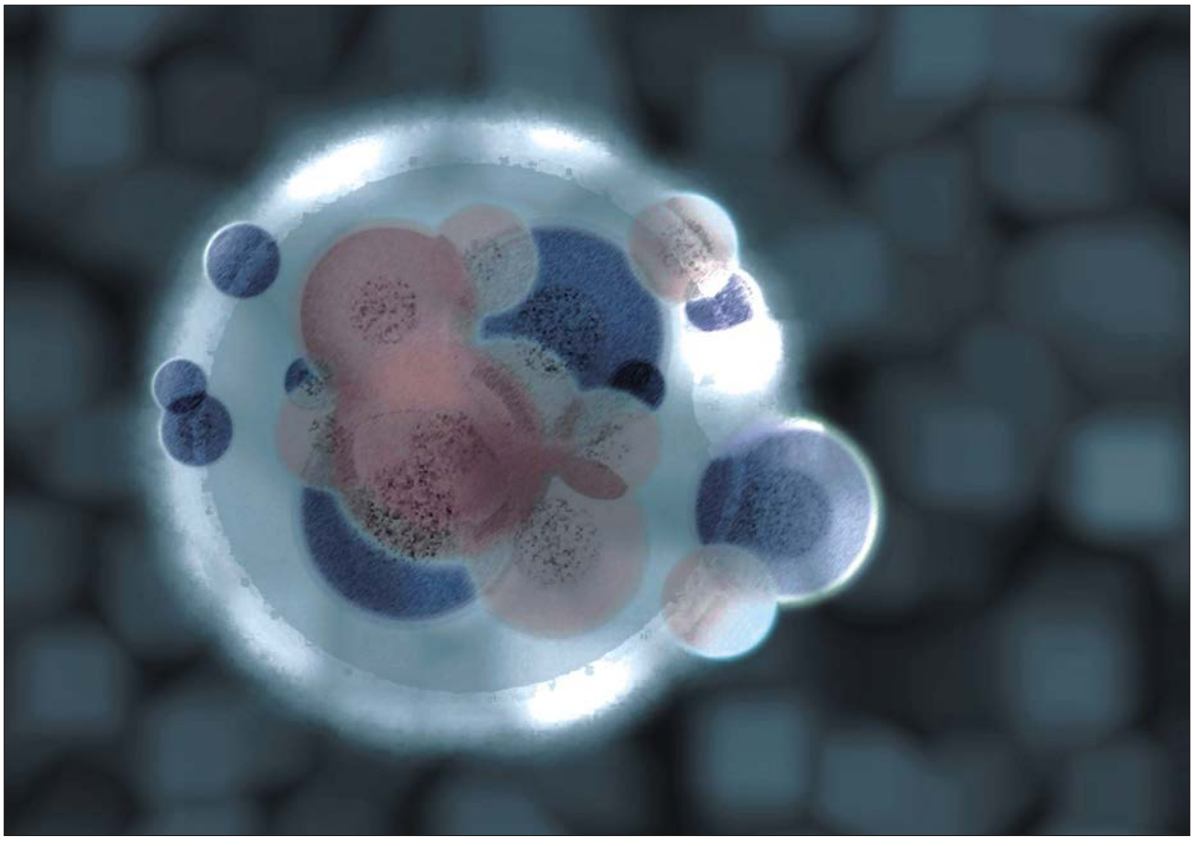

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 9 (8): 872-877, 2009
ISSN 1727-3048

X-Rays Irradiation Produced Dual Effects on the Constituents of Medicinal Plants Extracts

