resistance [3]. Globally, diabetes is a rapidly growing medical condition, and by 2045, there will be 700 million diabetics [2]. The most prevalent kind of disease, known as T2DM, is characterized by IR and hyperglycemia associated with disruption of carbohydrate, lipid, and protein metabolism and have severe consequences, including long-term complications in many vital organs [4]. Biguanides and sulfonylureas are antidiabetic agents associated with severe adverse drug reactions such as weight gain, gastrointestinal disturbances, and liver and kidney damage. There is a growing interest in herbal therapy owing to its safety, cost-effectiveness, and convenient availability [1].

Olea europaea L., an evergreen tree belonging to the Oleaceae family and occupying a total area of 10.8 million hectares, has been cultivated in forty-one different countries, mainly in the countries of the Mediterranean Basin [5]. The olive oil industry generates large amounts of waste, especially during the agricultural phase, i.e., harvesting and oil production tasks. These by-products are mostly olive pulp, pits, effluent, and leaves that can hurt the environment when not processed properly. One of the by-products is olive leaves, which are produced by pruning and harvesting olive trees [6]. The olive leaf extracts are of special interest for their therapeutic effects and have different classes of bio-phenols including phenolic acids, phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside,

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luteolin 4-O-glucoside), and secoiridoids (oleuropein) [7]. These active constituents have antioxidative, antimicrobial, antiviral, anti-atherogenic, cardio-protective, antihypertensive, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities [6].

Olive leaves are highly susceptible to microbial contamination due to the medium (water and soil) in which they grow. Harvesting, storage, and processing may cause additional contamination and microbial growth that can affect the efficacy and stability of active compounds of the plants during storage [8]. Food irradiation is a processing method used to expose food to ionizing radiation to boost the food's safety and shelf life while also decontaminating it. Ionizing radiation provides a safe substitute for other methods of food decontamination since it is very successful at rendering microorganisms in a variety of vegetables inactive. Numerous studies have been conducted to investigate the effects of radiation on fresh fruit and vegetables to control pests and diseases, as well as delay the ripening process [9]. Gamma-irradiation is well-known as a decontamination method for reducing post-harvest food losses, ensuring hygienic quality, and facilitating a wider trade in foodstuffs [8].

In a way of using one of the modern food processing techniques to improve the hygienic quality and facilitate a wider trade for olive leaves, this study aimed to evaluate impact of gamma-irradiation on total phenolic and total flavonoid contents, antioxidant capacity, and concentration of phenolic compounds of olive leaves The study also aimed to assess the potential hypoglycemic effect of gamma-irradiated olive leaf extract in rats with alloxan-induced diabetes to provide a safe treatment for diabetics without side effects.

MATERIALS AND METHODS

All experiments were carried out in 2022 at the Egyptian Atomic Energy Authority's Food irradiation department. Olea europaea leaves were purchased from Siwa Oasis (Egypt). The leaves were cleaned and rinsed with sterile water and air dried. The Sigma Chemical Co. (St. Louis, MO, United States agency) supplied the chemicals and reagents.

Gamma irradiation treatment

Dried *Olea europaea* leaves powder was transferred into polyethylene bags and treated with gamma rays at the dose of 5 kGy, using Indian Gamma Cell (Ge 4000 A) 60Co source at a dose rate of 0.717 kGy/h (duration = 83 min) the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Determination of total phenolic and flavonoid contents, antioxidant capacity of raw and gamma-irradiated olive leaves

Total phenolic contents were measured by the folin-Ciocalteu method. Briefly, 1ml of each extract (in the concentration of 1mg/ml) was mixed with 5ml of folin-ciocalteu reagent (formerly diluted tenfold with distilled water) and stood at room temperature for 10min. Then 4 ml sodium bicarbonate solution (75g/l) was added. The mixture was allowed to stand for a further half-hour withinside the darkish at room temperature, and absorbance was measured at 765nm using a Uv/Vis spectrophotometer (SPECORD 210 plus). Total phenolic contents were quantified by a calibration curve obtained from measuring the absorbance of four known concentrations of gallic acid (GA) standard (25–50–70–100–200 µg/l). The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract [10].

The determination of total flavonoids was performed according to the colorimetric assay of Kim *et al*. [11]. Distilled water (4 ml) was added to 1 ml of raw and gamma-irradiated olive leaf extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes had been incubated at ambient temperature (25°C) for five min, and then 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the volume of the reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed, and the absorbance of the pink color developed was determined at 510 nm. Aqueous solutions of known Catechin concentrations in the range of 50 – 100 ppm were used for calibration and the results were expressed as mg catechin equivalents (CEQ)/ g sample.

