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Evaluation of Gamma Ray Effects on Total Phenolic Contents, Antioxidants and Antibacterial Activities of *Cichorium Intybus* L

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ABSTRACT

The efficacy of gamma irradiation is well versed as a method of decontamination for food and herbal resources. In the present study, *Cichorium intybus* L. roots were irradiated at multiple doses of 1, 2, 4, 6, 8 and 10 kGy by a cobalt-60 irradiator. The irradiated and non-irradiated control samples were evaluated for total phenolic contents, chlorogenic acid assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging properties, ferric reducing power assay and antimicrobial activities. The results revealed that radiation treatment at dose levels of 1, 2 and 10 kGy showed no effects on chlorogenic acid contents, antibacterial activities and scavenging properties. On the other hand, radiation treatment at dose levels of 4, 6 and 8 kGy significantly increased chlorogenic acid contents, antibacterial activities and scavenging properties. Similarly, no effects were observed on the antibacterial activities at radiation dose levels of 1, 2 and 10 kGy, while samples irradiated at 4, 6 and 8 kGy showed significant rise in the antibacterial activities against *E.coli*. Further, significant, rise in the phenolic contents was recorded at 4, 6 and 8 kGy radiation doses. On the contrary, phenolic contents showed decrease at dose level of 10 kGy. The present study concluded that gamma ray has strong potential to stimulate antioxidant as well as antibacterial activities of *Cichorium intybus* L.

Keywords: Antibacterial activity, *Cichorium intybus* L., DPPH scavenging activity, Gamma radiation, Phenolic content

INTRODUCTION

The preservative effects of various plants and herbs confirm the presence of antioxidants and antimicrobial constituents. Recently, interest has been increased considerably for the search of naturally occurring antioxidants. These could be consumed as diet or could be utilized for medicinal purposes. Moreover, these plants are quite helpful in replacing the synthetic antioxidants that are associated with risks of carcinogenicity.

Chicory is a root of a vegetable whose green leafy tops are consumed as salads in diet. Recently pharmacological actions of chicory have attracted the attention of many researchers. Recent studies revealed that chicory induces hypocholesterolemia [1], protects against hepatocellular damage and inhibits lipid peroxidation [2-4]. Moreover, chicory root has been also reported to induce anti-hyperglycaemic effects [5] and has confirmed role in regulation of appetite [6]. Important phytochemicals are distributed throughout the chicory plant but the important antioxidant properties are present specifically in the roots [87]. Heimler *et al*. (2009) confirmed chicory roots for their polyphenol contents and antiradical activities [8]. HPLC/DAD/MS analyses identified 5 hydroxycinnamic acids and e8 flavonoids (quercetin, kaempferol, luteolin and apigenin glycosides) in chicory plant [9]. The DPPH radical scavenging activity of polyphenols-rich fraction of chicory has been recently reported [8]. Further, the pharmacological investigation of the root and leaf fraction of this plant revealed immunomodulator, antitumor and anticancer properties [10]. Moreover, the water, ethanol and ethyl acetate extracts of chicory root have been conformed earlier for the antibacterial activities [11].

Food irradiation is a process of exposing food to ionizing radiations, such as gamma rays (emitted from radioisotopes ^{60}Co and ^{137}Cs), or high-energy electrons and

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X-rays produced by machine sources [12]. Gamma irradiation has been recognized as a reliable and safe method for improving the nutritional value and inactivation of anti-nutritional factors in food products [13,14]. In 1981, the US Food and Drug Administration (FDA) concluded that food irradiated at 10 kGy or less could be considered safe for human consumption [15]. Some studies have confirmed elevated antioxidant activities in gamma-irradiated products [16-20]. Gharaghani *et al.* (2008) reported that gamma irradiation has strong potential for improving the nutritional quality of canola meal for broiler chickens [21]. Further, the gamma irradiation of soybean meal and canola meal resulted in reduced degradation of proteins [22,23]. However, there is a paucity of information with regard to gamma irradiation effects on bioactive properties of plants. So, this study was carried out to evaluate the effects of gamma ray irradiation on phenolic contents, antioxidants and antibacterial activity of *Cichorium intybus* L. root.