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Abstract: The objective of this study is to show the effect of free radicals induced by fixed dose rate of X-rays radiation on the chemical constituents of some medicinal plants; barks of *Cinnamomum verum* (cinnamon), leaves of *Salvia officinalis* (sage) and *Camellia sinensis* (green tea). Four extracts (1%) were prepared for each medicinal plant; hydro-distilled, aqueous, ethanol and methanol. Each extract was subjected to X-rays radiation at rate of 1.9 Gy min^{-1}. The UV-Visible spectra, physiochemical properties and biochemical constituents of each non irradiated and irradiated extracts were determined. The results showed that the effect of irradiation on the hydro-distilled and aqueous extract differed from alcohol extract. Favorable effect of irradiation was observed on the green tea extract. Considerable loses of total polyphenols and flavonoids quantities were observed in aqueous extracts. X-rays radiation remarkably induced degradation of allantoin and a slight changes in release nitrogen species. In conclusion X-ray radiation of medicinal plants in solutions produced dual effect in terms of improving and degrading the active ingredients depending on the extracted solution as well as the native constituents of each medicinal plant.

Key words: X-rays, irradiation, medicinal plants

INTRODUCTION

Ionizing radiations induced generation of reactive oxygen species in form of hydroxyl (·OH), hydrogen (·H), singlet oxygen (O_2 ($^1\Delta_g$)) and peroxyl radicals (ROO) that follows a cascade of events (Tarpan *et al*., 2008). Detection of free radical reactions induced by low linear-energy-transfer irradiation in an aqueous solution is recently demonstrated (Matsumoto *et al*., 2009). The extent of these reactions are depended on the radiation energy. The radioprotective activity of plant and herbs may be mediated through several mechanisms, since they are complex mixtures of many chemicals. The leading mechanisms for herbal radioprotection included scavenging of radiation-induced free radicals by polyphenols and elevation of cellular antioxidants by plants and herbs in irradiated systems (Jagetia *et al*., 2004; Jagetia and Venkatesh, 2005, 2006).

Gamma irradiation is used to disinfect the herbs and spices for the purpose of storage. Irradiation of cinnamon and sage resulted in a general increase of quinone radical content and significant loss of total antioxidants (Calucci *et al*., 2003). Moreover, gamma-irradiation did not bring about any distinct qualitative or quantitative chemical changes of water soluble constituents and water insoluble steam volatile oils based on spectrophotometric analysis of cinnamon extracts (Josimoviæ and Cudina, 1987). Methanol-water fraction separated from sage acetone oleoresin had the highest antioxidant activity in terms of the formation of primary and secondary oxidation products (Bandoniene *et al*., 2001). Lee *et al* (2008) demonstrated that total polyphenols or a mixture of green tea polyphenols were more effective than individual catechin in protection the mice against gamma-radiation in terms of reducing apoptotic cells. (-)-Epigallocatechin-3-gallate which is abundant in green tea protected the keratinocytes from UVB-induced photo-damage and hydrogen peroxide-induced oxidative stress via scavenging of free radicals (Huang *et al*., 2007).

Recently Kim *et al*. (2009) found that ionizing radiation enhancing the free radical scavenging activity and polyphenols which are the major by-product in cooking drip extracts from Hizikia fusiformis.

The objective of this study is to test the hypothesis that fixed dose of X-rays irradiation, via releasing free radicals in solution, will alter the constituents of some

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medicinal plants; barks of *Cinnamomum verum* (cinnamon), leaves of *Salvia officinalis* (sage) and *Camellia sinensis* (green tea) and this alteration is related to the nature of prepared extract.

MATERIALS AND METHODS

This study was conducted in Department of Pharmacology and Department of Physiology/Medical Physics, College of Medicine, Al-Mustansiriya University in cooperation with X-ray Unit in Al-Yarmouk teaching hospital in Baghdad, Iraq during May 2009. Three medicinal plants; *Cinnamomum verum* (cinnamon) bark, *Salvia officinalis* (sage) leaves and *Camellia sinensis* (green tea) leaves were investigated in this study. They were obtained from local sources, grinded mechanically and sieved prior to their extraction.

