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Effects of γ-irradiation on free radicals, active components and toxicity of Turmeric rhizomes

Piyanuch Thongphasuk[1*] and Jarunee Thongphasuk[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Patumthani 12000, Thailand
[2]Research and Development Division, Thailand Institute of Nuclear Technology (Public Organization), Nakornnayok 26120, Thailand

*Corresponding author; E-mail: piyanut.t@rsu.ac.th

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Abstract

Gamma irradiation is a method utilized to improve safety of medicinal herbs by inactivation of microorganisms. Since irradiation may also affect active compounds and toxicity of the irradiated herbs, the objective of this research is to study the effects of γ-irradiation (10 and 25 kGy) on turmeric (*Curcuma longa* L.). GC-MS, HPLC and Electron paramagnetic resonance spectroscopy (EPR) were used to determine volatile oil content, curcuminoid content and free radicals, respectively. Total phenolic content and free radical scavenging activity were investigated by spectroscopic techniques. Toxicity was determined by Toxi-Chromo Test. The results showed that γ-irradiation at the doses of 10 and 25 kGy significantly ($P < 0.05$) increased free radicals. However, volatile oil content, curcuminoid content, total phenolic content, free radical scavenging activity and toxicity were not significantly ($P > 0.05$) affected by the irradiation doses.

Keywords: *gamma irradiation, turmeric, volatile oil, free radicals, antioxidant activity, total phenolic content*

บทคัดย่อ

การฉายรังสีแกมมาเป็นวิธีหนึ่งที่ถูกนำมาใช้เพื่อปรับปรุงคุณภาพของสมุนไพรให้มีความปลอดภัยโดยการทำลายจุลินทรีย์ แต่เนื่องจากการฉายรังสีอาจมีผลต่อสารสำคัญและพิษในสมุนไพร จุดประสงค์ของการวิจัยนี้ได้แก่ศึกษาผลของรังสีแกมมา (10 และ 25 กิโลเกรย์) ในขมิ้นชัน โดยการใช้ GC-MS, HPLC และ อิเล็กตรอนพาราแมกเนติก (electron paramagnetic resonance spectroscopy; EPR) ในการวิเคราะห์น้ำมันระเหยง่าย ปริมาณสารในกลุ่มเคอร์คูมินอยด์ และอนุมูลอิสระ ตามลำดับ ศึกษาปริมาณฟีนอลิกรวม และ ฤทธิ์ต้านอนุมูลอิสระ โดยเทคนิคทางสเปกโทรสโคปี ตรวจสอบความเป็นพิษโดยท็อกสิกโครโมเทสท์ (Toxi-Chromo Test) ผลการทดลองแสดงว่า การฉายรังสีแกมมา 10 และ 25 กิโลเกรย์เหนี่ยวนำให้เกิดอนุมูลอิสระในสมุนไพรเพิ่มขึ้นอย่างมีนัยสำคัญ ($P < 0.05$) อย่างไรก็ตามการฉายรังสีแกมมาไม่มีผลอย่างมีนัยสำคัญ ($P > 0.05$) ต่อองค์ประกอบทางเคมีของน้ำมันระเหยง่าย สารในกลุ่มเคอร์คูมินอยด์ ปริมาณฟีนอลิกรวม ฤทธิ์ต้านอนุมูลอิสระ และ ไม่ก่อให้เกิดความเป็นพิษ

คำสำคัญ: รังสีแกมมา ,ขมิ้นชัน, น้ำมันระเหยง่าย, อนุมูลอิสระ, ฤทธิ์ต้านอนุมูลอิสระ, ปริมาณฟีนอลิกรวม

1. Introduction

Gamma irradiation is an effective and safe process for decreasing or eliminating harmful bacteria in plants and medicinal herbs. It also reduces insects and parasites. However, irradiation may affect active components, biological activities and toxicity of the irradiated herbs.

