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CITROFLAVONOIDS and FLAVAN-3-OLS
High radioprotective capacity and prospects for health

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INTRODUCTION

Oxidative stress is reported to be involved in many physiopathological conditions. Taking this concept as a starting point, it is natural consider the possibility of correcting or preventing such conditions by using exogenous antioxidants. Among these, some classes of phytochemical compounds normally present in diets, such as polyphenols, represent particular interesting groups of antioxidants which can reduce peroxidative processes in tissues through different mechanisms (**1-3**). The Mediterranean diet, rich in fresh fruits and vegetables, has been associated with a lower incidence of cardiovascular disease and cancer, partly because of its high proportion of bioactive compounds such as vitamins, flavonoids and polyphenols.

Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-γ-pyrone structure which have been reported to act as antioxidants in various biological systems (**4,5**). They are present in a wide variety of edible Mediterranean plants especially *Citrus* and *Vitis* species. Five types of flavonoids, i.e. flavanones, flavones, flavonols, flavan-3-ols and anthocyanins occur in these two species (**6-9**).

Recent studies on the quantitative distribution of these flavonoids in citrus have shown that the 7-O-glycosylflavanones are the most abundant flavonoids in all species of the genus, whose aglycones are intermediates in the biosynthetic pathway (**10, 11**). Although flavones and flavonols are found in low concentrations in citrus tissues, these types of flavonoids have been shown to be powerful antioxidants and free radical scavengers (**12**). Recently, these flavonoids have attracted attention as potentially important dietary cancer chemoprotective agents (**13-16**). In addition, the possible antitumour action of certain flavonoids has also generated interest. Citrus flavonoids, due to their antioxidant properties and their ability to absorb UV light, may act at all stages of the carcinogenic process: i.e. damage to the DNA (or initiation step), tumour growth (or promotion step) and invasion (or proliferative step) (**4,17**).

Furfural Español has developed a combination of citroflavonoids and flavan-3-ols* (Cf complex) with high radioprotective capacity.

Flavan-3-ols can be obtained from various botanical sources and the difference is in the varying concentration in the different plants and the specific profile of procyanidin compounds (polymerization degree, esterification degree, *etc*) (**18-20**).

Flavan-3-ols present in Cf complex are polymers formed by more than 12 monomers of the flavan-3-ol skeleton, obtained from the rare

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* ***Nutrafur, Citroflavan-3-ol Health Protector*** is a product developed by *Furfural Español*

Spanish *vitis* species, the highest polymerization degree found in any plant source (**21**). The polymerization degree of flavan-3-ols are characteristic of the raw material used, but they can be changed and/or modified by the procedures used in extraction of these polyphenols, as well as in the subsequent operations of purification and/or crystallization. In addition, these flavan-3-ols have an advantage over flavan-3-ol extracts from other plants since they contain compounds esterified with gallic acid. These procyanidin-esters have been recently described as the most active substances *versus* free radical damage (**22-24**).

It is important to note the relation that exists between the molecular profile of procyanidin compounds and their physical properties, and their biological and health effects. In fact, flavan-3-ols ranging from monomers to trimers show less activity in capillary protection, and antiulcer properties in gastric tissues than do polymeric flavan-3-ols (**23, 25-28**). UV light, X-rays and γ-rays generate hydroxyl radicals in organisms and induce cellular DNA damage which leads to mutations and chromosomal aberrations (**29,30**). Flavonoids and flavan-3-ols are radical scavengers (**4,31,32**) and protect against the DNA damage induced by radiation (**5,33**).

Properties and applications of flavonoids and flavan-3-ols

The health-related properties of citrus flavonoids and flavan-3-ols, based on their antioxidant activity, have been found to include anticancer, antiviral and anti-inflammatory activities, capillary fragility effects and human platelet aggregation inhibition (**4,22**). Free radical formation is associated with the normal natural metabolism of aerobic cells. The oxygen consumption inherent in cell growth leads to the generation of a series of oxygen free radicals, which are the most abundant and characteristic species in the phenomenon known as 'oxidative stress'. The interaction of these species with molecules of a lipid nature produces new radicals: hydroperoxides and different peroxides. This group of radicals (superoxide, hydroxyl and lipoid peroxides) may interact with biological systems in a clearly cytotoxic manner. These species interact with such life essential molecules as nucleic acids and proteins, in which they produce oxidative reactions involving alterations and protein exchange, a fundamental process whose efficacy is subordinate to the functional activity of potential repair systems (**3,34**).