Extracts preparation: Hydro-distilled extracts of *Cinnamomum verum* (cinnamon) bark, *Salvia officinalis* (sage) leaves and *Camellia sinensis* (green tea) leaves were prepared by simple distillation. In brief 1 g of dried fine powder, of each herb, in 100 mL distilled water (1%) was boiled and the vapor separated and condensed to obtain clear colorless liquid that was more concentrated in the more volatile components. Also, aqueous, ethanol and methanol extracts were prepared. A 1 g dried fine powder was extracted with 100 mL of distilled water (aqueous extract), absolute ethanol or methanol i.e., (1%) for 24 h in dark place at room temperature (25°C). The extraction was followed by filtration. The UV-Visible spectra is used to demonstrate the presence of active ingredients in the extracts. The UV-Visible spectra of full strength hydro-distilled extract and 1:80 v/v aqueous, ethanol or methanol/distilled water extracts were obtained by scanning the extract using UV-Visible spectrophotometer (Aquarius, France, Cecil series with scanning ability). The survival fraction of active ingredients was calculated by: irradiated Optic Density (OD)/non radiated Optic Density (OD)

Radiation of medicinal herb extracts: A total number of 24 tubes (10 mL) contained extracts solutions within 20×20 cm were exposed to conventional X-ray radiation with the following specifications: X-ray tube distance from upper level of extract was 80 cm, accelerated potential 120 KpV, effective energy of X-ray at 120 kVp was calculated using Hubbell's tables and it was 30.976 KeV and the absorbed dose 1.9 Gy min^{-1} at room temperature 22°C. The UV-Visible spectra of each herbal extract was obtained by scanning the extract using UV-Visible spectrophotometer.

Physiochemical properties of medicinal herb extracts: The physiochemical properties of each extract (irradiated and non irradiated) including pH, conductivity (µS/cm) and total dissolved salts (TDS) (ppm) were determined using pH/°C/ EC/ TDS meter.

Determination of the amount of total polyphenolic compounds: This was carried out as described previously (Chandler and Dodds, 1993). Briefly 1 mL of each herbal extract was mixed with 5 mL distilled water and 0.5 mL of Folin-Ciocalteu reagent (50%). Then allowed the mixture to stand and after 5 min, 1 mL of Na_2CO_3 (5%) was added. Subsequently the mixture was shaken for 1 h at room temperature in dark place. Afterward the absorbance was measured at 725 nm. Gallic acid was used as the standard for calibration curve and phenolic content were expressed as µg gallic acid equivalent/mg dry weight. Experiments were performed in triplicate.

Quantification of total flavonoids: The method is based on the quantification of the yellow color produced by the interaction of flavonoids with $AlCl_3$ reagent (Lamaison and Carnet, 1990; Jagadish *et al*., 2009). Aliquots of 1.5 mL of extracts were added to equal volumes of a solution of 2% $AlCl_3 \bullet 6H_2O$ (2 g in 100 mL methanol). The mixture was vigorously shaken and absorbance at 367 nm was read after 10 min of incubation. The flavonoids content was calibrated using the linear equation based on the calibration curve quercetin. Flavonoids content was expressed as µg quercetin equivalent/mg dry weight. Experiments were performed in triplicate.

Determination of allantoin: This was carried out as described previously (Vrbaski *et al*., 1978) using Ehrlich's reagent, which consisted from 1 g ρ-dimethylaminobenzaldehyde (ρDMAB) in a mixture of 25 mL concentrated HCl and 75 mL methanol. One milliliter of each extract was mixed with Elrich's reagent (1:2 v/v), incubated at room temperature and read the absorbance at 440 nm. The allantoin content was calibrated using the linear equation based on the standard allantoin calibration curve. Experiments were performed in triplicate.