MATERIALS and METHODS

Sample Collection

The roots of *Cichorium intybus* L. were collected in the month of January 2016 from the region of Boluk (Latitude 28º 13' 52", Longitude 57º 30' 45") Jiroft, Kerman, Iran.

Gamma Irradiation

The fresh *Cichorium intybus* L. roots were collected and were washed under running tap water. The samples of *C. intybus* were then subjected to multiple gamma radiation doses (1, 2, 4, 6, 8 and 10 kGy) using a ^{60}Co gamma source (Gamma cell 220) (at dose rate of 4.70 kGy/h) at Atomic Energy Organization of Iran. The irradiations were carried out at room temperature. This was followed by the shade drying of the irradiated and non-irradiated samples at room temperature. After drying they were transformed into a fine powder in a mechanic grinder. The powdered materials were packed using polyethylene bags and were stored at 4 °C for further use.

Phytochemical Analysis

Extract preparation. Briefly, 100 mg of powdered roots were placed separately in a clean, flat-bottomed glass container and soaked in 95% ethanol. This was followed by extraction for 48-72 h and the resulting extract was passed through a filter paper (Whatman No. 1.). The obtained ethanolic extract was then concentrated using rotary evaporator at 45 ºC. After evaporation, the solvent and the extracts were scraped out, weighed and stored at 4 ºC.

Estimation of total phenolic contents. Phenolic levels in root were tested using Folin- Ciocalteu's reagent. 1.5 ml of 95% ethanol and 5 ml of distilled water were added to 1 ml of sample, Further, 0.5 ml of 50% Folin's reagent (dissolved in distal water) and 1 ml of 5% Na_2CO_3 (dissolve in distilled water) were added to the above mixture and was vortexed. The mixture was allowed to stand in the dark for 1 h. The blue color formation as a result of oxidation of the Folin-Ciocalteu reagent by extract was read at 760 nm against a blank reagent. Samples were quantified using various concentrations of 3,4,5-trihydroxybenzoic acid (gallic acid) (0.01- 0.1 mg ml^{-1}) as a standard curve. Total phenolic content was expressed as milligram gallic acid equivalent per gram of dried weight (mg gallic acid/gr dw) [24].

DPPH method of total antioxidant capacity assessment. DPPH is also exploited widely to evaluate the antioxidant activity of plant extracts and foods. The free radical scavenging activity of *C. intybus* ethanolic extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH [25]. 3 ml of 200 µg ml^{-1} extract in ethanol was mixed with 1 ml of 0.1 mM DPPH in ethanol solution. The solution was incubated at 37 ºC for 30 min. The antioxidant activity was measured by observation of the decrease in the absorption at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\%\text{Inhibition} = ((A_o - A_t)/A_o \times 100)$$

Where A_o was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract.

Ferric reducing antioxidant power (FRAP) assay.

The ability of chicory root extract to reduce ferric ions was measured using the method described by Benzie and Strain [26]. The FRAP reagent was formed by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ (2,4-6 tripyridyl triazine) solution and 20 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1 in volume. Samples were then added to 3ml of FRAP reagent and the reaction mixture was incubated at 37 ºC for 30 min. The increase in absorbance at 593 nm was measured. The antioxidant capacity was measured on the basis of ability to reduce ferric ions of sample. It was calculated from the linear calibration curve and was expressed as µmol Fe (II)/gr dw.

