PROTECTIVE EFFECTS OF SEVERAL PLANT POLYPHENOLS AGAINST CHROMOSOMAL DAMAGE INDUCED IN VIVO BY X-RAYS. COMPARATIVE STUDY VERSUS DIOSMIN AND RUTIN*

XA0101760

M. Alcaraz[1], B. Rosa[1], J. Castillo[2], O. Benavente-García[2], J. Lorente[2], V. Vicente[3], M. Canteras[4]

[1]Radiology and Physical Medicine Department of Furfural Español S.A., Murcia, Spain
[2]Research and Development Department of Furfural Español S.A., Murcia, Spain
[3]Pathology Department of Furfural Español S.A., Murcia, Spain
[4]Biostatistical Department, Faculty of Medicine, University of Murcia, Murcia, Spain

Abstract

Protective effects of grape (Vitis *vinifera*) seed (GSE), *Citrus spp.* fruits (CE) and olive (*Olea europaea* L.) leaf (OL) extracts, the flavonoids diosmin and rutin, widely used as pharmaceuticals, and dimethylsulphoxide (DMSO) against chromosomal damage induced by X-rays were determined by using the micronucleus test for anticlastogenic activity. The reduction of the frequency of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow of mouse exposed to X-rays was examined. The most effective compounds were, in order: GSE ≈ CE > rutin ≈ DMSO ≈ OL > diosmin. These results suggest a correlation between the antioxidant and anticlastogenic activity of these polyphenolic extracts.

1. Introduction

The micronucleus test "in vivo" is a method devised primarily for screening chemicals for chromosome-breaking effects. The test substances are normally applied sub-acutely to small mammals, and the effect is read in direct smears from bone marrow. The micronucleus assay on mouse bone marrow polychromatic erythrocytes, originally developed by Schmidt (1975) [1], is probably the most frequently used in vivo short-term genotoxicity tests. Bone marrow micronucleated erythrocytes provide a simple and rapid method for detection of chromosomal damage by chemical and physical agents [1-4]. For this reason, micronuclei have been widely used to detect chromosomal breakage and chromosome lagging "in vivo" and "in vitro" [2-4].

2. Materials and methods

Plant Materia: Grape Seeds Extract (GSE) was obtained from four different varieties of *V. vinifera* grapes selected in different areas of the community of Murcia (Spain): "Macabeo" and "Airen" are white grapes and "Tempranillo" and "Monastrel" are red grapes. The grapes were picked at their optimum commercial maturity.

Citrus Fruit Extract (CE) was obtained from immature fruits of several characteristic cultivars from the region of Murcia from three *Citrus* species: *Citrus limonia, Citrus paradisi* and *Citrus aurantium*. The fruits were harvested from the trees by natural abscission during the initial phase of the fruit growth.

Olive Leaf Extract (OL) was obtained from *Olea europaea* L. leaves of five cultivars: Villalonga, Alfafarenca, Picual, Cornicabra and Blanqueta from the regions of Andalucia and Murcia. The leaves were collected when the olive fruits were picked at their usual commercial time.

* This work was supported by a grant from the European Union (grant no. 1FD97-0576).

Chemical Reagents:

Diosmin and rutin were obtained from Extrasynthèse S.A. (Genay, France). DMSO was obtained from Merck (Darmstadt, Germany). Fetal calf serum was obtained from Sigma Chemical Co. (Madrid, Spain).

Extraction and HPLC Analysis of Polyphenolic Compounds from Plant Material

The methods to obtain and quantify the grape seed (GSE), citrus (CE) and olive leaf (OL) extracts have been described previously.

Animals

Adult male Swiss albino mice, 9-12 weeks of age, weighing approximately 25 g were used from our animal colony (license 300030-2A). All mice were acclimatized for at least one week prior to dosing. They were maintained under constant environmental conditions with 12/12 h light/dark cycle. They were fed standard granulated chow (Rodent toxicology diet®, BYK Universal Beekay Feeds, France) and given drinking water ad libitum. Each group consisted of 6 mice.

Chemicals and Treatment

The polyphenolic extracts were administered orally. All solutions were freshly prepared immediately before treatment of the animals. GSE, CE, and OL were dissolved in 0.2 % drinking water and administered during 5 days before the X-irradiation. DMSO was dissolved in water (50 g/100 mL). Diosmin and rutin were dissolved in DMSO (300 mg/mL). DMSO, diosmin and rutin were injected in a single dose of 0.6 mL directly into the gastric lumen 6 h before the X-irradiation.

