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X-rays radiation directly produced favorable and harmful effects on the constituents of different medicinal plants

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ABSTRACT

The effect of ionizing radiation on the constituents of solid materials of medicinal plants was studied in few reports. The present study was performed to investigate the direct effect of 1.9Gy/min X-rays radiation on the dry leaves of *Camellia sinensis* (green tea), *Salvia officinalis* (sage), barks of *Cinnamomum verum* (cinnamon) and tuberous of *Zingiber officinale Rosc*. (ginger). Three extracts (1%) were prepared for each medicinal plant; aqueous, ethanol and methanol. The UV-Visible spectra, and biochemical constituents of each non irradiated and irradiated extract were determined. The results showed that X-rays radiation induced remarkable changes in UV-Visible spectra of irradiated compared with non irradiated medicinal plants. This effect was well observed with irradiated green tea leaves. Irradiated medicinal plants lost considerable percents of allantoin and higher percents of flavonoids as well as total polyphenols were lost from irradiated ginger and cinnamon. Irradiated medicinal plants were superior than non irradiated in releasing nitric oxide. It concludes that irradiated medicinal plants carried favorable and harmful effects on their constituents and their favorable effects can be clinically as well as experimentally applied.

Keywords: Medicinal plants, X-ray radiation

INTRODUCTION

Polyphenol" refers to one or more of a class compounds comprising a plurality of hydroxyl groups attached to one or more aromatic groups. They have synthetic, medicinal and industrial value (for example as antioxidants, antimicrobials, pigments and/or UV-absorbers) (1) . They are found to be potential candidates for use in treating or preventing diseases as diverse as heart ailments, ulcer formation, bacterial infection, mutagenesis and neural disorders (2). In addition polyphenols generally have poor oxidation stability (3).

Flavonoids, a class of phenolic compounds widely distributed in plants, can protect the organism against reactive oxygen species and present multiple biological effects, including liver protection, antithrombotic, anticancer and immuno-stimulant activities (4–6). Flavonoids exhibit the highest antiradical property towards hydroxyl radical, peroxyl and superoxide anion (7). The realization that flavonoids form novel compounds following their reaction with free radicals and other oxidant species produced at sites of inflammation has further increased the range of compounds (8,9). On the other side, Allantoin , an anti-aging substance, can be found in few plants and are responsible for wound healing (10,11). Allantoin indirectly contributes to the oxidative stress theory in acute vitiligo via epidermal xanthine oxidase enzyme (12). In vivo, few plants are found to increase the vascular blood flow , possibly via releasing nitric oxide (13,14).

Ionizing radiation is known to stimulate the generation of oxygen radicals which destabilize organic molecules resulting in a decrease of the system's antioxidant potential. Brandstetter et al (15) demonstrated that gamma-irradiation at 10 kGy showed insignificant effect on the total polyphenols and antioxidant activity of sage. Also, irradiation of cinnamon and ginger up to 10 kGy did not show significant differences in the antioxidant activity with respect to the non-irradiated (16). On the other hand irradiated green tea polyphenol at 40 kGy by γ-ray inhibited the collagenase activity of human fibroblast and possessing anti- wrinkle effect compared with non irradiated polyphenol (17). Also, Green tea derived- polyphenol epigallocatechin-3-gallate inhibited the UVB-induced expression of inducible-nitric oxide synthase mRNA and generation of nitric oxide in HaCaT cells (18). In vivo, About two-thirds of X-ray and γ-ray damage to cells is caused by indirect action. Knowing that water radiolysis, the predominant effect of ionizing radiation in organisms, induces reactive oxygen formation. Animal studies show that whole-body exposure to X-ray irradiation decreases tissue concentrations antioxidants (19).

Thus, in this study, an attempt was made to answer the following questions: does acute superficial low dose external irradiation significantly modify the activity of antioxidants and nitrogen reactive species of dry leaves of *Camellia sinensis* (green tea), *Salvia officinalis* (sage), barks of *Cinnamomum verum* (cinnamon) and tuberous of *Zingiber officinale Rosc.* (ginger)? and (ii) do some medicinal plants show the same response with respect to ionizing radiation?

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. Four medicinal plants; *Cinnamomum verum* (cinnamon) bark, *Salvia officinalis* (sage) leaves, *Camellia sinensis* (green tea) leaves and tuberous of *Zingiber officinale Rosc.* (ginger) were investigated in this study. They were obtained from local sources, grinded mechanically and sieved prior to their extraction.

Radiation of herbal extracts

A total number of four containers contained dry fine powder of cinnamon barks , sage leaves, green tea leaves and ginger tuberous within 10 x 10cm 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 (calculated effective energy 30.976 KeV and the absorbed dose 1.9 Gy/min), at room temperature 22°C.