Antibacterial Assay of Plant Extract

Test organism. The test organisms used (including both gram positive as well as gram negative strains) for the study were *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Disc diffusion method. The antibacterial activity of the *C. intybus* root extract was determined by the disc diffusion method [27]. Antibacterial activities performed against both Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *E. faecalis*) bacteria. The test organisms were subcultured in 5% sheep blood agar plate (BAP) for 24 h at (35 ± 2) ºC. The colonies were inoculated in normal saline solution. The turbidity was then adjusted to 0.5 McFarland standard giving a final inoculum of 1.5×10^8 CFU/ml. About 100 µl of inoculum of test organism was spread on Mueller-Hinton agar plate (Remel™, Thermo Fisher Scientific, USA). The discs (6 mm in diameter) were impregnated with 10 mg ml^{-1} extract dissolved in 10% sterile dimethyl sulfoxide (DMSO) were placed (30 µl/disc) on the inoculated agar. The plate was incubated at 37 ºC and the pattern of inhibition zones was determined after 24 h. The discs containing solvent DMSO without any test compounds served as a negative control. Rifampicin (30 µl/disc) (Oxoid) was used as positive control. Antibacterial activities were evaluated by measurement of the diameters of zones of inhibition in mm.

Extraction and HPLC Characterization of Chlorogenic Acid

Extraction and characterization of chlorogenic acid were performed by the method of Li *et al.* with slight modifications [28]. Briefly, a sample of 0.1 g of *C. intybus* root powder was added into a beaker containing 5 ml of ethanol and was kept for 24 h in dark place. The extraction was then carried out with ultrasound treatment for a period of an h. The extraction was performed by centrifugation at 14500 rpm for 30 min and the upper phase was filtered by C18 column. The solvent was concentrated in an incubator to evaporate the ethanol and then 200 µl ethanol was added. The mixture was injected into HPLC column. The HPLC conditions were as follows:mobile phase:mixture solution of water-ethyl acetate-acetic acid (95/5:4/1:0/3), flow rate: 1 ml min^{-1} and detection wavelength: 280 nm.

Statistical Analyses

The statistical analyses were performed using SPSS version 19. One-way ANOVA test was used for statistical analyses followed by a post hoc test namely least significant difference (LSD) test. All data were presented as means ±SD for at least three replications for each prepared sample.

RESULTS

DPPH Radical Scavenging Activity

Figure 1 shows effect of gamma irradiation on DPPH radical scavenging activity. No significant changes were observed between control and irradiated samples at dose levels of 1, 2 and 10 kGy. Moreover, gamma irradiation at dose levels of 4, 6 and 8 kGy significantly increased (respectably as $p < 0.01$, $p < 0.001$ and $p < 0.001$) the radical scavenging activity of *C. intybus*.

Ferric Reducing Antioxidant Power

The gamma irradiations at dose levels of 4, 6 and 8 kGy significantly increased ferric reducing antioxidant power activities of chicory roots in comparison to control group (Fig. 2). On the other hand, FRAP activities at dose levels of 1, 2 and 10 kGy showed no change.

Total Phenolic Contents

The results are expressed as mg gallic acid/g dry weight of extract in Fig. 3. For radiation-processed samples, the data showed significant rise in the total phenolic contents in comparison to control at dose levels of 4 ($p < 0.01$), 6 ($p < 0.001$) and 8 kGy ($p < 0.001$). Further, no significant