Research into the mechanisms by which these free radicals can be blocked and/or scavenged is, therefore, of manifest interest. In this respect, phenolic compounds, particularly flavonoids and flavan-3-ols, have been shown to posses an important antioxidant activity towards these radicals, which is principally based on their structural characteristics. Together with an ability to capture electrons, such characteristics impart great stability to the flavonoid radical formed by means of tautomeric dislocation, which prevents the propagating chain reactions of these oxygen free radicals (**4,32**).

The active compounds of Cf complex are valued as therapeutic agents in the treatment of vascular disorders, such as collagen instability in the arterial wall, arterially localized histamine formation and cholesterol oxidation (**35-38**). They show anti-inflammatory (**39,40**), antihypertensive (**41**), antiviral (**28,42,43**), vitamin C and E sparing (**35,44**), antimutagenic (**17,45,46**) activities, and inhibit some undesirable enzymatic activities (**23,47**).

Radioprotective Activity

Based on the good antioxidant and radical scavenger properties of Cf complex, *Furfural Español* has developed a new application for this product as radioprotector against UV, X- and γ-radiation (patent pending). Ionizing radiation cause a high degree of *in vivo* hydroxyl radical generation by homolytic cleavage of body water or from the endogenous hydrogen peroxide formed by reduction of the superoxide anion by the Haber-Weiss or Fenton mechanism (**48**). The hydroxyl radical is the most cytotoxic radical of all those so far described, with an estimated half-life of about 10^{-9}s (**48**).

Its high reactivity leads to an immediate reaction at the place where it is generated. When hydroxyl radical generation is massive, as it is during UV, X- or γ-irradiation, the cytotoxic effect is not merely local but may result in intracellular and extracellular propagation. This increases the interaction of these radicals with phospholipid structures, inducing peroxidation processes that increase hydroxyl radical activity in DNA oxidative damage (**4**,**34**).

In these oxidative stress conditions, when the endogenous antioxidant systems are defective or insufficient, exogenous agents with a strong-radical scavenging capacity must be used. This capacity depends on high absolute reactivity against different radicals or the high stability of the intermediate aroxyl radical formed (**4**).

Vitamin C is considered to be one of the most powerful and least toxic natural antioxidants (**49**).

DMSO (dimethylsulfoxide) and PTU (propylthiouracil) can be considered as radioprotective agents according to structural and experimental data (**50**).

PTU and other thiouracil compounds, in particular, have been shown to have a chemical radioprotective effect against X-irradiation on the thyroid gland of rats, acting through inhibition of thyroid peroxidase with a consequent decrease of oxygenation and thyroid metabolism (**51**).

DMSO is a classic radical scavenger, with a capacity for in vitro hydroxyl radical scavenging higher than that shown by many flavonoids, i.e. 30% higher than quercetin (aglycon of rutin), 40% higher than diosmin and 50% higher than (+)-catechin (**52**,**53**).

However, when applied in radioprotective doses, in the absence of any subsequent irradiation, these S-containing compounds are highly toxic in animals (**54-56**).

Flavonoids are excellent hydroxyl scavengers (**4**,**48**, **52**,**53**,**57**), their effectiveness obviously depending on their structure.

It is known that this capacity to inhibit hydroxyl radicals is based principally on the binary substitution model in the B-ring (*o*-dihydroxy or catechol structure) and, to a lesser extent, on the presence of a 3-OH group in the flavonoid skeleton (**4**).

This greater activity of compounds with a catechol structure can be attributed to the stability of the flavonoid radical generated in the process.

We studied the activity of Cf complex, GSP (grape seed polyphenols), ascorbic acid, DMSO, PTU, and the most used flavonoids in pharmaceuticals, rutin and diosmin, as radioprotective agents.

MATERIALS and METHODS

Chemicals and Reagents

The following chemicals and reagents were used: (+)-catechin, quercetin, rutin, diosmin and luteolin-7-glucoside (Extrasynthèse, Genay, France), ascorbic acid, ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt), Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid), manganese dioxide, fetal calf serum, 6n-propyl-2-thiouracil-6c (PTU), F-10 medium, foetal bovine serum, phytohaemagglutinin, penicillin/streptomycin and cytochalasin B (all from Sigma, Madrid, Spain), DMSO (Merck, Darmstadt, Germany).