Exposure to X-rays

The mice were whole-body X-irradiated using CGR apparatus with radioscopy (General Electric, Spain). During exposure to X-rays, the animals were placed in a well-ventilated acrylic box. Irradiation conditions: 120 kV, 1.4 mA, filter 2.5 mm Al, exposure rate of 2cGy/min, FDO 100 cm. The mice were exposed to a single dose of 48 cGy. The X-ray exposure was established by means of thermoluminescent dosimeters (TLDs) (GR-200®, Conqueror Electronics Technology Co. Ltd, China). The TLDs were supplied and measured by CIEMAT (Ministry of Industry and Energy, Spain)

Bone Marrow Preparation and Staining

Two femurs were removed from each mouse 24 h after X-irradiation, and bone marrow samples were taken. The bone marrow cells were dispersed by gently pipetting and then collected by centrifugation at 1,000 rpm for 5 min at 4°C. Cell pellet was resuspended in one drop of fetal calf serum and bone marrow smears (two slides per mouse) were prepared. The slides were coded to avoid observation bias. After 24 h air-drying, the smears were stained with May-Grünwald/Giemsa[48, 49]. With this method polychromatic erythrocytes (PCEs) stain reddish-blue and normochromatic erythrocytes (NCEs) stain orangey, while nuclear material is a dark purple colour. The number of micronucleated polychromatic erythrocytes (MnPCEs) among 2,000 PCEs per mouse (1,000 PCEs per slide) was determined. The slides were examined at 1,000x magnification using a Zeiss light microscope (Oberkochen, Germany).

Statistical Evaluation

Differences in the frequency per animal of MnPCEs per 1,000 PCEs were tested by analysis of variance and evaluated using Student's t-test.

3. Results

The data presented (Figure 1) show that whole-body exposure to 48 cGy of X-rays results in a substantial increase in the frequency of MnPCEs in comparison with that occurring spontaneously ($p<0.001$). There is a significant reduction of frequency of MnPCEs in all pre-treated, irradiated groups compared with the control and irradiated group.

Figure 1 shows the influence of treatments on the frequencies of MnPCEs in the bone marrow of animals non-irradiated and irradiated, permitting thus to compare the potential toxicity of each treatment vs. their anticlastogenic activity. Diosmin, rutin, GSE, CE and OL show very low levels of MnPCEs generation, similar in respect to non-irradiated control data, while the sulphur-containing compound, DMSO, presents higher genotoxicity levels (>5 MNPCEs/ 1000 PCEs) than the other compounds studied. Also, Figure 1 shows the influence of X-irradiation on the frequencies of MnPCEs in mouse bone marrow. There is a significant reduction of frequency of MnPCEs in the pre-treated groups compared with the irradiated control group. The order of treatments with respect to the minor level of MnPCEs generated after irradiation is: GSE ≈ CE < rutin ≈ DMSO ≈ OL < diosmin (at least $p<0.05$ in each one of the steps represented).

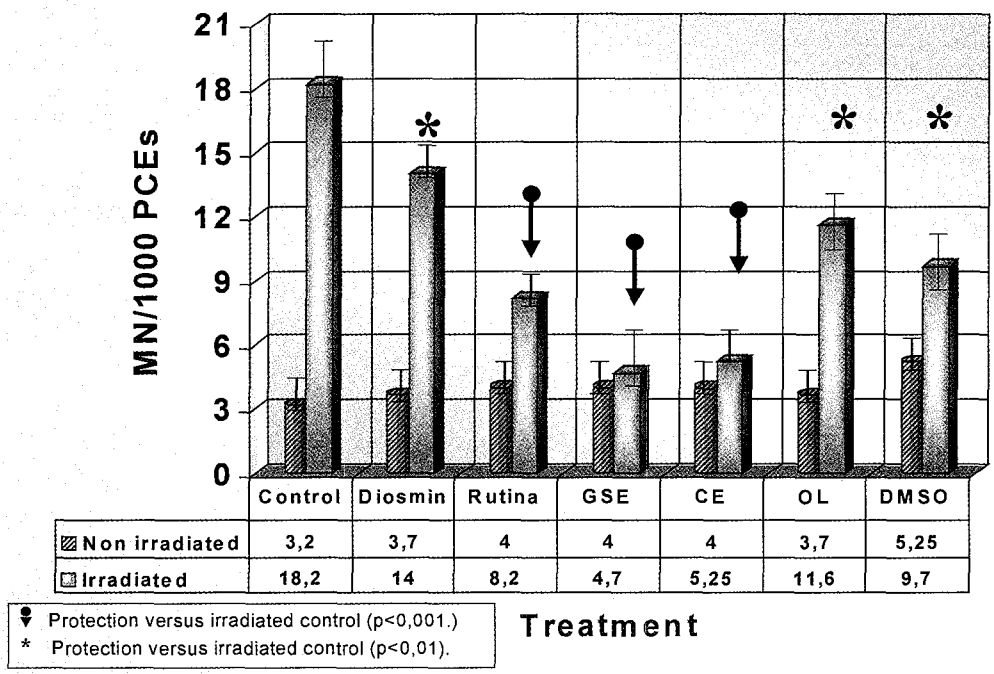
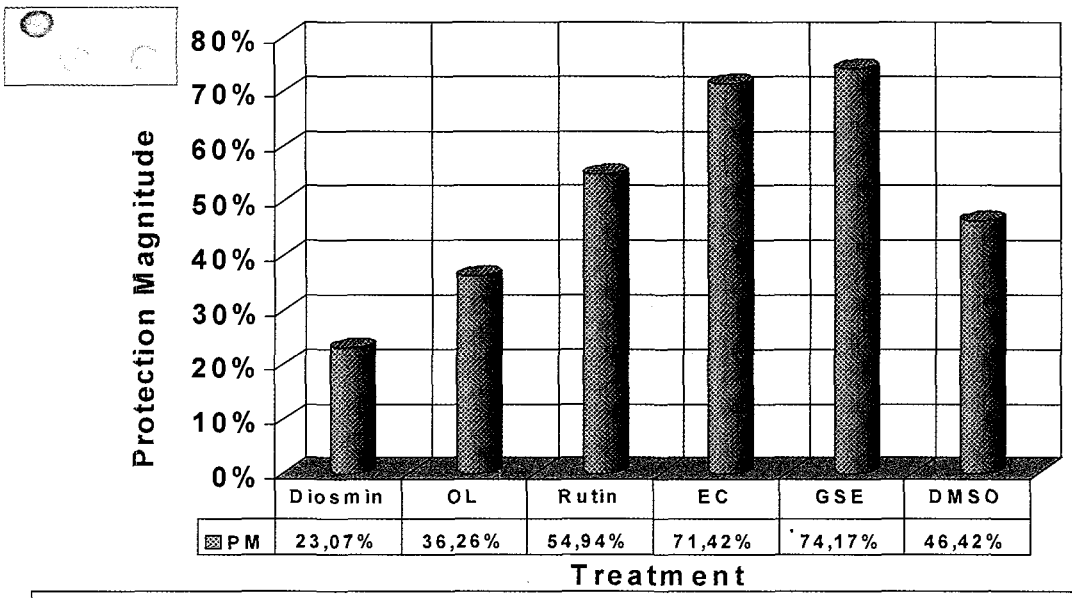