Nitric oxide assay: Nitric oxide donating activity was determined as describe by Newaz *et al*. (2003) using Griess's reagent. Briefly, 3 mL of each extract (1: 2 v/v distilled water) was added to 50 µL HCl (6.5 M) and 50 µL sulfunalic acid (37.5 mM). After incubation of 10 min, 50 µL naphthylethylenediamine hydrochloride (12.5 mM) was added and incubated for further 30 min, centrifuged for 10 min at 3000 rpm. The reference nitric oxide donating compound was 5 mM sodium nitroprusside. The absorbance was immediately recorded at 540 nm. Experiments were performed in triplicate.

Statistical analysis: The results are presented as absolute numbers, percents and Mean±SD. The data are analyzed by unpaired, two tailed Student's "t" test taking p= 0.05 as the lowest limit of significance.

RESULTS

UV-Visible spectra of aqueous extracts of cinnamon revealed that the peak at wavelength of 286 nm was augmented by X-rays radiation. This effect is not observed with ethanol and methanol extracts (Table 1). The peaks at 272.5-274 nm that observed with green extracts also increased by irradiation in aqueous extract while they decreased in ethanol and methanol extracts (Table 1). The survival fractions for this active ingredient were 0.818 and 0.593 for ethanol and methanol extracts respectively. There was no specific peak that observed in hydro-distilled and aqueous extracts of sage. As with cinnamon, the ethanol and methanol extracts of sage showed specific peaks ranged 282-286.5 nm that were slightly affected by irradiation (Table 1). The survival fractions were 0.852 and 0.988 for ethanol and methanol extracts, respectively.

Irradiated extracts did not show visually changes in the color from non-irradiated extracts. The effect of radiation on the pH of extract followed a mirror image fashion. Radiation reduced the pH of hydro-distilled and aqueous extracts while the pH was increased in solvents extracts (ethanol or methanol) in all studied herbs (Table 2). Neither hydro-distilled nor ethanol extracts were affected by radiation in respect to the conductivity and total dissolve salts (Table 2). Radiation reduced the conductivity and the total dissolved salts in both aqueous and methanol extracts (Table 2).

Total polyphenols of sage resisted the effect of radiation compared with green tea or cinnamon. Polyphenols of methanol extract for each medicinal plant used in this study were more resistant to the effect of radiation compared with other extracts (Table 3).

Flavonoids were more susceptible to radiation in hydro-distilled or aqueous compared with ethanol and methanol extracts. Sage flavonoids were more resistant to the radiation compared with green tea or cinnamon.

Small quantity of allantoin was detected in the investigated medicinal plants and they were vulnerable to degradation by radiation (Table 3). The effect of radiation on the allantoin in ethanol or methanol is less than that observed with hydro-distilled or aqueous extracts. Again the generation of nitric oxide was more stable in methanol extract compared with ethanol or aqueous extracts.

Table 1: UV-Visible spectra of medicinal plants extract before and after X-ray irradiation at 1.9 Gy min^{-1}

	Hydro-distilled		Aqueous		Ethanol		Methanol	
	Wavelength (nm)	Absorbance (OD)	Wavelength (nm)	Absorbance (OD)	Wavelength (nm)	Absorbance (OD)	Wavelength (nm)	Absorbance (OD)
Cinnamon								
Not irradiated	207, 292	0.192, 0.542	202.5, 286.5	1.132, 0.456	191.5, 287	1.242, 0.524	191.5, 287	1.361, 0.675
Irradiated	191, 291.5	0.688, 0.548	202.5, 286	1.288, 0.548	192, 287	1.288, 0.500	202, 286	1.523, 0.669
Green tea								
Not irradiated	195, 273	0.308, 0.079	273, 501.5	0.652, 0.049	206.5, 273.5, 415.5, 671	2.488, 0.371, 0.041, 0.024	191, 274, 671.5	2.398, 0.990,0.055
Irradiated	195, 256	0.445, 0.192	272.5	0.708	191.5, 205.5, 272.5, 415.5, 671	1.470, 1.964, 0.300, 0.046, 0.030	273.5, 671.5	0.588, 0.032
Sage								
Not irradiated	199	0.474	195	0.528	192, 285.5, 435, 669	1.155, 0.142, 0.037, 0.025	199, 286.5, 434, 668	1.411, 0.258, 0.041, 0.024
Irradiated	194.5, 254.5	1.121, 0.474	191.5	0.718	191.5, 282, 433.5,668.5	1.027, 0.121, 0.040, 0.025	191.5, 286, 435, 669	1.153, 0.255, 0.105, 0.094