The antioxidant capacity of samples was determined using 1,1–diphenyl-2- picrylhydrazyl (DPPH) by radical scavenging activity. DPPH was weighed as 0.024 g and dissolved in 100 mL methanol in a flask. 0.25 mL extract, 2.5 mL DPPH, and 2.5 mL methanol were added into a test tube and were kept in the dark for 1 h. For the control, methanol was used instead of extract. As described by Benavente et al., measurements were made using a UV-visible spectrophotometer (SPECORD 210 plus) at 517 nm [12]. The radical scavenging activity of the samples was calculated using the following formula.

Antioxidant activity % = [1- (Absorbance of sample / Absorbance of control)] × 100

HPLC analysis of the phenolic compound contents:

HPLC analysis was carried out according to Sati *et al.* [13] by using HPLC Hewllet Packared (series 1050) equipped with an auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280nm, and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards. The results were expressed in mg/g of water extract.

Preparation of olive leaf extracts

Either raw or gamma-irradiated olive leaf powder (5.5 gm) was soaked in boiled distilled water (100

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ml) and covered for ten minutes, then cooled to room temperature and filtered. The extract was given orally by using the stomach tube with a dose of (1ml/100 gm /body weight/ day) which is equivalent to the therapeutic human dose (500mg) [14].

Animals and biochemical Assay

Animals

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) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on a stock rodent diet and tap water that were allowed ad libitum. All animal procedures were carried out following the Research Ethics Committee for experimental studies (Human & Animal subjects) at the National Centre for Radiation Research and Technology (REC-NCRRT), Egyptian Atomic Energy Authority (Cairo, Egypt) (6A/23, 30-1-2023) and conformed to the CIOMS and ICLAS International Guiding Principles for Biomedical Involving Animals 2012.

Administration of alloxan

Male albino rats were made diabetic by injecting them with alloxan monohydrate dissolved in saline intraperitoneally with a dosage of 150mg/kg B. WT. [15]. Alloxan can induce fatal hypoglycemia because of massive pancreatic insulin release; therefore, 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 by the method of Trinder [16]. Experimental animals exhibited fasting blood glucose levels in the range of 200 to 250 mg/dl.

Grouping of animals

The animals were randomly divided into 4 groups, each consisting of 7 rats; Group C: rats fed on a balanced diet and served as control, Group D: Diabetic group, Group D + ROLE: diabetic rats received raw olive leaf powder aqueous extract (ROLE) orally at dose 1ml/100 gm /body weight/ day [14] for 8 weeks and Group D + GOLE: diabetic rats treated with oral γ-irradiated olive leaf powder aqueous extract (GOLE) (1ml/100 gm /body weight/ day) for 8 weeks.

At the end of the experiment, animals from each group were sacrificed 24 hrs. post 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 by the method of Trinder [16] and insulin hormone was determined by a radioimmunoassay kit supplied by Diasari, Italy. The homeostatic index of insulin resistance (HOMA-IR) was calculated using the equation: [fasting insulin concentration × fasting glucose concentration × 0.05551] /22.5 according to Wallace *et al*. [17]. α-Amylase activity was measured with an Alpha amylase assay kit by using the spectrophotometric method. An insoluble dye-coupled substrate amylose azure was cleaved by α-amylase into soluble colored products and the color intensity in the sample was measured at 595 nm. α-Glucosidase activity was measured with an Alpha glucosidase assay kit by using the spectrophotometric method as specified by the supplier. α-glucosidase reacts with 4-nitrophenyl α-D-glucopyranoside and a yellow complex is formed and was measured at 405 nm.

Hepatic hexokinase activity was measured by the method of Brandstrup *et al*. [18], glucose 6-phosphate dehydrogenase was measured by Ells and Kirkman [19], pyruvate kinase was measured by the method of Pogson and Denton [20], glucose 6-phosphatase was measured by the method of Koide and Oda [21], and fructose 1,6-bisphosphatase activity was measured by Gancedo and Gancedo [22]. The glycogen content in the liver and enzymes such as glycogen synthetase and glycogen phosphorylase were measured using previously described methods by Morales *et al*. [23], Leloir and Goldemberg [24], and Cornblath *et al*. [25], respectively. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel [26] and serum γ-glutamyl transferase (GGT) was assessed according to Rosalki [27]. Determination of creatinine, urea, and uric acid was assayed in serum by the methods of Murray [28], Fawcett and Scott [29], and Fossati *et al*. [30], respectively.

