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Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention

Debasis Bagchi [a,*], Manashi Bagchi [a], Sidney J. Stohs [a], Dipak K. Das [b], Sidhartha D. Ray [c], Charles A. Kuszynski [d], Shantaram S. Joshi [d], Harry G. Pruess [e]

[a] *Department of Pharmaceutical and Administrative Sciences, Creighton University School of Pharmacy & Allied Health Professions, 2500 California Plaza, Omaha, NE 68178, USA*

[b] *Department of Surgery, University of Connecticut School of Medicine, Farmington, CT 06030, USA*

[c] *Department of Pharmacology, Toxicology and Medicinal Chemistry, Arnold & Marie Schwarz College of Pharmacy, Long Island University, Brooklyn, NY 11201, USA*

[d] *Department of Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198, USA*

[e] *Department of Medicine, Georgetown University Medical Center, Washington, DC 20007, USA*

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Abstract

Free radicals have been implicated in over a hundred disease conditions in humans, including arthritis, hemorrhagic shock, atherosclerosis, advancing age, ischemia and reperfusion injury of many organs, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis, and AIDS. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes. A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. However, the structure-activity relationship, bioavailability and therapeutic efficacy of the antioxidants differ extensively. Oligomeric proanthocyanidins, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. We have assessed the concentration- or dose-dependent free radical scavenging ability of a novel IH636 grape seed proanthocyanidin extract (GSPE) both in vitro and in vivo models, and compared the free radical scavenging ability of GSPE with vitamins C, E and β-carotene. These experiments demonstrated that GSPE is highly bioavailable and provides significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and β-carotene. GSPE was also shown to demonstrate cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells. The comparative protective effects of GSPE, vitamins C and E were examined on tobacco-induced oxidative stress and apoptotic cell death in human oral keratinocytes. Oxidative tissue damage was determined by lipid peroxidation and DNA fragmentation, while apoptotic cell death

* Corresponding author.

E-mail address: debsis@bluejay.creighton.edu. (D. Bagchi).

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was assessed by flow cytometry. GSPE provided significantly better protection as compared to vitamins C and E, singly and in combination. GSPE also demonstrated excellent protection against acetaminophen overdose-induced liver and kidney damage by regulating *bcl*-X_L gene, DNA damage and presumably by reducing oxidative stress. GSPE demonstrated excellent protection against myocardial ischemia-reperfusion injury and myocardial infarction in rats. GSPE was also shown to upregulate bcl_2 gene and downregulate the oncogene c-myc. Topical application of GSPE enhances sun protection factor in human volunteers, as well as supplementation of GSPE ameliorates chronic pancreatitis in humans. These results demonstrate that GSPE provides excellent protection against oxidative stress and free radical-mediated tissue injury.

Keywords: Free radicals; Human diseases; Grape seed proanthocyanidin

1. Introduction

1.1. Free radicals, human diseases and antioxidants

Occupational exposure to chemically and structurally diverse environmental pollutants including pesticides, toxic chemical wastes, direct and second hand cigarette smoke, gasoline exhaust, urban air pollutants ozone and radiation, and physical stress, produce similar toxic effects on human health. These environmental pollutants have been demonstrated to produce enormous amounts of free radicals, resulting in oxidative deterioration of lipids, proteins and DNA, activation of procarcinogens, inhibition of cellular and antioxidant defense systems, depletion of sulfhydryls, altered calcium homeostasis, changes in gene expression and induction of abnormal proteins, and contribute significantly to human disease pathophysiology (Herman, 1982; Ames, 1992; Kehrer, 1993; Stohs and Bagchi, 1995). Antioxidants/free radical scavengers function as inhibitors at both initiation and promotion/propagation/transformation stages of tumor promotion/carcinogenesis and protect cells against oxidative damage (Halliwell et al., 1992). The potential role of the antioxidant vitamins such as vitamin C and E, β-carotene and proanthocyanidins, antioxidant minerals such as zinc and selenium, and antioxidant enzymes such as glutathione, superoxide dismutase and catalase, have been extensively studied in the prevention of numerous degenerative diseases including tumor growth and carcinogenesis (Halliwell et al., 1992). The following features are considered to evaluate the therapeutic potential of a given antioxidant:

1. Absorption and bioavailability.
2. Effective dose, safety and toxicity.
3. Distribution in cells, tissues and extracellular fluids.
4. Free radical scavenging ability.
5. Metal chelating activity.
6. Effects on gene expression.
7. Interaction with cellular antioxidants/antioxidant enzymes.
8. Detoxification of carcinogenic metabolites.