Grape seed extracts, GSE1 and GSE2, were obtained from European and Chinese commercial sources, respectively.

Naringin, grape seed polyphenols (GSP) and Cf complex were manufactured by Nutrafur-Furfural Español S.A.

The active principles of Cf complex are the following: polymeric flavan-3-ols, 23%, flavanones (neoeriocit- rin, naringin, neohesperidin), 25%, flavones (apigenin and rhoifolin) and flavonols (kaempferol), 8%.

In addition, the amounts of flavan-3-ols longer than tetramers present in the studied grape seed products are the following: GSE_1, 35%, GSE_2, 17%, GSP, 7% and Cf complex, 0.8%.

Figure 1 shows the chemical structures of the main flavanones, flavones, flavonols and polymeric flavan-3-ols present in Cf complex.

The methods used to extract and identify the grape seed products and Cf complex have been described previously (**5**,**10**,**19**,**34-38**).

Trolox Equivalent Antioxidant Capacity (TEAC)

The antioxidant capacity was measured using the method of Miller *et al* (**39**) based on the abilities of different substances to scavenge the $ABTS^{\bullet+}$ radical cation, compared with a standard antioxidant (Trolox) in a dose-response curve as described in previous papers (**5, 12**).

The compounds and extracts studied were dissolved in DMSO to a concentration of 50 mM.

After addition of 1 ml of $ABTS^{\bullet+}$ solution to aliquots of Trolox or the phenolic and reference compounds (1 to 100 ml, depending on the activity of the particular compound) the solutions were vortexed for exactly 30 seconds and the absorbance at 734 nm was taken exactly 1 min after initiation of mixing in a Unicam UV-2 spectrophotometer (Cambridge, UK) at 30ºC.

PBS blanks and DMSO blanks were run in each assay. The dose-response curve for Trolox consisted of plotting the absorbance at 734 nm as a percentage of the absorbance of the uninhibited radical cation (blank) and was based on triplicate determinations.

The activities of different compounds were assayed at four different concentrations, which had been determined to be within the range of the dose-response curve. Each concentration was analysed in triplicate. By reference to the Trolox dose-response curve, the mean Trolox equivalent antioxidant capacity (TEAC) value was derived for each compound.

TEAC, the unit of activity, is defined as the concentration (mM) of Trolox having the equivalent antioxidant capacity of a 1.0 mM solution of the substance under investigation.

Evaluation of X-ray radioprotective activity (in vivo)

Animals

The adult male Swiss mice (supplied by Hallan breeder and maintained in the Animal house of the University of Murcia, license nº 30030-2A) used in the experiments weighed between 27 and 30 g.

All mice were acclimatized for at least one week prior to dosing in constant environmental conditions with a 12 h/12 h light/dark cycle (20ºC and 50% relative humidity).

They were fed standard granulated chow (supplied by Purina) and given drinking water ad libitum. Each group consisted of 5-7 mice.

Animals used in this work were treated according to the Biological Council Guidelines on the Use of Living Animals in Scientific Investigations.

Chemicals and Treatments

The substances used in these experiments were: diosmin, rutin, DMSO, GSP, Cf complex, PTU and ascorbic acid. All the solutions were freshly prepared immediately before treatment of the animals. GSP, Cf complex, PTU, and ascorbic acid, were dissolved in drinking water to a final concentrations of 2% and administered for 5 days before the X-irradiation.

The mean total water consumption measured during the 5-day period was 8.75 ml for GSP, Cf complex and PTU groups of mice and 10 ml for the ascorbic acid group.

DMSO was dissolved in distilled water to a final concentration of 50 g/100 ml. Rutin and diosmin were dissolved in pure DMSO to a final concentration of 300 mg/ml each.

DMSO, rutin and diosmin were administered 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 aquipment 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 2 cGy/min, target distance 100 cm. The mice were exposed to a single dose of 48 cGy, and X-ray exposure was measured by means of a thermoluminescence dosimeter (TLD) (GR-200, Conqueror Electronics Technology Co. Ltd, China).