Statistical analysis

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

RESULT

The results revealed that the total phenolic and total flavonoid contents and antioxidant activity in olive leaves were significantly increased under the

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effect of gamma-irradiation (5kGy) treatment by percent changes of 31.74%, 15.02%, and 13%, respectively (table 1).

Table 1. Total phenolic and total flavonoid contents and antioxidant capacity of raw and gamma-irradiated olive leaves.

Radiation dose (kGy)	Total phenolic (mg GAE /g extract)	Total flavonoid contents (mg CEQ /g extract)	Antioxidative activity (%)
0.0	146.5±0.82	84.33±0.39	80.9 ± 0.7
5.0	193 ± 0.92	97± 0.73	91.42 ± 0.8

Values are means of three replicates (± SD), GAE: gallic acid equivalents, CEQ: catechin equivalents

The results of HPLC analysis in table 2 revealed that olive leaves have different types of phenolic compounds. Hydroxytyrosol, hydroxytyrosol-1-βglucoside, rutin hydrate, and tyrosol were identified as the most abundant phenolic compounds present in olive leaves. The values of these phenolic compounds were significantly increased by gamma-irradiation (5 kGy) by percent changes of 33.3%, 142.1%,23.1%, and 50%, respectively.

Table 2. Quantification of phenolic compounds in the raw and gamma-irradiated samples (mg/g water extract).

Compound	Raw	Gamma Irradiated (5 KGy)	% Change
Hydroxytyrosol-1-β-glucoside1	9.5 ± 0.7	23 ± 2.1	142.1
Hydroxytyrosol	24 ±1.7	36 ± 1.6	33.3
Oleuropein aglycon isomer	3.10 ± 0.3	8.81 ± 0.7	184.19
Tyrosol	6.1 ± 0.4	12.2 ± 0.8	50
Oleuropein aglycon isomer	1.55 ± 0.07	3.12± 0.06	101.29
Caffeic acid	0.59 ± 0.03	1.17± 0.01	98.3
β-Hydroxyverbascoside isomer	1.72 ± 0.06	3.4 ± 0.11	49.41
Vanillin	1.28 ± 0.03	2.41± 0.06	88.2
Syringic acid	0.89 ± 0.03	3.12± 0.17	250.5
Verbascoside	2.33 ± 0.04	3.6 ± 0.21	54.5
4-hydroxyphenyl acetic acid	1.64 ± 0.09	4.33 ± 0.17	164.02
Luteolin-7-O-rutinoside	3.16 ± 0.12	5.22 ± 0.32	65.18
Rutin hydrate	7.68± 0.14	9.46± 0.35	23.1

Administration of alloxan produced diabetes in all animals which was confirmed by a significant increase in serum glucose level, HOMA-IR, and activity of α-amylase and α-Glucosidase. In addition, alloxan-induced upregulation in the activity of hepatic glucose 6-phosphatase, fructose 1,6-bisphosphatase, and glycogen phosphorylase compared to control and other treated groups (tables 3-5).

Alloxan administration resulted in a reduction in the serum level of insulin, a decline in glycogen storage, and downregulation in the activity of hepatic hexokinase, glucose 6-phosphate dehydrogenase, pyruvate kinase, and glycogen synthase compared to control and other treated groups (tables 3-5).

Table 3. Effect of raw and γ-irradiated olive leaf aqueous extract on the serum level of glucose, insulin, homeostatic index of insulin resistance, and activity of α-amylase and α-glucosidase in alloxan-induced-diabetic rats.

Parameters	Control	Diabetic	Diab. + ROLE	Diab. + GOLE
Glucose(mg/dl)	87.65±4.37^{d}	294.36±6.32^{a}	139.27±4.25^{b}	120.45±3.42^{c}
Insulin(μU/ml)	17.62±1.36^{a}	5.61±0.67^{d}	11.29±0.57^{c}	13.31±1.12^{b}
HOMA-IR	3.81±0. 31^{c}	4.07±0.56^{a}	3.87±0.29^{c}	3.95±0.22^{b}
α-Amylase (U/L)	1259±89^{d}	1932±96^{a}	1389±92^{b}	1301±81^{c}
α-Glucosidase (mU/mL)	3158±120^{d}	3462±153^{a}	3364±104^{b}	3132±122^{c}

HOMA-IR, homeostatic index of insulin resistance, was calculated as [fasting insulin x fasting glucose concentration x 0.05551]/22.5. ROLE: raw olive leaf powder aqueous extract, GOLE: γ-irradiated olive leaf powder aqueous extract. Values are expressed as means ± S.E. (n = 7). Values in the same row with different superscripts are significantly different at P < 0.05.