Extracts preparation

Extracts of leaves of cinnamon barks, sage leaves, green tea leaves, and tuberous of ginger were prepared. A 1 g dried herbal fine powder was extracted with 100 mL of distilled water (aqueous extract), absolute ethanol or methanol i.e. (1%) for 24 hours in dark place at room temperature 25°C. The extraction was followed by filtration. The UV-visible spectra of 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).

Determination of the amount of total polyphenolic compounds

This was carried out as described previously (20). Briefly 1 mL of each 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 minutes 1 mL of Na_2CO_3 (5%) was added. Subsequently the mixture was shaken for 1 hour 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.

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 (21). Aliquots of 1.5 mL of extracts were added to equal volumes of a solution of 2% $AlCl_3 \cdot 6H_2O$ (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 were calibrated using the linear equation base on the calibration curve quercetin. Flavonoids content were expressed as µg quercetin equivalent/mg dry weight.

Determination of allantoin

This was carried out as described previously (22) using Ehrlich's reagent, which consists of 1 g *p*-dimethylaminobenzaldehyde (ϱDMAB) in a mixture of 25 mL concentrated HCl and 75 mL methanol. 1 mL of each extract was mixed with Elrich's reagent (1:2 v/v), incubated at room temperature and read the absorbance at 440nm. The allantoin content was calibrated using the

linear equation based on the standard allantoin calibration curve.

Nitric oxide assay

Nitric oxide donating activity was determined as describe by Newaz et al (23) using Griess's reagent. Briefly, 3 mL of each extract (1: 2 v/v distilled water) was added to 50µL HCl (6.5M) and 50µL sulfunalic acid (37.5mM), After incubation of 10 min, 50µL naphthylethylenediamine hydrochloride (12.5 mM) was added and incubated for further 30 min, centrifuged for 10 minutes at 3000 rpm. The reference nitric oxide donating compound was 5 mM sodium nitroprusside. The absorbance was immediately recorded at 540nm. Experiments were performed in triplicate.

Statistical analysis

The results are presented as absolute numbers and percents.

RESULTS

UV- Visible spectra of investigated medicinal plants showed that X-rays radiation induced changes in the constituents of aqueous extracts in term of shifting peaks or appearance of new peaks that were not observed in non irradiated extracts (Table 1). The active ingredient peak at 273 nm was observed in irradiated aqueous green tea extract. X-rays radiation produced decrease in the peaks of active ingredients of ethanol extract of cinnamon, sage and ginger but not green tea (Table 1). An optic density of 0.371 at 273.5 nm of non irradiated ethanol extract of green tea was augmented to 0.520 after radiation. Irradiated medicinal plants extracted with methanol showed decrease in the peaks of active ingredients except the sage which showed a slight increase in the optic density of peak detected at 286 nm (Table 1).

Irradiated medicinal plants resulted in degradation in allantoin particularly with aqueous extract which amounted 46%, 70%, 85% and 90% loss for cinnamon, sage, green tea and ginger respectively (Table 2). The lower percents of allantaoin degradation were observed with medicinal plants extracted with methanol (Table 2). Irradiated ginger and cinnamon showed higher percent of degradation of flavonoids in all extracts compared with sage and green tea which ranged 91–98.5% with ginger and 56–89% with cinnamon (Table 2). A constant finding of increase contents of flavonoids in irradiated sage was observed in all extracts (Table 2). Table 2 showed that the changes in total polyphenols of irradiated cinnamon and green tea for all extracts were similar to that observed with flavonoids while the changes in the total polyphenols of irradiated sage and ginger were inconstant. The interesting observation was the ability of irradiated medicinal plant to generate nitric oxide in all tested extracts that was clearly observed after extraction with ethanol or methanol (Table 2).

DISCUSSION

This preliminary study was designed to explore the direct effect of low dose superficial X-rays radiation on solids materials of medicinal plants. Several findings emerged from this study. First, the alterations in the constituents of medicinal plants as demonstrated by UV-visible spectra are not related to the oxidative damage mediated by the ionization of water (radiolysis) and that free radicals are

Table 1. UV-Visible spectra of medicinal plants extract before and after X-ray irradiation at 1.9 Gy/min