Table 2: Physio-chemical properties of medicinal plants extract before and after X-ray irradiation at 1.9 Gy min^{-1}

	Hydro-distilled			Aqueous			Ethanol			Methanol		
	pH	C	TDS	pH	C	TDS	pH	C	TDS	pH	C	TDS
Cinnamon												
Not irradiated	5.8	0010	0000	4.6	0080	0030	4.6	0000	0000	5.0	0010	0000
Irradiated	5.3	0020	0010	4.5	0140	0070	6.1	0000	0000	5.5	0010	0000
Green tea												
Not irradiated	6.3	0000	0000	5.1	0430	0210	5.9	0000	0000	5.9	0000	0000
Irradiated	5.9	0010	0000	4.9	0040	0190	7.1	0000	0000	6.5	0000	0000
Sage												
Not irradiated	5.5	0000	0000	4.9	0960	0470	5.2	0000	0000	5.2	0080	0040
Irradiated	5.1	0000	0000	4.6	0820	0410	5.5	0000	0000	5.4	0060	0030

C: Conductivity (μS cm^{-1}), TDS: Total dissolved salts (ppm)

Table 3: Effect of X-ray irradiation (1.9 Gy min^{-1}) on the constituents related to antioxidants and free radicals of medicinal plants

	Hydro-distilled				Aqueous			
	Allantoin	Flavonoids	Total polyphenols	Nitric oxide	Allantoin	Flavonoids	Total polyphenols	Nitric oxide
Cinnamon								
Not irradiated	0.33±0.05	0	0	0.040±0.008	2.50±0.3	21.60±2.1	128.70±5.1	0.120±0.009
Irradiated	0.33±0.05	0	0	0.048±0.01	1.40±0.2†	3.70±1.1*	50.90±3.2*	0.110±0.006
Green tea								
Not irradiated	1.33±0.08	0	0	0.040±0.008	3.88±0.4	21.60±2.6	518.50±7.8	0.068±0.01
Irradiated	0.68±0.1*	0	0	0.020±0.005**	0.83±0.2*	4.63±1.1*	170.30±4.9*	0.038±0.01**
Sage								
Not irradiated	0.53±0.08	0	18.5±2.0	0.072±0.01	3.98±0.2	10.40±1.9	12.50±2.0	0.180±0.03
Irradiated	0.3±0.07***	0	0*	0.080±0.01	1.68±0.3*	6.72±1.6	5.74±0.9†	0.124±0.01**
	Ethanol				Methanol			
	Allantoin	Flavonoids	Total polyphenols	Nitric oxide	Allantoin	Flavonoids	Total polyphenols	Nitric oxide
Cinnamon								
Not irradiated	6.78±1.1	12.7±2.1	185.2±6.5	0.42±0.1	8.28±1.7	21.64±2.0	270.3±6.2	0.460±0.15
Irradiated	5.28±0.9	8.3±1.8	116.7±5.8*	0.38±0.08	7.48±2.0	8.08±1.2*	200.0±5.8*	0.440±0.12
Green tea								
Not irradiated	11.78±2.1	20.5±2.7	481.4±7.0	0.26±0.05	16.28±2.2	21.10±1.9	296.3±8.9	0.264±0.02
Irradiated	7.28±1.6**	16.0±2.3	325.9±4.6*	0.26±0.07	14.28±3.1	20.50±2.4	518.5±9.2*	0.264±0.02
Sage								
Not irradiated	14.78±2.9	21.1±2.4	185.2±5.3	0.38±0.06	13.28±2.2	21.07±2.6	212.9±4.7	0.420±0.08
Irradiated	10.78±2.5	20.5±2.2	120.4±4.9*	0.34±0.07	8.98±2.0†	21.64±1.1	157.4±3.1*	0.380±0.01