Table 4. Effect of raw and γ-irradiated olive leaf aqueous extract on the activity of hepatic carbohydrate metabolic enzymes in alloxan-induced-diabetic rats.

Parameters	Control	Diabetic	Diab.+ ROLE	Diab.+ GOLE
Hexokinase (μmol of Pi/mg protein)	0.44 ± 0.03^{a}	0.16 ± 0.03^{d}	0.32 ± 0.02^{c}	0.36 ± 0.04^{b}
glucose 6-phosphate dehydrogenase (μmol of Pi/mg protein)	4.75 ± 0.06^{a}	2.11 ± 0.02^{d}	3.29 ± 0.04^{c}	3.86 ± 0.05^{b}
pyruvate kinase (μmol of Pi/mg protein)	178.55 ± 1.8^{a}	69.62 ± 1.14^{d}	123.16 ± 1.4^{c}	160.92 ± 1.2^{b}
glucose 6-phosphatase (μmol of Pi/mg protein)	0.19 ± 0.04^{d}	0.45 ± 0.03^{a}	0.33 ± 0.02^{b}	0.25 ± 0.04^{c}
fructose 1,6-bisphosphatase (μmol of Pi/mg protein)	0.40 ± 0.05^{d}	0.79 ± 0.07^{a}	0.56 ± 0.03^{b}	0.46 ± 0.04^{c}

ROLE: raw olive leaf powder aqueous extract, GOLE: γ-irradiated olive leaf powder aqueous extract. Values are expressed as means ± S.E. (n=7). Values in the same row with different superscripts are significantly different at P< 0.05.

Treatment of rats with alloxan along with either ROLE or GOLE induced significant hypoglycemia, hyperinsulinemia, and reduced HOMA-IR and the activity of α-amylase and α-glucosidase compared to the diabetic group. Also, administration of either ROLE or GOLE with alloxan reverses the action of alloxan on the activity of the carbohydrate metabolic enzymes evidenced by a significant elevation in the hepatic glycogen storage and the activity of hepatic hexokinase, glucose 6-phosphate dehydrogenase, pyruvate kinase and glycogen synthase associated with downregulation in the activity of hepatic glucose 6-phosphatase, fructose 1,6-bisphosphatase, and glycogen phosphorylase compared to diabetic-group.

Table 5. Effect of raw and γ-irradiated olive leaf aqueous extract on the level of hepatic glycogen and the activity of glycogen synthase and phosphorylase in alloxan-induced-diabetic rats.

Parameters	Control	Diabetic	Diab.+ ROLE	Diab.+ GOLE
Glycogen (mg/100 g tissue)	51.42± 3.4^{a}	21.82± 2.1^{d}	39.32± 3.2^{c}	46.22± 2.5^{b}
Glycogen synthase (mmol of UDP formed/h/mg protein)	758.76± 14.3^{a}	484.93± 8.4^{d}	594.29± 12.3^{c}	635.86 ±11.5^{b}
Glycogen phosphorylase (mmol Pi liberated/h/mg protein)	609.42± 14.1^{d}	965.19± 18.7^{a}	780.42± 11.2^{b}	732.16 ±10.6^{c}

ROLE: raw olive leaf powder aqueous extract, GOLE: γ-irradiated olive leaf powder aqueous extract. Values are expressed as means ± S.E. (n=7). Values in the same row with different superscripts are significantly different at P < 0.05.

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The activity of the liver marker enzymes (AST, ALT, and γGT) and the level of creatinine, uric acid, and urea were significantly elevated due to the injection of alloxan into rats in comparison with normal controls. Whereas treatment of rats with alloxan and either ROLE or GOLE led to significant improvement in the liver and kidney function of diabetic rats compared to treatment with alloxan alone (table 6).

Table 6. Effect of raw and γ-irradiated olive leaf aqueous extract on the activity of some hepatic enzymes and the level of urea, uric acid, and creatinine in alloxan-induced-diabetic rats.