Bone marrow preparation, staining and slide analysis

The animals were killed by cervical dislocation about 24 h after X irradiation, and bone marrow samples were taken. The two femurs were removed from each mouse. Both the proximal and distal ends of the femur were cut off and the bone marrow cells were gently flushed out with fetal calf serum. These cells were dispersed by gently pipetting and collected by centrifugation at 1000 rpm for 5 min at 4ºC. The cell pellet was resuspended in a small volume of fetal calf serum and bone marrow smears (two slides per mouse) were prepared. The slides were coded to be read in blind. After 24 h air-drying, the smears were stained with May-Grünwald/Giemsa (**64**,**65**). With this method polychromatic erythrocytes (PCEs) stain reddish-blue and normochromatic erythrocytes (NCEs) stain orange, while nuclear material is dark purple.

The number of micronucleated polychromatic erythrocytes (MnPCEs) per 1000 PCEs per mouse (500 PCEs per slide) was determined. The slides were examined at 1000x magnification using a Zeiss light microscope (Oberkochen, Germany).

Evaluation of g-ray radioprotective activity (in vitro)

Blood samples and irradiation procedure.

Heparinized samples of human peripheral blood were obtained from two healthy young, non-smoking female donors. GSE_1, GSE_2, GSP and Cf complex were dissolved in 5% aqueous DMSO at 12.5 mg/ml; 25 ml of this solution was added to 2 ml of human blood and mixed before g-irradiation. The blood samples were exposed at room temperature for 40 seconds to ^{137}Cs γ-rays with an Irradiator IBL 437 C (CIS, France) at a dose of 2 Gy ± 3%. The dose rate was 5 cGy/second. The γ-ray exposure was established by means of TLDs which were supplied by- and measured by CIEMAT (Ministry of Industry and Energy, Spain).

Micronucleus assay

The micronucleus assay was carried out on human lymphocyte cultures according to an adaptation of the method of Fenech and Morley (**66**,**67**) as follows. Whole blood (1ml) was cultured at 37°C for 72 h in 9 ml of F-10 medium, 15% foetal bovine serum, 1.6% phytohaemagglutinin and 1% penicillin/streptomycin. At 44 h after initiation of the lymphocyte culture, cytokinesis was blocked by the addition of cytochalasin B in a concentration of 3μg/ml. At 72 h, the lymphocytes were treated with hypotonic solution (0.075 M KCl) for 3 min and fixed using methanol:acetic acid (3:1). Air-dried preparations were made and slides stained with May-Grünwald/Giemsa.

The number of micronuclei (MN) in cytokinesis-blocked cells (CB cells) was scored using a Zeiss light microscope (Oberkochem, Germany) at 400x magnification to survey the slides and 1000x magnification to detect the presence or absence of MN in the cells.

At least 500 cells were examined per slide.

Magnitude of protection (%) = $[(F_{control} - F_{treated})/F_{control}] \times 100$

where $F_{control}$ = frequency of MN in irradiated animals or blood lymphocytes and $F_{treated}$ = frequency of MN in animals or blood lymphocytes treated before the X- or γ-ray irradiation (**49**).

Statistical analysis (in vivo and in vitro)

Differences in the frequency of samples were tested by analysis of variance and evaluated using Student's t-test.

Evaluation of UV radioprotective activity (in vitro)

Human cutaneous explants were obtained and prepared by the Toxicology Department of Antonio Puig S.A. (Barcelona, Spain).

The Cf complex was dissolved in distilled water to a final concentration of 2% w/v and uniformely distributed on the explant (2 ml solution). After 30 min, the control and treated explants were irradiated using a UV-B Vilber-Lourmat VL-6M lamp at 197, 246 and 308 mJ/cm².

After UV-B radiation, the explants were incubated in a 5% CO_2 atmo-

sphere for 24 h at 37°C, and stained with haematoxylin/eosin.

The number of sunburn cells/cm was determined in 10 histological sections at 400x magnification using a Zeiss light microscope.

The magnitude of protection was evaluated using the following formula:

$$\%\text{protection} = [(SBC_{Ir} - SBC_{Ir\ prod})/SBC_{Ir}] \times 100$$

where SBCIr is the number of sunburn cells/cm in irradiated explants without treatment, and SBCIr prod. is the number of sunburn cells/cm in irradiated explants treated with Cf complex.