The results (Mean±SD) are expressed as µg mg^{-1} dry weight for allantoin, flavonoids and total polyphenols and as µmol nitric oxide/mg dry weight. *p<0.001, **p<0.01, ***p<0.02, †p<0.05 compared with corresponding non irradiated samples

DISCUSSION

Each medicinal herb responded to X-rays radiation by changes in O.D. of UV-Visible spectra in different pattern and a decrease in pH values for all aqueous medicinal herbs while the pH values increased in irradiated herbs extracted by alcohols. These changes are accompanied with alteration in the total polyphenols, flavonoids and allantoin.

There is no doubt that X-rays radiation induced changes in the active ingredients of medicinal herbs in solutions. Each medicinal plant and each extract showed specific response to radiation. X-rays radiation improved the active ingredients that were observed in UV-Visible scan of aqueous extract at 272.5-274 nm. This finding is in agreement with Kim *et al*. (2006) who demonstrated that the chemical properties of green tea are significantly affected by irradiation and resulted in high quality green tea. There is evidence that the active ingredients of green tea act as radiosensitizer, i.e. it promotes the effect of ionizing radiation on the target cells (McLaughlin *et al*., 2006).

The same finding is obtained with cinnamon aqueous extract (Turgis *et al*., 2008). Regarding alcohols extracts, the active ingredients of each medicinal plants are adversely affected by irradiation. The possible explanation for this observation is related to the radiolysis of the constituents of each medicinal herbal extract as well as the quantum of generated hydroxyl radicals (Ma *et al*., 2007). In general, the active ingredients of the tested medicinal herbs evident by UV-peaks resist the effect of radiolysis particularly the aqueous extract.

There is evidence that phenol contents of medicinal plants are increased when they were irradiated with ultraviolet B (Kumari *et al*., 2009) while gamma and electron beam ionizing radiation did not induce any detectable qualitative or quantitative significant changes in the contents and yields of essential oils immediately after ionizing radiation of some medicinal herbs (Haddad *et al*., 2007). In this study only green tea methanol extract showed increase total polyphenol contents after irradiation with low energy ionizing radiation. Flavonoids in methanol extract of medicinal plants relatively resist the effect of ionizing radiation compared with other extracts. This finding supports the early investigations that flavonoids are instable in aqueous solution (Scalia and Mezzena, 2009). Moreover, Kozlowski *et al*. (2007) reported that radiolysis of flavonoids in ethanol or methanol solution resulted in the formation of new antioxidants depsides.

Although radiolytically-generated hydroxyl radical caused oxidation of urate to allantoin (Grootveld *et al*., 1999), the present study showed that the allantoin level is reduced rather than increased. The explanation of this observation is possibly related to the scavenging effect of polyphenols towards hydroxyl radicals resulted in insufficient level of hydroxyl radical to oxidize the uric acid. In this study, radiation induces minor changes in nitric oxide level, a contradictory finding to others who demonstrates increased reactive oxygen species (Balabanli *et al*., 2006) and reactive nitrogen species (Matsumoto *et al*., 2007). The possible explanation for this difference is related to the methodology of the study including *in vitro* study, irradiated aqueous solution, fixed

low dose of X-rays irradiation…etc. One of the limitation of the study is to test the effect of several ionizing radiations other than X-rays and to test different absorbed doses. It concludes that X-ray radiation of medicinal plants in solutions produces dual effect in terms of improving and degrading the active ingredients depending on the extracted solution as well as the native constituents of each medicinal plant.

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