Turmeric (*Curcuma longa* L.) is a plant of the ginger family, the Zingiberaceae. Turmeric is widely used as a food coloring and ingredient in curry powder, and also used in traditional drugs. It has various medicinal properties, such as antioxidant, anti-inflammatory, anti-bacteria, and anti-parasitic activities and also has been shown to inhibits carcinogenesis (Arajo & Leon, 2001; Surh, & Chun, 2007).

The effects of irradiation on active components and biological activities of medicinal plants were reviewed (Thongphasuk & Thongphasuk, 2012). Inconsistent results of the effects of irradiation on volatile oil content which contribute to their typical aroma of spices were reported. No qualitative or major quantitative changes were observed in the volatile oil constituents of irradiated clove, cardamom (Variyar, Bandyopadhyay, & Thomas, 1998), ginger (Variyar, Gholap, & Thomas, 1997) and saffron (Zareena, Variyar, Gholap, & Bongirwar, 2001). Whereas,

Farkas (1988) reported an increase in cinnamylacetate and eugenol content and decrease in cinnamaldehyde content of cinnamon bark samples after irradiation at 10 kGy. Salum , Araújo, Fanaro, Purgatto, and Villavicencio (2009) also found that increasing radiation doses promoted an increase in volatile oil loss in cinnamon. Furthermore, Variyar et al. (1998) reported a 6-fold increase in myristicin content of nutmeg irradiated at a dose of 10 kGy. An almost 50% reduction in the content of total glycosides in nutmeg was noted at a dose of 5 kGy (Ananthakumar, Variyar, & Sharma, 2006). Moreover, the lipid profiles of nutmeg were changed after irradiation at 5 kGy (Niyas, Variyar, Gholap, & Sharma, 2003).

The studies on the effects of irradiation on the phenolic content and antioxidant activities are also controversial. Significant increases of total phenolic content were found for nutmeg, clove (Variyar et al., 1998), black cumin seeds (Khattak, Simpson, & Ihasnullah, 2008) and velvet beans (Bhat, Sridhar, & Yokotani, 2007), whereas significant decreases of phenolic content were observed for dehydrated rosemary (Koseki, Villavicencio, Brito, Nahme, Sebastiao, & Rela, 2002) and tomato (Schindler, Solar, & Sontag, 2005). Khattak et al. (2008) and Variyar, Limaye, and Sharma (2004) reported an increased antioxidant level, whereas a research study conducted by Ahn, Kim, Kim, Kim, Yook, and Byun (2005) and Suhaj, Rácová, Polovka, and Brezová (2006) indicated a decrease of antioxidant levels.

Irradiation resulted in the increase of the free radicals in basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage (Calucci, Pinzino, Zandomeneghi, Capocchi, Ghiringhelli, Saviozzi, & Tozzi et al., 2003). The free radicals are produced by the oxidation of polyphenolic compounds which are ubiquitously present in plants (Horvathova, Suhaj, Polovka, Brezova, & Šimko, 2007)

A FAO/IAEA/WHO joint committee declared that food irradiated up to 10 kGy was safe to consume (WHO, 1999). Irradiation of food up to 10 kGy presents no toxicological hazard. Hence, toxicological testing is no longer required (Crawford, & Ruff, 1996). However, doses higher than 10 kGy may be used to reduce or eliminate certain pathogenic microorganisms in some medicinal herbs. While this level is above the levels deemed safe for consumption, benefits of these higher doses were also reported. Lee, Park, Son, Jo, Byun, and An (2007) demonstrated that gamma rays from cobalt-60 at the dose of 20 kGy improved color of the Sopoongsan (a medicinal prescription including 12 medicinal herbs). The problem of dark color could be decreased significantly with time saving and cost benefit compared to conventional color removal processes.

2. Objectives

Irradiation is used to inactivate microorganisms for improving the safety of herbs. However, active components and biological activities of the irradiated herbs have to be maintained and toxicity from high dose irradiation has to be determined. The objective of this study was to determine the effects of γ-irradiation (10 and 25 kGy) on volatile oil constituents, curcuminoid content, total phenolic content, and free radical scavenging activity of turmeric rhizomes. Furthermore, the effects of the irradiation on free radical induction and toxicity were also investigated.