Medicinal plants	Aqueous extract		Ethanol extract		Methanol extract	
	Wavelength	Optic density	Wavelength	Optic density	Wavelength	Optic density
Cinnamon						
Not irradiated	202.5, 286.5	1.132, 0.456	191.5, 287	1.242, 0.524	191.5, 287	1.361, 0.675
Irradiated	195, 281	0.641, 0.015	191.5, 203, 288	1.078, 0.840, 0.461	202.5, 286	1.416, 0.560
Sage						
Not irradiated	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	201.5, 270	0.568, 0.131	191.5, 281	0.729, 0.016	201, 286, 434.5, 669	1.533, 0.291,0.050, 0.023
Green tea						
Not irradiated	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	210, 273	2.833, 0.527	201, 273, 415.5, 671.5	2.914, 0.520, 0.024, 0.012	202, 273.5, 671	3.000, 0.896, 0.017
Ginger						
Not irradiated	195, 278.5	0.752, 0.053	191.5, 278	0.815, 0.040	191.5, 277, 578.5	0.741, 0.052, 0.003
Irradiated	203, 286	0.764, 0.316	191.5, 281	0.729, 0.016	191.5, 280	0.430, 0.020

Table 2. Effect of X-ray irradiation (1.9 Gy/min)on the antioxidant constituents of medicinal plants.

	Aqueous extract				Ethanol extract				Methanol extract			
	A	F	P	NO	A	F	P	NO	A	F	P	NO
Cinnamon												
Not irradiated	2.5	21.6	128.7	0.12	6.78	12.74	185.2	0.42	8.28	21.64	270.3	0.46
Irradiated	1.35	2.564	62.9	0.0	3.25	5.502	109.25	2.324	4.95	7.423	43.98	2.324
Sage												
Not irradiated	3.98	10.4	12.5	0.18	14.78	21.07	185.2	0.38	13.28	21.07	212.9	0.42
Irradiated	1.2	17.254	48.148	0.0	7.35	22.903	104.16	2.324	10.35	23.468	125	2.324
Green tea												
Not irradiated	3.88	21.64	518.5	0.068	11.78	20.5	481.4	0.26	16.28	21.1	296.3	0.264
Irradiated	0.6	15.559	269.3	0.524	5.85	20.644	291.66	2.124	8.35	22.903	250	1.724
Ginger												
Not irradiated	5.1	187.2	4.43	0	7	92.4	52.6	0.35	11.5	95.2	50.0	0.37
Irradiated	0.55	2.790	64.62	0.524	2.85	5.954	51.85	1.324	4.85	8.101	72.22	2.124

The results are expressed as µg/mg dry weight for allantoin (A), flavonoids (F)and total polyphenols (P), and as µmol nitric oxide (NO)/ mg dry weight

formed by reacting with dissolved oxygen (24). Fixed low dose ionizing radiation improved the active constituents of green tea demonstrated by UV-Visible spectra. This finding is in agreement with others who showed improvement in some quality and nutritive values of seeds irradiated by gamma rays up to 30 kGy (25). Second, the changes in the UV-visible spectra are not related to the nature of medicinal plants but to the extracted solvents. Li et al (26) reported that methanol produced a higher recovery of polyphenolic acid than pure water and the lower the carbon number of straight chain alcohol solvents, the higher the recoveries of the polyphenolic acids. This observation highlighted an important practical point that it is the value of irradiated green tea is higher than that of non-irradiated. Third, ionizing radiation induced changes in physiochemical properties and this led to degradation of allantoin and further subjected to hydrolysis when it extracted with water or alcohols solvents (27). This finding was observed in all irradiated medicinal plants of whatever solvent is used in extraction. Therefore, it is unlikely to use irradiated medicinal plants in pharmaceutical preparations that contained allantoin as an active ingredient. Also this finding was observed with flavonoids which lost their stability after ionization with X-rays. Several previous studies demonstrated the radioprotective effects of flavonoids against radiation but there was no report on the effect of ionizing radiation against flavonoids (28). The higher percent of flavonoids recovery in irradiated sage could be explained in term of availability of radioprotective substances in this medicinal plant which reduced the radiosensitivity effect of flovanoids towards radiation (29). The incompatibility in the recovery of flavonoids and polyphenols in this study is explained in term of extracted lipid soluble antioxidants as well as the method of determination of antioxidants (30). Fourth, X-rays radiation induced the release of nitric oxide from medicinal plants. There is an evidence that X-rays radiations induced the release of nitrogen radical species from the cellular elements but not from medicinal plants in vitro model (31). This observation is of great importance because it is possible to use irradiated medicinal plants as nitric oxide donor and utilize them in diseases with dysfunction of vascular endothelium. The limitations of the study are to isolate and determine each of polyhenols from irradiated medicinal plants and to study its biological effects in experimental model. It concludes that irradiated medicinal plants carried favorable and harmful effects on their constituents and their favorable effects can be clinically as well as experimentally applied.

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