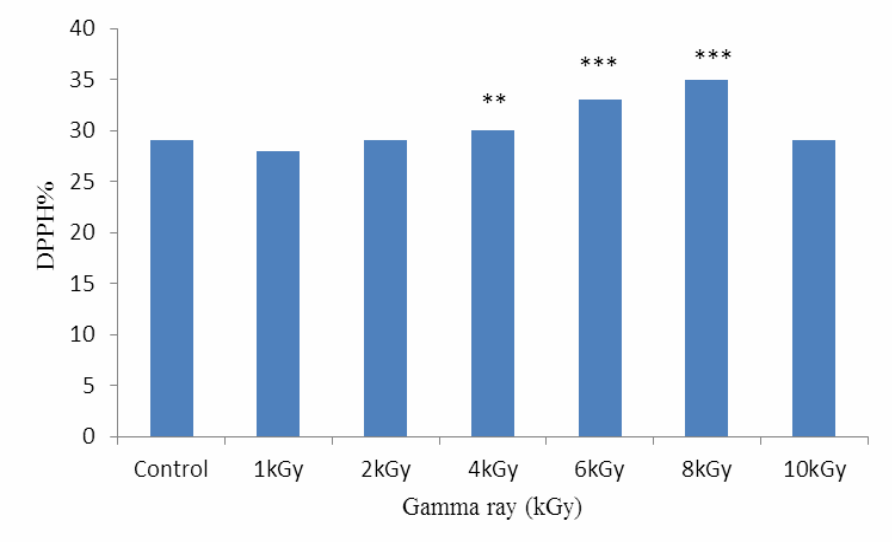

Fig. 1. Effects of gamma rays on the free radical scavenging activity of *C. intybus*. Data are presented as means ± SD. **, $P < 0.01$ and ***, $P < 0.001$ according to LSD test.

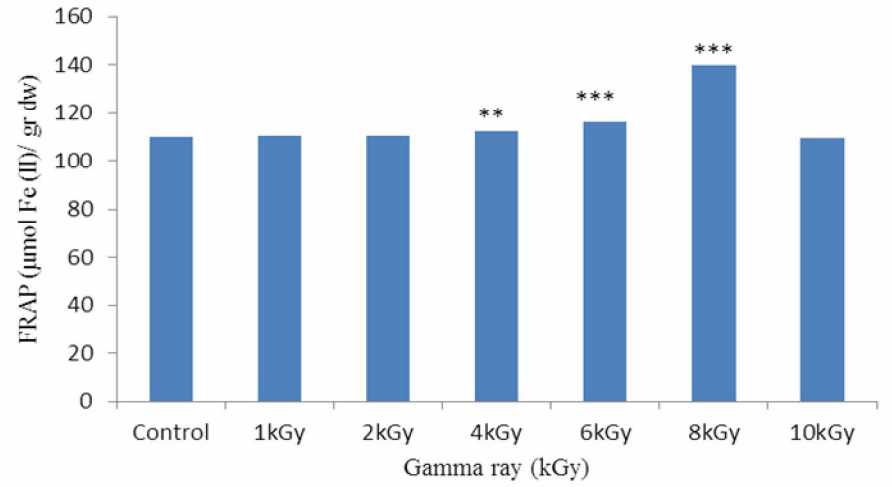

Fig. 2. Effects of gamma rays on the ferric reducing antioxidant power activities of *C. intybus*. Data are presented as means ± SD. **, $P < 0.01$ and ***, $P < 0.001$ according to LSD test.

changes in phenolic contents were noticed as the radiation dose levels of 1 and 2 kGy. Moreover, total phenolic contents of irradiated samples at dose level of 10 kGy revealed significant decrease ($p < 0.01$).

Chlorogenic Acid Assay

The gamma irradiation resulted in significant elevation (Fig. 4) in the levels of chlorogenic acid at doses 4 ($p < 0.01$), 6 ($p < 0.001$) and 8 kGy ($p < 0.001$). Furthermore, no significant changes were observed at 1, 2 and 10 kGy doses.

Antibacterial Activity of *C. Intybus* Roots Extract

The gamma irradiation increased the inhibition zone at dose levels of 4, 6 and 8 kGy (40.13 ± 2.12, 43.24 ± 2.21 and 50.4 ± 2.3 mm). Further, there were no changes in the antibacterial activity of *C. intybus* roots at dose levels of 1,

