Radio-protective Potential of Rosemary (Rosmarinus Officinalis) against Effects of Ionising Radiation

MARIA MARIS[1], DAN A. MARIS[1], SILVIU JIPA[2,3*], TRAIAN ZAHARESCU[3], LAURA MONICA GORGHIU[2]

[1] „Ovidius" University of Constanţa, Faculty of Dental Medicine, 7 Ilarie Horonca Str., 900684, Constanta, Romania

[2] Valahia University of Targoviste, Faculty of Sciences and Arts, 18-24 Unirii Av., 130082, Targoviste, România

[3] INCDIE ICPE CA, Department of Radiation Processing, 313 Splaiul Unirii, 030138, Bucharest, România

Several herbs have been screened for their antioxidative activity using chemiluminescence method. Rosemary contains phenolic diterpenes, phenolic acids and flavonoids which protect cells and their organic constituent molecules from free radical radiation- induced oxidative damage. Effects of 0.1 and 0.2 g/Kg body wt. of Rosemary extract on radiation – induced morbidity and mortality in mice exposed to 10Gy of gamma radiation were studied for the characterization of high efficiency protection exhibited by Rosemary extract.

Keywords: rosemary, chemoluminescence, antioxidative activity, ionizing radiation, morbidity, mortality

The human organisms could be exposed to ionizing radiation in clinical, military or industrial applications. Gamma irradiation causes oxidative stress generating reactive oxygen species such as hydroxyl ($HO^{\cdot}$) radicals, superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). These free radicals can easily damage the structural and functional components of cells such as lipids, proteins and nucleic acids, causing oxidative injury and disturbance in cellular metabolism. By definition, radioprotectors are chemical compounds that have the ability to reduce the oxidative effects of ionizing radiation on normal tissues.

Numerous studies have examined the radio-protective effects of antioxidant compounds, generally known as free radical scavengers, which protect cells from free radical damage. Various plant extracts such as Ginkgo biloba [1], Panax ginseng [2], Mentha piperita [3], Zingiber officinale [4], Camellia sinensis [5], Hippophae rhammoides [6], Allium sativum [7], Agaricus blazei a.s.o., have been investigated to evaluate their radio-protective effects.

The present study deals with the radio-protective activity of ethanolic extract prepared from Rosmarinus officinalis in terms of antioxidative activity and on Swiss albino mice post irradiation morbidness and death rate, respectively.

Experimental part

The dried Rosemary plant (10 g) and the extracting solvent (ethanol) were placed in an Erlenmeyer flask (250mL); the ratio of plant material and extracting solvent was 1:10 w/v.

Maceration was performed for 120 h at room temperature, by permanent shaking.

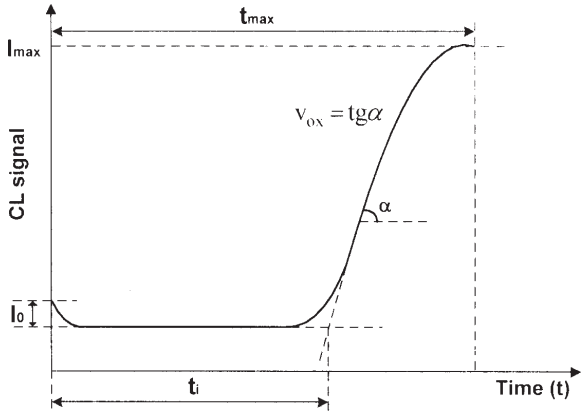

Fig. 1. Kinetic parameters from typical chemiluminogram

The liquid extract was separated from the plant material by filtration, and the solvent was evaporated under vacuum. The solid extract has been used for paraffin modification (0.25 wt %). Similarly, paraffin samples of extracts from other radioprotective extracts from plants have been obtained (fig. 1).

Isothermal chemiluminescence determinations were performed in air at 160^{0}C by a Lumipol-3 instrument. The meanings of kinetic that are evaluated in this paper are presented in figure 1 and table 1.

The radio-protective capability of the Rosemary extract was analysed on a batch of 60 white mice. All mice were adults, ages over 15 weeks, both genders, weight ranging between 20 and 30 g each. The location chosen for the experiment provided a constant environmental temperature of 21±2^{o}C, an atmospheric pressure of 759±4 mm Hg and natural lighting. For a period of four days the mice were given tetracycline to prevent infection.