RESULTS and DISCUSSION

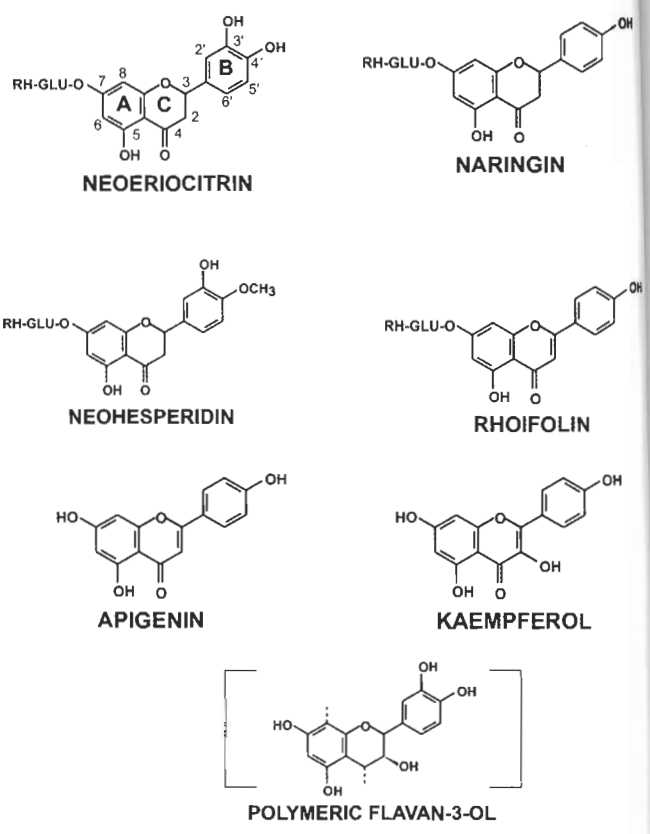
Antioxidant Activity

The Cf complex showed the highest antioxidant activity compared with that of the flavonoids quercetin, rutin, and diosmin and other reference compounds as determined by the extent of their abilities to scavenge the $ABTS^{\bullet+}$ radical cation (***Table 1***). The Cf complex showed the highest $ABTS^{\bullet+}$ scavenging capacity because of its high content of polymeric flavan-3-ols and citroflavonoids. The particular structure of these molecules permits the existence of a high number of conjugated structures between the 3-OH groups and the B-ring catechol groups (*o*-dihydroxy) of the flavan-3-ol skeleton (***Fig 1***), despite the minor concentration of flavones with 2,3-double bonds conjugated with the 4-oxo function.

Table 1 *Antioxidant activity: $ABTS^{\bullet+}$ radical cation scavenging ability.*

Compound	*TEAC (mM)*
Quercetin	*3.29 ± 0.12*
Rutin	*2.75 ± 0.05*
Diosmin	*1.14 ± 0.09*
Luteolin-7-glucoside	*0.71 ± 0.04*
Naringin	*0.35± 0.05*
Ascorbic acid	*1.12 ± 0.06*
(+)-catechin	*1.37 ± 0.07*
GSP	*8.21 ± 0.16*
Cf Complex	*9.71 ± 0.18*

Figura 1 *Chemical structures of the most abundat flavonoids in Cf complex*



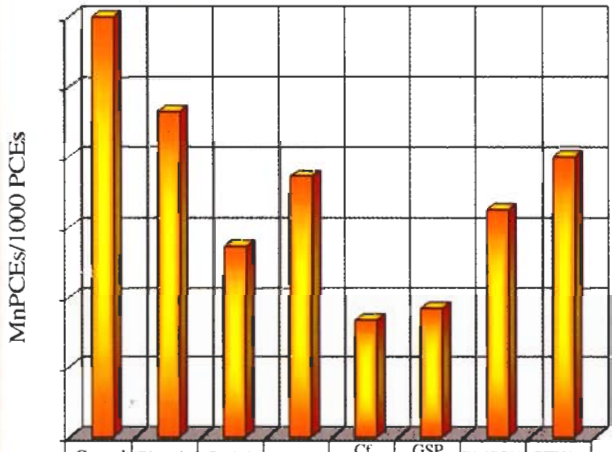
In addition, the presence of many C_4-C_8 linkages structurally increased the electron dislocation capacity of Cf complex and, consequently, its free radical scavenging capacity. Such activity is higher in Cf complex because the high number of associations between the free 3-OH group associated with the catechol structure in the B-ring (**2,5,12,31,32,63,68**) permits the stabilization of the aroxyl radical via electron dislocation through the A, B and C rings. This generates multiple mesomeric structures after hydrogen donation to the $ABTS^{\bullet+}$ radical cation.