3. Materials and methods

3.1 Sample irradiation

Spray-dried ethanolic extract of turmeric rhizomes were purchased from a local company in Bangkok. The samples were irradiated by gamma rays from cobalt-60 at the doses of 10 and 25 kGy (Gammacell 220; dose rate 12 kGy/h).

3.2 EPR measurements

All electron paramagnetic resonance (EPR) measurements were performed using a JEOL JES-RE2X (X band) spectrometer provided with a TE_{011} cylindrical resonant cavity. The samples were introduced into quartz tubes (4 mm inner tube diameter). Measurements were made at room temperature on the days of irradiation. Mn^{2+}/MgO was used for calibration with the g value of the fourth Mn^{2+} signal from the lowest magnetic field as g = 1.981, and that of the third Mn^{2+} signal as g = 2.034.

3.3 Analysis of volatile compounds by GC-MS

Volatile oil of turmeric rhizomes extract were obtained by steam distillation. The oil was diluted 1:100 in methanol before being injected into a gas chromatography-mass spectrometer (GC-MS). The injection volume was 1 µl with analyses were carried out using an Agilent Technologies (model 6890 N) mass spectrometer coupled to a Quadrupole mass

selective detector (model 5973 inert). The ionization voltage was 70eV. HP-Innowax capillary column (30m x 0.25 mm i.d., 0.25mm film thickness) was used for the separation. The oven temperature was programmed as 50-240° C at 4 °C/min.

3.4 Determination of curcuminoid content by HPLC

Quantification of curcuminoid was determined by high performance liquid chromatography (HPLC) (Boonchoong, Saohin, Kittijarukhajohn, Kriyasin, & Malithong, 2006). HPLC analysis was performed on JASCO 980 series chromatographic system. Separation was achieved on Phenomenex C18 column (250 mm x 4.5 mm, 5.0 µ) with an injection volume of 20 µL. A mixture consisting of 1% acetic acid and acetonitrile in the ratio of 45:55 (v/v) was used as mobile phase and was filtered before use through a 0.45µ membrane filter. The flow rate of mobile phase was maintained at 1.0 ml/ min. Detection was carried out at 425 nm at ambient temperature.

3.5 Determination of total phenolic content

The total phenolic content was determined by using the Folin-Ciocalteu method. Ten microliters of the extract (10 mg/ml) was added to 100 µl of 7% aqueous sodium carbonate solution and mixed well. Then 10 µl of Folin-Ciocalteu reagent was added to the mixture and the total volume made up to 250 µl using distilled water. After shaking, it was kept for 90 min and the absorbance of the blue complex formed was measured at 750 nm against a blank control. All spectrophotometric work was performed using a Benchmark plus microplate spectrophotometer (Bio-Rad Laboratories (UK) Ltd). The total phenolic content was calculated on the basis of a calibration curve of gallic acid. The results were expressed as gallic acid equivalents (mg) per 10 mg of dry weight of the extract.

3.6 Free radical-scavenging assay with DPPH

Evaluation of free radical scavenging activity as percent inhibition was performed by spectrophotometry. One hundred microliters of samples (10 mg/ml) were mixed with 100 µl of 0.022% 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in MeOH, and left standing for 30 min. Absorbance of the mixture was then determined at 517 nm and the percentage of activity was calculated.