1.2. Natural diet and antioxidants

Epidemiological studies have demonstrated that five to seven servings of fresh fruits and vegetables, and two glasses of red wine per day can lead to a prolonged healthy life (German, 1997). Vegetables, fruits and their seeds are rich sources of vitamins C and E, and β-carotene, and/or protease inhibitors, compounds which might protect the organism against cancer (Hocman, 1989). Several plants have been reported to contain compounds including bioflavonoids and proanthocyanidins, ellipticine and taxol, indole derivatives, dithiolthiones, phytoestrogens, etc, which exhibit chemopreventive and/or anticancer properties (Hocman, 1989; Teel, 1992). These chemoprotective components have been demonstrated to inhibit oxidative stress and chemically induced carcinogenesis by the following mechanisms (Hocman, 1989):

1. Modulation of metabolic functions so that toxins and carcinogens are not produced.
2. Enhancement of detoxification pathways.
3. Prevent interaction of the ultimate carcinogen with biological macromolecules.

1.3. Oligomeric proanthocyanidins as novel antioxidants

The biological, pharmacological and medicinal properties of the bioflavonoids and proanthocyanidins have been extensively reviewed (Shahidi and Wanasundara, 1992; Suzuki, 1993; Jovanovic et al., 1994; Rice-Evans et al., 1996). Besides the free radical scavenging and antioxidant activity, proanthocyanidins exhibit vasodilatory, anticarcinogenic, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immune-stimulating, anti-viral and estrogenic activities, as well as being inhibitors of the enzymes phospholipase A_2, cyclooxygenase and lipooxygenase (Salah et al., 1995; Rice-Evans et al., 1996). The chemical properties of proanthocyanidins in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers and singlet oxygen quenchers predicts their antioxidant activity (Chen et al., 1996; Rice-Evans et al., 1996). For a proanthocyanidin to be defined as an antioxidant it must satisfy two basic conditions: (i) when present in low concentrations relative to the substrate to be oxidized it can delay, retard, or prevent autooxidation or free radical-mediated oxidative injury; and (ii) the resulting product formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation (Shahidi and Wanasundara, 1992).

The total bioflavonoid/proanthocyanidin content in a typical fruit serving of 200 g is in the range of 50–500 mg, with apples having over 200 mg (Bravo, 1998). It has been demonstrated that proanthocyanidin content in the plasma can be maintained following regular intake of sufficient quantity of fresh fruits and vegetables or supplementation of bioavailable proanthocyanidins (Bravo, 1998). The absorption, distribution, metabolism and excretion of proanthocyanidins are governed by the chemical structures (Jimenez-Ramsey et al., 1994; Bravo, 1998). Distinct absorption and bioavailablity of various extractable proanthocyanidins, depending on their extractability with different solvents, was demonstrated by Jimenez-Ramsey et al. (1994). These authors demonstrated that proanthocyanidins soluble in water and ethanol are absorbed from the intestinal tract and extensively distributed in all tissues and plasma, while the proanthocyanidin fractions soluble in aqueous acetone but insoluble in water and ethanol are not at all bioavailable (Jimenez-Ramsey et al., 1994). Generally the dimer-, trimer- and tetrameric proanthocyanidins, also referred to as extractable proanthocyanidins or bioflavonoids, have been shown to be highly bioavailable and provide excellent health benefits. de Vries et al. (1998), Hollman and Katan (1998) have further demonstrated that flavonoid glycosides are more bioavailable as compared to the pure aglycone. These low molecular proanthocyanidins are also known as sustained release antioxidants, and can remain in the plasma and tissues for up to 7–10 days and exert antioxidant properties, which is mechanistically different from other water soluble antioxidants. On the contrary, high molecular weight polymeric non-extractable proanthocyanidins are very potent scavengers of peroxyl radicals, but these are not absorbable or bioavailable at all. However, these high molecular weight proanthocyanidins can exert their antioxidant activity in the digestive tract and protect lipids, proteins and carbohydrates from oxidative damage during digestion and spare soluble antioxidants (Hagerman et al., 1998). In this paper, we have demonstrated the protective ability and biological efficacy of a novel IH636 grape seed proanthocyanidin extract (GSPE) in selected in vitro and in vivo models as well as in human clinical studies.

2. Safety, free radical scavenging and biological efficacy of Activin, a novel IH636 grape seed proanthocyanidin extract (GSPE)

2.1. Occurrence

IH636 grape seed proanthocyanidin extract (GSPE, commercially available as ActiVin from InterHealth Nutraceuticals Incorporated, Benicia, CA) is a standardized water-ethanol extract from red grape seeds. It is worthwhile to mention that novel antioxidants including catechins and oligomeric proanthocyanidins (OPC) accumulate principally in the lignified portions of grape clus-

ters, especially in the seeds (Kovac et al., 1995). HPLC studies in conjunction with GC-MS demonstrate that GSPE contains monomeric, dimeric, trimeric, tetrameric and other oligomeric proanthocyanidin bioflavonoids.