Parameters	Control	Diabetic	Diab.+ ROLE	Diab.+ GOLE
AST (U/ml)	34.53±1.60 ^{d}	57.72±2.43 ^{a}	47.11±1.92 ^{b}	39.87±1.84 ^{c}
ALT (U/ml)	31.27±1.44 ^{d}	52.43±1.91 ^{a}	40.22±1.36 ^{b}	36.64±0.95 ^{c}
γGT (U/ml)	5.47±0.39 ^{d}	13.89±0.60 ^{a}	8.62±0.64 ^{b}	6.95±0.53 ^{c}
Urea (mg/dL)	40.9±1.7 ^{d}	85.2±3.4 ^{a}	58.6±2.9 ^{b}	47.9±1.18 ^{c}
Uric acid (mg/dL)	4.83±0.5 ^{d}	9.96±0.38 ^{a}	7.83±0.55 ^{b}	6.19±0.4 ^{c}
Creatinine (mg/dL)	0.7±0.01 ^{d}	3.7±0.11 ^{a}	1.32±0.14 ^{b}	1.10±0.22 ^{c}

DISCUSSION

Diabetes is a metabolic disorder that carries many complications if not managed properly and represents one of the major worldwide health problems [31]. Treating diabetes with medication is very expensive and the chances of side effects are high. Although olive leaves are environmentally harmful bio-substances from the olive oil industry, they contain significant amounts of bioactive compounds (Hydroxytyrosols and tyrosols, Secoridoid derivatives, phenolic acids, and flavonoids) that can be used in herbal medicine due to their antioxidants and medical activism [6]. Food processing by gamma irradiation is gaining importance as it provides longer storage periods without harming health [8].

The results of this study demonstrated that olive leaves under 5kGy of gamma-irradiation exhibited higher content of total phenolic and total flavonoid contents and antioxidant activity with values of 193 ±0.92 mg/g extract, 97± 0.73 mg chlorogenic acid /g extract and 91.42 ± 0.8 % compared to raw leaves (146.5 ± 0.82 mg/g extract, 84.33±0.39 rutin/g DW and 80.9 ± 0.7, respectively). Harrison and Were [32] suggested that the increment of total phenolic and total flavonoid contents under different levels of gamma-irradiation could be ascribed to the release of these compounds from glycosidic forms and the degradation of larger compounds into smaller ones by gamma-irradiation. The enhanced antioxidant capacity/activity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonialyase and peroxidase activity) or the increased extractability from the tissues as reported by the study of Althoman *et al.* [33]. El-Beltagi *et al.* [34] concluded that the elevation of flavonoid and phenolic at 5kGy in Sakouti and Bondoky dry date fruits, likely due to the enhancement of phenylalanine ammonialyase (PAL), tannin degradation, the release of compounds from its glycosidic form, and degradation of polymeric phenolic compounds into low molecular weight phenolic compounds. Khawory *et al.* [35] also demonstrated that gamma-irradiation of 3–13 kGy elevated the total phenolic content and antioxidant capacity of Gnetum gnemon and Khaya senegalensis leaves. The phenolic profile of olive leaves obtained by HPLC analysis revealed that hydroxytyrosol, hydroxytyrosol-1-βglucoside, rutin hydrate, and tyrosol were identified as the most abundant phenolic compounds present in olive leaves in agreement with the study of Madureira *et al.* [8]. The values of phenolic compounds in olive leaves were significantly increased after gamma radiation (5 kGy) compared to raw samples. The study of Madureira *et al.* [8] indicated that irradiation at 5 kGy increased the extractability of bioactive compounds from olive pomace by 2.4-fold compared to the non-irradiated ones. Also, Pereira *et al.* [36] demonstrated that the increase in phenolic concentrations on irradiated samples could be related to the release of these compounds from matrix structures, increasing their extractability.