3.7 Toxicity test using *E. coli* (K12 OR85) (Toxi-Chromo Test)

Toxi-Chromotest was performed according to the method of Schrock, James, Dindal, Willenberg, and Riggs (2006). The Toxi-Chromotest (Environmental Bio-Detection Products Inc., Ontario, Canada) is a bacterial assay based on the ability of toxic substances to inhibit the de novo synthesis of an inducible enzyme, beta-galactosidase, in a strain of the bacteria, *E. coli* (K12 OR85). To test for toxicity, the bacteria are mixed with a rehydration cocktail containing inducers of the enzyme beta-galactosidase and other factors. During the recovery phase, toxicants penetrate the cell walls of the bacteria and inhibit the de novo synthesis of the beta-galactosidase. The rate of production of the induced enzyme is detected by a reaction of the excreted enzyme with a chromogenic substrate in the bacterial suspension. Mercuric chloride in water was used as a positive control. The absorbances of the tests, blanks and controls were measured at 615 nm with a microplate reader.

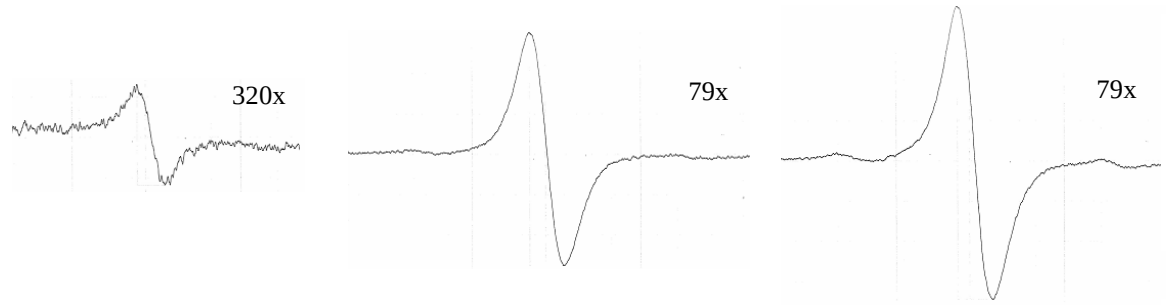
3.8 Statistical analysis

All measurements were performed in triplicate. Excel 2007 (Microsoft, Inc.) was used for t-test analysis. $P \leq 0.05$ was considered significant.

4. Results and discussion

4.1 Effects of gamma irradiation on free radical generation

The EPR spectra and the height of the samples were shown in Figure 1 and Table 1, respectively. Our results showed that on the day of the irradiation, non-irradiated turmeric showed a small singlet peak. EPR singlet signals at g = 2.006 of turmeric by γ-irradiation at the doses of 10 and 25 kGy were increased 10.0 and 12.6 times, respectively. Since our EPR signals at g = 2.006 obtained in solid state had similar characteristics as semiquinone radicals as previously reported by Calucci et al. (2003); Horvathova et al. (2007); Raffi, Yordanov, Chabane, Douifi, Gancheva, and Ivanova (2000); Tabner and Tabner (1991); Tabner and Tabner (1993); Yordanov and Gancheva (2000), it is likely that our EPR signals might be due to semiquinone radicals.



a) non-irradiated b) 10 kGy c) 25 kGy

Figure 1 EPR spectra of a) non-irradiated, b) 10 and c) 25 kGy irradiated turmeric samples with amplitude 320x, 79x, and 79x, respectively

Table 1 Free radical induction of control and irradiated turmeric

Radiation doses (kGy)	Free radical induction (height;amplitude 79x; mean ± SD)
0	239.2 ± 18.2
10	2,402.0 ± 38.2
25	3,003.0 ± 32.5

4.2 Effect of gamma irradiation on volatile oil

The results on effects of irradiation on volatile oil of turmeric are shown in Table 2. In this study, the major volatile compounds of turmeric oil were ar-turmerone, alpha-turmerone, beta-turmerone, beta-sesquiphellandrene and zingiberene. The volatile compounds of the irradiated turmeric were not significantly changed ($P > 0.05$) after irradiation. The results in our study were consistent with those reported earlier (Chatterjee, Variyar, Achyut, Padwal-Desai, & Bongirwar, 2000). Similar to our results, no qualitative or major quantitative changes were observed in volatile oil constituents of irradiated *Thymus vulgaris* L., *Eucalyptus radiate* D.C., and *Lavandula angustifolia* Mill. at a dose of 25 kGy (Haddad, Herent, Tilquin, & Quetin-Leclercq, 2007). Gamma irradiation upto a dose of 50 kGy did not bring about any detectable qualitative or quantitative changes in the aromatic constituents of spices (Maija, Merja, Pia, & Sinikka, 1990). Gamma radiation (10 kGy) did not induce any detectable qualitative or quantitative significant changes in the content and yields of volatile oil after radiation of clove and cardamom (Variyar et al., 1998).