Radioprotective Activity

The radioprotective activity against X-rays of Cf complex, GSP, ascorbic acid, DMSO, PTU, and rutin and diosmin was determined.

Figure 2 shows the effect of these treatments on the

Figura 2 *Effect of Cf complex and other orally-administered compounds on the frequency of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow smears of whole body X-irradiated mice*



	Control	Diosmin*	Rutin*	AA*	Cf complex**	GSP Nutrafur**	DMSOI*	PTU*
Dose (mg/)		180	180	200	175	175	200	175
Mean±S.D.	18.2±0.5	14.0±0.6	8.2±0.3	11.17±0.3	5.0±0.1	5.5±0.1	9.75±0.4	12.0±0.4
Protection		23.0%	54.9%	38.6%	72.5%	69.8%	46.4%	34.1%
(n) animals	7	5	5	6	6	6	5	6

Treatments

The data are the means and standard deviations a total of (n) animals obtained from 3 experiments
*, p<0.01; **, p<0.001 vs control
AA, ascorbic acid; DMSO, dimethylsulfoxide; PTU, propylthiouracil; GSP, grape seed polyphenols

number of micronucleated polychromatic erythrocytes (MnPCEs) in bone marrow of X-irradiated mice *in vivo*. The rank order of activity with respect to their ability to lower MnPCE generation after irradiation was: Cf complex > GSP > rutin > DMSO > ascorbic acid > PTU > diosmin.

Radioprotective activity and consequently anticlastogenic effectiveness of these treatments in terms of their percentage inhibition of MnPCE formation is also shown in *Figure 2*.

Table 2 *Micronuclei generated in Cytokinesis-blocked (CB) cells and magnitude of protection of different grape seed extracts after y-irradiation of human blood lymphocyte preparations in vitro*

Grape seed extract	*Micronuclei (MN/500 CB cells)*	*% Protection*
Control	32 ± 1.1	0
GSE1	29 ± 0.9	7
GSE2	26 ± 1.0	14
GSP	22 ± 0.7	28
Cf Complex	12 ± 0.5*	66

All coumpounds were present at a final concentration of 156.25 µg/ml of human blood
The data are the means and standard deviations of 3 experiments
*, p<0.001 vs or other treatments

In another study, performed *in vitro* on γ-ray irradiated human blood lymphocyte preparations, the effect of two commercial flavan-3-ol products, GSE_1 and GSE_2, in comparison with GSP and Cf complex was determined in order to clarify the influence of the polymerization degree on the antioxidant and anticlastogenic activity of flavan-3-ols. The results (***Table 2***) confirm that the maximum antioxidant and anticlastogenic activity of flavan-3-ol compounds is correlated with the higher content of polymers.

In addition, at the concentrations demonstrating radioprotective activity, these substances showed a lack of cytotoxicity, according to verified tests (**5,33**).

This datum increases the potential interest of these polyphenolic polymers as nutraceutical and pharmaceutical agents.

We also studied the UV-B photoprotective activity of Cf complex using human cutaneous explants, and as endpoint, the formation of sunburn cells (SBC).

The results showed that Cf complex inhibited UV-B light-induced SBC formation by 91%, 65% and 47% at 197, 267 and 308 mJ/cm^2, respectively with 40 mg of Cf Complex added uniformely to the explant.

SUMMARY and PERSPECTIVES

A combination of citroflavonoids and flavan-3-ols (Cf complex) has been developed which shows antioxidant and radical scavenger properties superior to those of several individual flavonoids and other reference compounds.

It was determined in *in vitro* and *in vivo* studies that Cf complex, in comparison with a series of other antioxidants, acted as a radioprotector against UV-B, X- and γ-ray

radiation which generate hydroxyl radicals in organisms and induce cellular DNA damage leading to mutations and chromosomal aberrations.