Table 1
CL PARAMETERS AND THEIR MEANING

Parameter	Significance
t_i	Oxidation induction time; the time during which the CL emission intensity is stationary due to the inhibition of the oxidation by the antioxidants
t_{max}	The time when CL intensity reaches the maximum value; it corresponds to the complete oxidation of the substrate
v_{ox}	Oxidation rate; it is determined as the slope of the CL curve on ascending part
I_{max}	CL maximum intensity
I_0	CL initial intensity

* email: jipasilviu@yahoo.comTel.: (+40) 01 / 3143508 int. 2153

The tested animals were divided into three experimental batches, each equal in number. The mice batches were kept in distinct conteiners, marked for identification.

For seven days the experimental batches were subject to a food diet in the following way:

- batch 1 – was given a standard diet for 7 days;
- batch 2 – the daily diet included and average Rosemary extract dose of 0.1 g / Kg body wt. for 7 days;
- batch 3 – the mice were given and average Rosemary extract dose of 0.2 g / Kg body wt. for 7 days.

After seven days, the mice batches were irradiated with ^{137}Cs γ radiation at dose of 10 Gy using a GAMATOR M-38-2 source. Then, the batches were monitored to observe the occurrence of morbidity and death rate.

Results and discussions

Figure 2 illustrates the isothermal chemiluminescence curves (160°C, air) of the paraffin stabilized with various plant extracts which prove radio-protection effect. The antioxidant efficiency in stabilizing an organic substrate is given by the increased value of the induction time as well as by the lower value of the oxidation rate in comparison with the unprotected sample. The curve describing the contribution of Rosemary to the inhibition of substrate oxidation presents the biggest induction time of the samples. This feature denotes the exceptional efficiency of Rosemary extract in the prevention of oxidation.

Table 2 shows the oxidation kinetic parameters of the samples presented in figure 2.

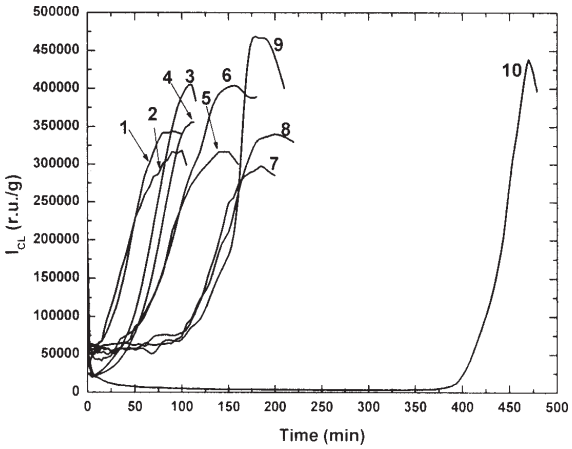

Fig. 2. Isothermal CL curves (160°C, air) for paraffin stabilized (0.25% wt%) with various plant extracts which prove a radioprotection effect: (1) control; (2) Garlic; (3) Ginseng; (4) Agaricus; (5) Mint; (6) Sea-buckthorn; (7) Ginkgo; (8) Ginger; (9) Green Tea; (10) Rosemary

The longer is the induction time, the stronger is the antioxidant activity. The analysis of the data in table 2 clearly shows that the Rosemary extract has the highest antioxidant activity. The researchers [9] showed that the antioxidant activity of the Rosemary extract is due, mainly (over 90%), to some phenolic diterpenes such as carnosic acid, carnosol, rosmanol, isorosmanol, rosmadial, epirrosmanol, rosmaridiphenol, rosmariquinone etc (fig. 3) [9]. Most of these diterpenes act as oxidative chain breakers.

Carnosic acid has its potent antioxidant activity based on oxidation cascade (fig. 4): after its molecules interacted with oxidizing material, it is turned to carnosol. Carnosol also extracts a free radical becoming rosmanol. Rosmanol continues the free radical scavenging until galdosol is formed, and further continues the scavenging process [10, 11].