4.3 Effect of gamma irradiation on curcuminoid content determined by HPLC

From HPLC analysis, we found that no substantial quantitative changes ($P > 0.05$) in curcuminoid content (curcumin, demethoxy-curcumin, and bisdemethoxycurcumin) of irradiated turmeric as compared to the equivalent non-irradiated ones (Figure 2).

Table 2 Volatile oil content (Mean ± SE) in untreated and gamma irradiated turmeric samples on the day of the irradiation

RT	Volatile oil	Relative percentage		
		Non-irradiated	irradiated 10 kGy	irradiated 25 kGy
5.27	limonene	0.400 ±0.098	-	0.197±0.067
5.40	1,8-cineole	0.460±0.191	0.583±0.162	0.723±0.194
7.11	alpha-terpinolene	0.277±0.105	0.340±0.087	0.663±0.202
15.49	trans-caryophyllene	1.060±0.219	1.043±0.145	0.950±0.606
17.79	trans-beta-farnesene	0.407±0.075	0.360±0.035	0.463±0.091
18.28	gamma-curcumene	0.343±0.059	0.257±0.023	0.390±0.080
19.18	zingiberene	5.820±0.991	5.140±0.392	6.053±1.025
19.30	beta-bisabolene	0.987±0.184	0.850±0.062	1.080±0.185
20.45	beta-sesquiphellandrene	6.287±0.939	5.403±0.320	6.657±1.000
20.55	ar-curcumene	2.007±0.345	1.867±0.172	2.387±0.400
29.06	zingiberenol	0.577±0.104	0.477±0.047	0.597±0.055
30.60	ar-turmerone	32.123±1.824	34.060±0.596	31.037±1.625
31.32	ar-turmerol	1.090±0.040	0.840±0.098	1.177±0.124
31.89	alpha-turmerone	15.767±1.150	18.247±0.171	15.003±1.290
32.19	beta-turmerone	12.617±0.886	13.553±0.152	12.527±0.875
33.14	alpha-oxobisabolene	1.443±0.626	2.023±0.057	1.230±0.418
33.62	trans-alpha-atlantone	1.850±0.781	2.067±0.237	1.567±0.464