Cf complex inhibited by 72% the formation of micronucleated polychromatic erythrocytes in mouse bone marrow induced by whole body X-irradiation with a rank order of activity with respect to other treatments being: Cf complex > GSP > rutin > DMSO > ascorbic acid > PTU > diosmin. The photoprotective activity of Cf complex against UV-B irradiation of human cutaneous explants *in vitro* was determined using as endpoint, the formation of sunburn cells.

Cf complex inhibited UV-B light-induced sunburn cell formation by 91%, 65% and 47% at 197, 267 and 308 mJ/cm^2.

In another study, performed *in vitro* on γ-ray irradiated human blood lymphocyte preparations, Cf complex was more effective than some commercial grape seed extract flavan-3-ol products in inhibiting the formation of micronuclei, lending support to the notion that the antioxidant and anticlastogenic activity of flavan-3-ol compounds present in the Cf complex is correlated with their higher content in polymers.

With a view to health related properties, Cf complex can be used not only as an antioxidant or radical scavenger but also as a dietetic or nutraceutic, especially in the prevention of cancer, cardiovascular and inflammatory related diseases, as described below.

Antimutagenic effects

Due to their absorption of light, flavonoids can protect against DNA damage. This effect is one of the physiological functions attributed to flavonoids in the plant kingdom (**69**), although it may be generalized to animal cells, particularly those of mammals. Recent experiments in template plasmid DNA irradiated with UV-B light, showed the protective effect of naringenin and rutin against UV-induced DNA damage (**70**).

Flavonoids may also protect DNA by interacting directly with carcinogens that have escaped detoxification processes, as occurs in the chromosome aberrations induced by bleomycin (**71**).

Antiproliferative effects

Test-diet treated animals showed a reduction in lipid peroxides and cytochrome P450. *In vitro*, [^{3}H]-thymidine incorporation showed that flavonoids inhibited DNA synthesis in fibrosarcoma cells. Similarly, citroflavonoids inhibit processes that are believed to represent non-specific markers of tumour promotion: epidermal ornithine decarboxylase induction, accelerated incorporation of ^{32}P inorganic phosphate into membrane phospholipid and activated protein kinase C (**72**).

In vitro, flavonoids display an antiproliferative effect on various human neoplasic cell lines, for example myeloid and lymphoid leukaemia cells (**73**), gastric cancer cells (**74**), ovarian cancer cells (**75**), prostate cancer cells (**76**), squamous cell carcinoma (**13**) and melanoma cells (**17**).

Effects on capillary fragility

Several studies indicate that certain flavonoids may have a protective and therapeutic effect in coronary heart disease. Capillary damage includes increased permeability, seepage of blood and plasma constituents into the tissues, followed by an inflammatory reaction. Some investigators have related the increase in vascular tone observed *in vivo* after treatment with these drugs to the inhibition of amine reuptake where the flavonoids act as antagonists of plasma membrane amine transporters (**77**). The vasodilatory mechanism of flavonoids seems to be the inhibition of protein kinase C (**78**).

Effects on coronary heart disease

Citroflavonoids show an antiadhesive and antiaggregation action against red cell clumping (**79**). Other authors have reported that flavonoids are effective inhibitors of platelet adhesion, aggregation and serotonin secretion although the degree of inhibition depends on the type of inducer and on the flavonoid structure (**72**). *In vitro*, flavonoids inhibit the oxidation of low-density lipoprotein (LDL) and reduce thrombotic tendencies.

Flavonoid intake has been inversely and significantly associated with death from coronary heart disease and showed an inverse relation with the incidence of myocardial infarction (**1**). There is evidence that free-radical oxidation of LDL plays an important part in atherogenesis.

On the other hand, citrus flavonoids exert an apparent regulatory action on erythrocyte aggregation and concentration (hematocrit), the two major factors affecting blood viscosity and flow (**80**).

Anti-inflammatory, antiallergic and analgesic effects

The possible activity of flavonoids in anti-inflammatory and antiallergic responses is well documented by Gabor (**50**). Recent studies on citroflavonoids (**51,81-83**) have shown the antiinflammatory dose-dependent activity of apigenin, apigenin-7glucoside and other flavonoids, and their influence on the metabolism of arachidonic acid and histamine release.

These flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cycloxygenase and phospholipase A2.

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