In the present work, alloxan has been used to induce diabetic rats and either ROLE or GOLE to ameliorate the diabetic complications induced by alloxan. Elevation of glucose levels, the activity of α-amylase and α-glucosidase, and upregulation of the activity of hepatic glucose 6-phosphatase, fructose 1,6-bisphosphatase, and glycogen phosphorylase have been observed in diabetic control rats in comparison with normal control rats in this experiment. Also, alloxan-induced diabetes by reducing the serum level of insulin, the decline in glycogen storage, and downregulation in the activity of hepatic hexokinase, glucose 6-phosphate dehydrogenase, pyruvate kinase, and glycogen synthase compared to control and other treated groups. Hamden *et al.* [37] reported that hyperglycemia induced by alloxan could be due to the inhibition of insulin secretion and damage to β-cells. Alloxan provokes the increase of glycemia through a selective cytotoxic effect on pancreatic β-cells through the generation of free radicals and destruction of pancreatic β-cells [31]. Pancreatic *α*-Amylase is responsible for the breakdown of starch into oligosaccharides which are further broken down by α-Glucosidase into glucose. Alloxan-induced elevation of these enzymes' activity results in enters of a high amount of glucose into the bloodstream upon absorption and leads to a raised level of post-prandial hyperglycemia [38].

Moreover, the reduction of hepatic glycogen in diabetic rats could be attributed to the downregulation of glycogen synthetase activity and increased activity of glucose 6- phosphatase and

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glycogen phosphorylase as obtained in this study. Thus, alloxan could induce hyperglycemia by upregulation of the activity of enzymes that are responsible for increasing rates of gluconeogenesis and glycogenolysis [39].

On the other side, co-administration of either ROLE or GOLE along with alloxan shows antidiabetic activity by reducing the serum glucose level and activity of hepatic enzymes responsible for gluconeogenesis in addition to increasing the serum insulin level, glycogen storage, and the activity of hepatic hexokinase, glucose 6-phosphate dehydrogenase, pyruvate kinase, and glycogen synthase compared to diabetic-group. The hypoglycemic effect of olive leaf extract (OLE) could be related to enhancing peripheral uptake of glucose and improving glucose-stimulated insulin secretion from the existing pancreatic β-cells [40]. Elsaid *et al*. [41] suggested that the possible antidiabetic effects of OLE could be related to the presence of phenolic compounds, flavonoids, secoiridoids, and secoiridoid glycosides that have antioxidant and antidiabetic activity. Also, these active constituents have radical scavenging activity potential and can induce the regeneration of pancreatic β-cells and inhibition of oligosaccharides and polysaccharides digestion by reducing *α*-amylase and α-glucosidase activity [41].

Furthermore, the antidiabetic and hypoglycaemic activity of hydroxytyrosol, oleuropein, and their Secoiridoids derivatives were evidenced by their ability to protect pancreatic cells from progressive damage, enhance insulin secretion by several mechanisms, increase the activity of some enzymes implicated in glucose metabolisms such as hexokinase and pyruvate kinase, and scavenge free radical and protect β-cells pancreatic cells from oxidative damage [42]. Several studies indicated that oleuropein present in olive leaves might be beneficial for the prevention of diabetes and control of hyperglycemia by improving glucose transport and intracellular metabolism, increasing insulin sensitivity, and facilitating insulin secretion by pancreatic β-cells [43].

Additionally, in this study, the activity of the liver marker enzymes (AST, ALT and γGT) and the level of creatinine, uric acid, and urea in the serum of diabetic rats were significantly elevated in comparison with normal controls. The increase in the levels of liver enzymes may be interpreted because of alloxan-induced hepatocyte damage or changes in their membrane permeability indicating hepatocellular damage [40]. The elevation of uric acid by alloxan could be due to a reduction in the total protein level that led to muscle wasting and an increased release of purine, the main source of uric acid [42]. Laaboudi *et al*. [42] indicated that diabetic hyperglycemia-induced renal dysfunction and diabetic nephropathy were observed by the elevation of the serum's urea and creatinine due to increasing protein catabolism in the liver and plasma. Treatment of rats with alloxan and either ROLE or GOLE led to significant improvement in the liver and kidney function of diabetic rats compared to treatment with alloxan alone. The antioxidant and anti-inflammatory nature of OLE might have contributed to the improvement of liver and kidney function tests and histopathological changes by reducing alloxan-induced lesions in the liver and kidney [44]. Ghanam *et al*. [45] reported that a reduction in AST and ALT levels by olive extract could be related to the anti-inflammatory activity of olive polyphenols that allowed regulation of transaminase levels.

CONCLUSION

This work showed that the hypoglycemic and anti-diabetic effect of the gamma-irradiated olive leaf extract was more significant than that observed by using the raw olive leaf extract. This observation could be explained by the effectiveness of gamma-irradiation (5 kGy) in improving the antioxidant activity and increasing the concentration of phenolic compounds and flavonoids in the olive leaf.

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