Figure 1. Influence of treatments and X-rays irradiation on the frequencies of MnPCEs in mouse bone marrow (irradiated and non-irradiated)

The radioprotective effects, and consequently the anticlastogenic activity of the different treatments used, were established according to the increase in the MnPCE level in animals after irradiation and their relation with this level in control animals, obtaining a percentage value that shows the level of protection of each treatment. Figure 2 shows the values of these protection capacities, the GSE-pre-treated group being the most effective protection against in vivo chromosomal damage and cytotoxicity induced by X-rays. The order of effectiveness was: GSE ≈ CE > rutin ≈ DMSO ≈ OL > diosmin.



Magnitude of protection (%) = $((F_{control} - F_{treated}) / F_{control}) \times 100$.
Where $F_{control}$ = frequency of MnPCEs in irradiated animals.
$F_{treated}$ = frequency of MnPCEs in animals treated before the X-ray irradiation (diosmin, rutin, GSE, CE and OL)[69].

Figure 2. Protection magnitude of different treatments in relation to irradiation with X-rays.

References

[1] SCHMIDT, W,. The micronucleus test, Mutation Research 31 (1975) 9-15.

[2] HEDDLE, J A, SALAMONE,M F, Chromosomal aberrations and bone marrow toxicity, Environmental Health Perspectives 39 (1981) 23-27.

[3] ALMASSY,Z, KREPINSKY,A B, BIANCO, A, KOTELES, G C, The present state and perspectives of micronucleus assay in radiation protection. Review, Applied Radiation Isotopes 38 (1987) 241-249.

[4] MAZUR,L, Induction of micronucleated erythrocytes by MEA, AET, WR-2721 and x-rays, Mutation Research 334 (1995). 317-322.

[5] CASTILLO, J, BENAVENTE-GARCIA, O, DEL RIO, J A,. Naringin and neohesperidin levels during development of leaves, flower buds, and fruits of Citrus aurantium, Plant Physiology 99 (1992) 67-73.

[6] CASTILLO, J, BENAVENTE-GARCIA, O, DEL RIO, J A,. Hesperetin 7-0-Glucoside and prurin in Citrus Species (C.aurantium and C. paradisi). A study of their quantitative distribution in immature fruits and as immediate precursors of neohesperidin and naringin in Citrus aurantium, Journal of Agricultural and Food.Chemistry 41 (1993). 1920-1924.

[7] BENAVENTE-GARCIA, O, CASTILLO, J, LORENTE, J, ORTUÑO, A, DEL RIO, J A , Antioxidant activity of phenolics extracted from Olea europaea L. Leaves, Food Chemistry 68 (2000) 457-462.

[8] SARMA, L, KESAVAN, P C, Protective effects of vitamin C and E against γ-ray induced chromosomal damage in mouse, International Journal of Radiation Biology 63 (1993) 759-764.