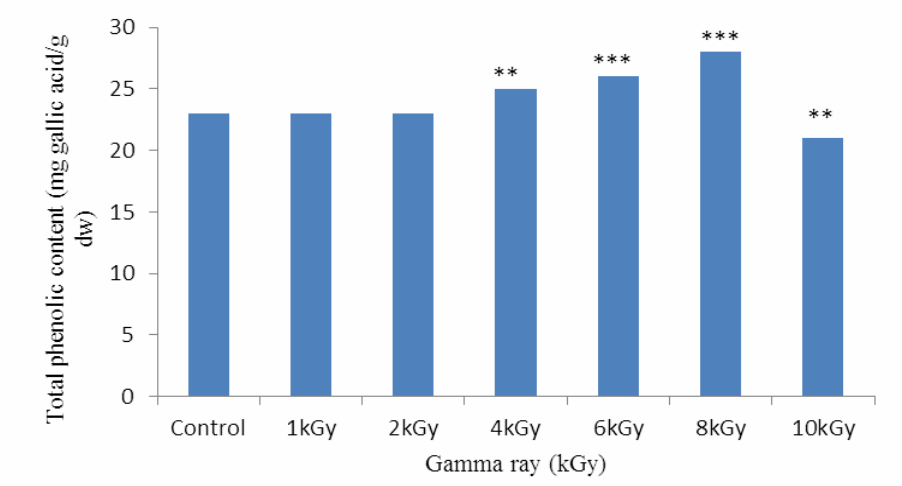

Fig. 3. Effects of gamma rays on the total phenolic contents of *C. intybus*. Data are presented as means ± SD. **, $P < 0.01$ and ***, $P < 0.001$ according to LSD test.

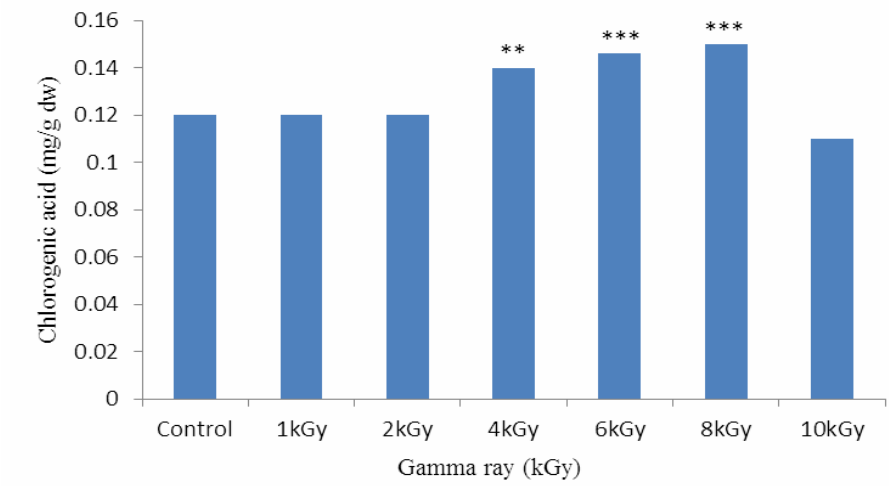

Fig. 4. Effects of gamma rays on the chlorogenic acid contents of C. *intybus*. Data are presented as means ± SD. **, $P < 0.01$ and ***, $P < 0.001$ according to LSD test.

2 and 10 kGy. Also. Chicory roots extract showed no antibacterial activities against *P. aeruginosa* and Gram-positive (*S. aureus* and *E. faecalis*) bacteria.

DISCISSIONS

There is an urgent need to ascertain link (if any) between the gamma irradiation of chicory roots and their antioxidant activities. There are multiple methods to evaluate the free radical scavenging abilities and antioxidant activities of ethanol extract of chicory roots. We evaluated the total phenolic content, DPPH free radical scavenging, and FRAP in the present study. The results confirmed that phenolic contents, antioxidants and antibacterial activities of *C. intybus* were significantly affected by gamma irradiation.