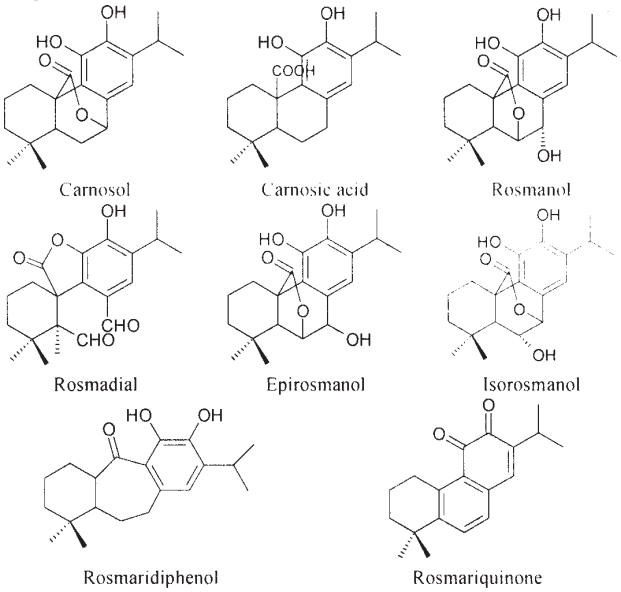

Fig. 3. Phenolic diterpenes in Rosemary composition

The caffeic and rosmarinic acids associated with flavonoids represent other important source of Rosemary antioxidants.

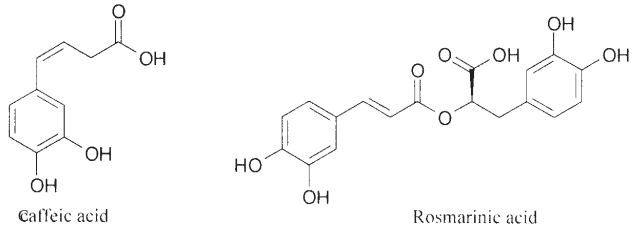

Table 2
KINETIC OXIDATION PARAMETERS (CL) FOR PARAFFIN DOPED WITH VARIOUS RADIOPROTECTIVE PLANT EXTRACTS

Extract	t_i (min)	t_{max} (min)	v_{ox} (r.u./g·min)	I_{max} (r.u./g)
control	16	52	68000	340000
Rosemary (Rosmarinus officinalis)	423	470	64280	442000
Green Tea (Camellia sinensis)	150	175	65800	460000
Ginger (Zingiber officinale)	107	183	50900	339900
Ginkgo (Ginkgo biloba)	108	180	53100	291000
Sea-buckthorn (Hippophae rhamnoides)	55	144	55220	400000
Peppermint (Mentha piperita)	54	140	55000	315000
Agaricus (Agaricus blazei)	50	100	57236	350000
Ginseng (Panax ginseng)	38	98	59616	360000
Garlic (Allium sativum)	26	75	60100	330000

The reactions of these compounds with peroxy radicals follow the scheme:

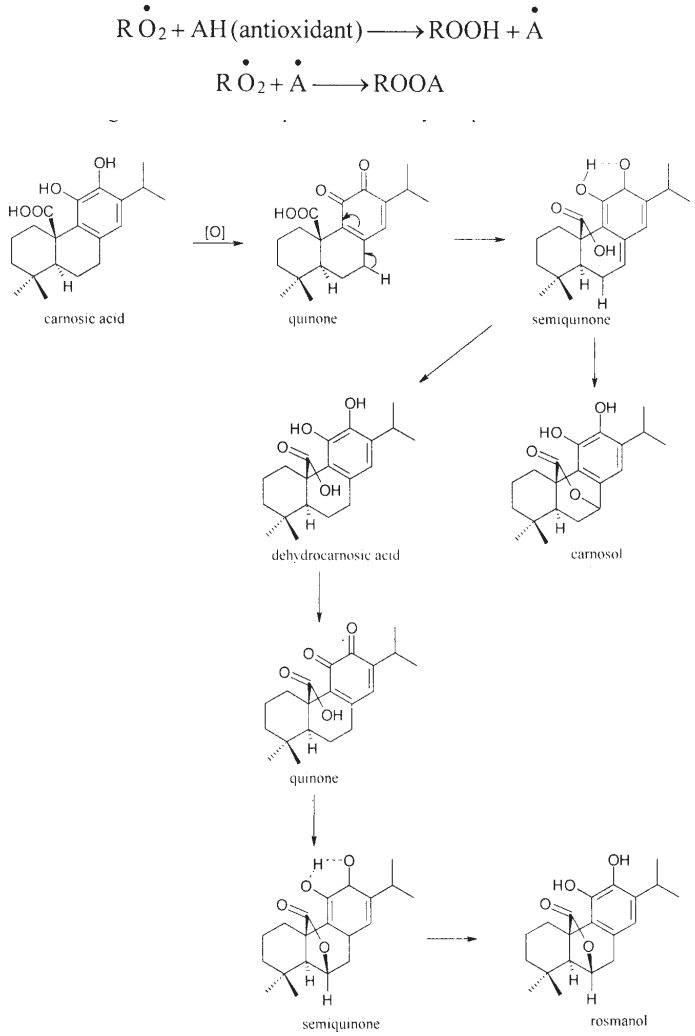

Figure 4. Oxidation cascade reactions of carnosic acid

The outstanding antioxidant activity of Rosemary extract is based on its radio-protective capability which results from the morbidity and mortality analysis of the mice fed with Rosemary extract and further irradiated with γ radiation of ^{137}Cs at the dose of 10 Gy. Morbidity watched out for the pathological skin modifications (hair loss) and the appearance of toxic signs (diarrhoea) with the mice batches under study. Due to the increase in the Rosemary extract amount given to the mice batches, a delay in the occurrence time of mice morbidity and mortality was recorded as shown in figure 5.

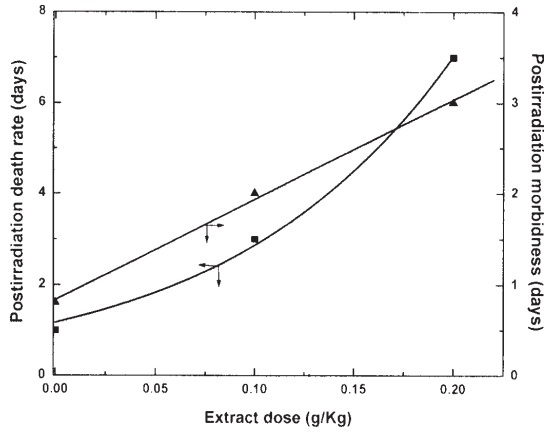

Fig. 5. The delay in the mice morbidity and mortality after γ ^{137}Cs (10Gy) irradiation due to the increase of the administrated Rosemary dose

The ionizing radiation occurred during exposure induce the lipid peroxidation chain reactions, which damage DNA and cell. The phenolic antioxidant contents in the composition of Rosemary extract, taken before irradiation, significantly reduce lipid peroxidation [12] (scheme 1).

Another hypothesis considers the increased level of glutathione in the blood for Rosemary pre-treated animals; this prevents lipid peroxidation [12-15]. Rosemary extract proved to be an effective radio-protector in animals exposed to gamma irradiation dose of 10 Gy.

INITIATION: Lipid + R$^\bullet$ (or HO$^\bullet$) → (Lipid)$^\bullet$

PROPAGATION: (Lipid)$^\bullet$ + O_2 → Lipid-OO$^\bullet$
Lipid-OO$^\bullet$ + Lipid → Lipid-OOH + (Lipid)$^\bullet$

TERMINATION: (Lipid)$^\bullet$ + (Lipid)$^\bullet$ → Lipid- Lipid
Lipid-OO$^\bullet$ + (Lipid)$^\bullet$ → Lipid-OO-Lipid

TRAPPING: (Lipid)$^\bullet$ + Antioxidant → Lipid + (Antioxidant)$^\bullet$

Scheme 1 Lipid oxidation

Conclusions

Rosemary extract shows a remarkable activity in stabilization of organic substrate.

Rosemary extract could offer protection against the effects of ionizing radiation because of their ability to scavenge free radicals.

Phenolic diterpenes, caffeic and rosmarinic acids associated with flavonoids in Rosemary extract suppress lipid peroxidation and stop oxidative DNA damage and so it may be useful as radio-protective agent.

Chemiluminescence has proved its versatility in fast and accurate assessment degradation study.

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