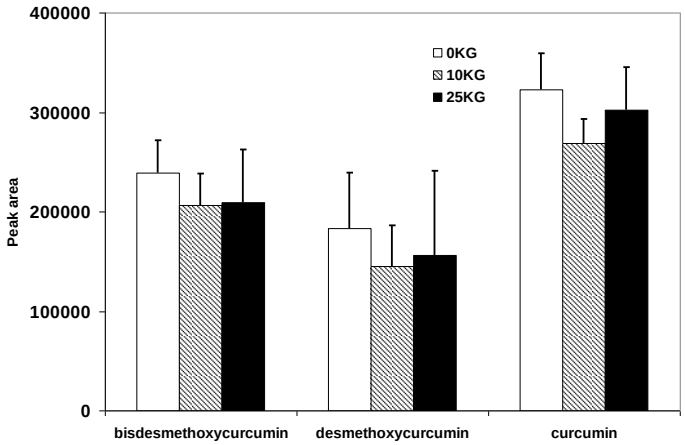


Figure 2 Effect of gamma irradiation on curcuminoid content of turmeric samples

4.4 Effects of gamma irradiation on total phenolic content

The results of total phenolic content expressed as mg equivalents of gallic acid/10 mg dry weight of extract are given in Table 3. No significant changes ($P > 0.05$) in phenolic content were observed following 10 and 25 kGy gamma irradiation. There are reports wherein irradiation treatment did not show any significant effect on total phenolic content. For example, gamma irradiation doses up to 30 kGy did not induce any significant changes in flavonoids, tannins, phenolic content of artichoke and sweet basil (Koseki et al., 2002). Research on the effect of gamma irradiation on spices showed that some spices such as cardamom and cinnamon did not exhibit greater phenolic content with irradiation (Variyar et al., 1998). Conversely, the ability of gamma irradiation to increase phenolic content in plant material has also been observed in many herbs and spices.

Study on the effect of gamma irradiation on spices showed that clove and nutmeg had increased phenolic content with irradiation (Variyar et al., 1998). The differences in effects were attributed to the different phenolic compounds present in the various spices. These increases in phenolic content were associated with the degradation of tannins as a result of irradiation treatment (Variyar et al., 1998; Khattak et al., 2008). Clove and nutmeg have appreciable amounts of hydrolysable tannins, which may be more susceptible to gamma-irradiation compared with condensed tannins present in cinnamon and other spices (Khattak et al., 2008). The increased phenolic content in gamma-irradiated almond skin extract could be attributed to the release of phenolic compounds from glycosidic components and degradation of larger phenolic compounds into smaller ones by gamma irradiation (Harrison & Were, 2007). Moreover, enhancement in the total phenolic content on exposure to gamma radiation has been reported in fresh-cut vegetables (Romaine, iceberg lettuce, endive) (Fan, 2005), carrot and kale juice (Song, Kim, Jo, Lee, Kim, & Byun, 2006), *Citrus unshiu* pomaces (Kim, Lee, Lee, Nam, & Lee, 2008), Rosemary (Pe´rez, Caldero´n, & Croci, 2007), Niger seeds (Khattak et al., 2008) and velvet beans (Bhat et al., 2007). However, for other plant materials, diverse effects of radiation on the phenolic content have been reported. Koseki et al. (2002) reported a decrease in the amount of total phenolic compounds in dehydrated rosemary after irradiation doses of between 10 and 30 kGy, as compared to the controls. The gamma-irradiation treatment (2, 4, and 6 kGy) markedly reduced the concentration of the phenolic compounds of tomato (Schindler et al., 2005). The difference in the effect of radiation on total phenolic content may be due to plant type, environmental and geographical conditions, dose of gamma irradiation, extraction solvent, extraction procedures, type of phenolic compounds, sample state such as solid or dry, temperature, etc (Khattak et al., 2008).

Table 3 Total phenolic content and free radical scavenging activity of control and irradiated turmeric

Radiation doses (kGy)	Gallic acid equivalent (mg per 10 mg of dry weight; mean ± SD)	% Inhibition of DPPH (mean ± SD)
0	0.0730 ± 0.0011	65.98 ± 1.34
10	0.0727 ± 0.0016	68.43 ± 1.45
25	0.0730 ± 0.0007	65.90 ± 2.60