Phenolic compounds present in medicinal have confirmed antioxidant potential [29,30]. Reactive oxygen radical species (ROS) produced in normal cellular metabolism have both beneficial and harmful to living systems [28]. A recent study suggested that the antioxidant

capacity of vegetables is by virtue of their high levels of phenolic compounds [31]. *C. intybus* also contained high phenolic contents especially in leaves as well as roots [32]. Further, gamma irradiation at dose levels of 4, 6 and 8 kGy, caused significant elevation in the phenolic contents. The above increase in phenolic contents could be owed to the ability of gamma irradiation to stimulate activity of phenylalanine [33]. Our results are in sync with an earlier study that also reported rise in phenolic contents in *Pterocarpus santalinus* L after gamma ray treatment [34]. Similar results were observed in the T. arjuna seedlings, in cauliflower, cinnamon and clove exposed to increasing radiation doses [35-36]. Camargo *et al*. (2011) observed a linear increase of phenolic compounds in peanuts at doses of 5.0 and 7.5 kGy. On the other hand, we also observed a significant decrease in phenolic contents at dose of 10 kGy as compared with non-irradiated chicory roots. Similar decrease in the phenolic contents at a similar radiation dose was reported earlier [37]. Some studies did not exhibit any change in phenolic contents upon irradiation [38]. De Toledo *et al*., found that the effects of ionizing irradiation on tannin and phenolic compounds were dose dependent [39]. In contrast to the present study, Harrison and Were reported an increase in phenolic contents in almond skin extracts irradiated at dose greater than 12.7 kGy [17]. They suggested this increase in phenolic content might be due to the release of phenolic compounds from glycosidic components and degradation of larger phenolic compound into smaller ones by gamma irradiation. So, above variable responses of gamma irradiation on phenolic contents could be justified by the fact that variable doses of gamma irradiation on chicory root have variable effects on phenolic contents.

Several studies in recent past on plant materials confirmed gamma irradiation stimulated antioxidant properties [40,41]. In the present study irradiation at doses of 4, 6 and 8 kGy significantly increased antioxidant properties in comparison with non-irradiated samples. Phenolic compounds are a class of antioxidants that act as free radical scavengers [42]. This compound contains hydroxyl functional groups, which are responsible for the antioxidant effect in plants [43]. Our results are in sync with an earlier study by Adhitia *et al*. (2017) that reported rise in phenolic contents as well as antioxidant activity of *Peperomia pellucida* at dose of 5 kGy [44]. Fan (2005) also reported enhancement in the phenolic contents and antioxidant capacities after irradiation with gamma rays [45]. So, observed rise in the antioxidant activity in the present might be the result of high total phenolic accumulation following dose dependent radiation treatment.

It is an established fact that chlorogenic acid containing plant resources have strong antibacterial activities [46]. Chlorogenic acid, the ester of caffeic acid with quinic acid, is one of the most abundant polyphenols in human diet and has been reported to decrease the incidence of chemical carcinogenesis in several animal models of cancer [47,48]. The number and position of hydroxyl groups is the key factor in the antimicrobial activity of phenolic compounds. With the increase in the number of hydroxyl groups, the antimicrobial activity increases as well [49]. The phenolic compounds inhibit enzyme activity *via* their sulfhydryl groups or through nonspecific interactions with proteins. Chlorogenic acid is the main polyphenol compound isolated from ethanolic extract of chicory root [50]. *E. coli* bacterial pathogen has been reported to be highly susceptible to chlorogenic acid as observed in our earlier study [51]. On the other hand, Mollan and Allen (1996) also studied the effects of gamma irradiation on the antibacterial activity of honey and noticed no significant changes on antibacterial changes up to 25 kGy [52]. Furthermore, we noticed significant increase in antibacterial activity post gamma treatment at dose levels of 4, 6 and 8 kGy. This could be owed to rise in the chlorogenic acid contents of *C. intybus* root extract and are responsible for the antibacterial activity of medicinal plants.

CONCLUSIONS

In conclusion, the present study indicated radiation treatment up to 8 kGy is safe and beneficial for *C. intybus* root. However, radiation at higher doses causes degradation or changes in chemical structures of some biologically active important ingredients. Therefore, additional studies are required for concrete conclusions.

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