4.5 Effects of gamma irradiation on free radical-scavenging activity

The radical-scavenging activities of the samples were 65.98%, 68.49% and 65.90% for non-irradiated, 10 and 25 kGy irradiated turmeric samples, respectively. No significant difference ($P > 0.05$) was found in the scavenging activity of control and radiation-processed samples at 10 and 25 kGy (Table 3). Our results are in accordance with earlier reports (Chatterjee, Desai, & Thomas, 1999). According to a Murcia, Egea, Romojaro, Parras, Jime´nez, and Martı´nez-Tome´ (2004) report, irradiated dessert spices (cinnamon, ginger, nutmeg, anise, vanilla, licorice, mint) did not show significant modifications in their scavenging activity as a result of irradiation at doses of 1, 3, 5, and 10 kGy. Gamma-irradiation treatment (at 2.5 and 20 kGy) did not cause any significant differences in antioxidant capacity of methanol extracts of freeze-dried mushrooms (Huang & Mau, 2006). However, some reports showed different results for the effect of gamma irradiation on antioxidant properties. A research study conducted by Ahn et al. (2005) indicated that immediately after irradiation at 2 kGy, the radical scavenging ability of Chinese cabbage was reduced. Suhaj et al. (2006) also found that the black pepper scavenging ability of DPPH radicals decreased with gamma irradiation doses from 5 to 30 kGy over a storage period up to five months. Moreover, Lampart-Szczapa, Korczak, Nogala-Kalucka, and Zawirska-Wojtasiak (2003) revealed that increased doses of irradiation decreased the antioxidant activity of lupin seed extracts. On the other hand, Khattak et al. (2008) found that scavenging ability on DPPH radicals was increased in Niger seed (*Nigella sativa* L.) extracts following irradiation at 2 and 16 kGy. DPPH free radical-scavenging activity of soybean increased after doses of gamma-irradiation at 0.5 to 5 kGy (Variyar et al., 2004). Radiation treatments have been shown to either increase or decrease antioxidant content of fresh plant produce, which is dependent on the dose delivered, exposure time, the technological criteria, the specific type of produce, raw material used and solvents used for extraction (Alothman, Bhat, & Karim, 2009).

4.6 Effects of gamma irradiation on toxicity

Our results as shown in Figure 3 demonstrated that toxic effects increased with increasing concentrations of the samples but were not significantly ($P > 0.05$) affected by the irradiation doses. In addition, our data suggest that the irradiation-induced free radicals did not cause amplification of the toxicity.

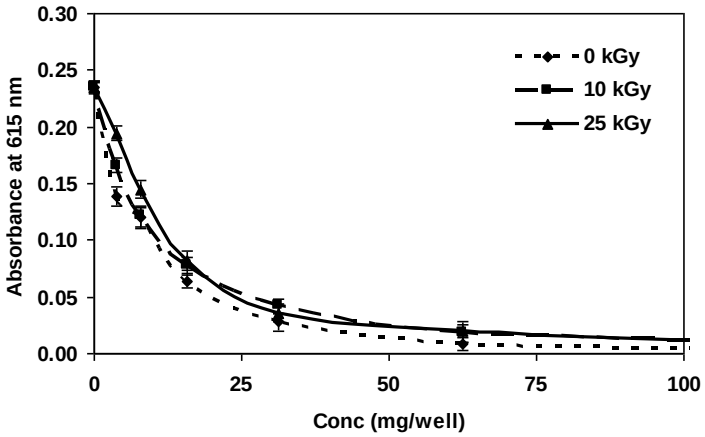


Figure 3 Toxicity of non-irradiated, 10 and 25 kGy irradiated turmeric samples

Our results showed that γ- irradiation at the doses of 10 and 25 kGy significantly increased free radicals ($P < 0.05$). However, the volatile oil content, curcuminoid content, total phenolic content, free radical scavenging activity and toxicity were not significantly ($P > 0.05$) affected by the irradiation doses and the increased free radicals. Free radicals are not unique to radiation processing, common processing such as roasting, heating, pounding, and crushing can also generate free radicals similar to irradiation (Bhat et al., 2007; Bhushan, Bhat, & Sharma, 2003; Fan, & Sokorai, 2008). The radiolytic products which classified as carcinogens such as benzene, furan and 2-alkylcyclobutanones are found from both irradiation and cooking processes. Therefore these doses of irradiation and the resulting changes in free radical formation, phenolic content and radical scavenging activities should not be viewed with alarm and may prove to be considered as safe as 10 kGy doses.

5. Conclusion

The conclusion of this study is that gamma irradiation at the dose of 10 and 25 kGy induced free radicals, but did not affect volatile oil content, curcuminoid content, total phenolic content, free radical scavenging activity and toxicity of irradiated turmeric.

6. Acknowledgements

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