2.2. Safety and toxicity studies

To determine the safety of GSPE, a series of toxicity studies were conducted on GSPE in compliance with the U.S. Environmental Protection Agency and Toxic Substances Control Act Health Effects Test Guidelines, 40 CFR 798.4500. The following studies demonstrate the safety of GSPE.

2.2.1. Acute oral toxicity study

The LD_{50} of GSPE was found herein to be greater than 5000 mg/kg body weight when administered once orally via gastric intubation to fasted male and female albino rats. There were no gross findings at the scheduled necropsy.

2.2.2. Acute dermal toxicity study

GSPE was administered once dermally at a dose of 2000 mg/kg body weight to the clipped, intact skin of five male and five female albino rats for a 24-h exposure period under semi-occlusive dressing. There were no deaths or test material-related clinical findings, body weight changes or gross necropsy findings. GSPE induced very slight to slight erythema and desquamation on all animals. With the exception of desquamation noted on three animals, all dermal responses completely subsided by day 12 or earlier. Thus, LD_{50} of GSPE was found to be greater that 2000 mg/kg body weight when administered once for 24 h to the clipped, intact skin. In addition, 2000 mg/kg body weight dosage was found to be a no-observed-effect-level (NOEL) for systemic toxicity.

2.2.3. Primary dermal irritation study

Single 0.5 g doses of GSPE were applied to the clipped, intact skin of six New Zealand white rabbits under semi-occlusive dressings for a 4-h exposure period. Application sites were evaluated in accordance with the method of Draize at 30–60 min and 24, 48 and 72 h after bandage removal and once daily thereafter through day 12 if irritation persisted. The primary irritation index was calculated to be 2.7, a descriptive rating classification of moderately irritating.

2.2.4. Primary eye irritation study in albino rabbits

Single 85 mg doses of GSPE were instilled into the lower conjunctival sac of the right eye of six rabbits. The eyelids were held closed for approximately 1 s and released. The ocular reactions were examined in accordance with the method of Draize at 1, 24, 48 and 72 h after closing and on days 4, 7 and 14. The maximum average score was 16.7 at 24 h post-instillation. All irritation was reversible and completely subsided by day 14.

2.3. Free radical scavenging and antioxidant efficacy study

Following in vitro and in vivo assays were conducted to determine the biological efficacy of GSPE.

2.3.1. In vitro free radical scavenging assay

The free radical scavenging abilities (RSA) of GSPE, vitamin E and vitamin C against biochemically generated superoxide anion and hydroxyl radical were assessed in vitro at varying concentrations via cytochrome *c* reduction and chemiluminescence response. Chemiluminescence is a general assay for the production of reactive oxygen species, while cytochrome *c* reduction is a specific assay for superoxide anion. At 50 mg/l, GSPE demonstrated 84 and 98% greater RSA against superoxide anion and hydroxyl radical, respectively, as compared to natural vitamin E and at 100 mg/l, GSPE demonstrated 439 and 575% greater RSA against superoxide anion and hydroxyl radical, respectively, as compared to vitamin C (Bagchi et al., 1997). In a more recent study, we have demonstrated the superior peroxyl radical scavenging ability of GSPE as compared to Trolox (Sato et al., 1999).

2.3.2. Laser scanning confocal microscopy study

This novel state-of-the-art technique was used to assess the overall intracellular oxidized states of cultured macrophage J774A.1 and neuroactive

PC-12 adrenal pheochromocytoma cells following incubation with H_2O_2 and/or GSPE at an excitation wavelength of 513 nm using 2,7-dichlorofluorescein diacetate as the probe. Approximately 5.8- and 4.5-fold increases in fluorescence intensity were observed following incubation of J774A.1 and PC-12 cells with 0.5 mM H_2O_2 for 24 h, respectively. Pretreatment with 50 mg/l and 100 mg/l GSPE decreased H_2O_2-induced fluorescence intensity by 36 and 70% (Fig. 1), respectively, in the J774A.1 cells, and 50 and 70%, respectively, in the PC-12 cells (Fig. 2). Thus, GSPE provided significant protection against H_2O_2-induced oxidative damage to these cells (Bagchi et al., 1998).

2.3.3. Protection against tobacco-induced oxidative stress and apoptotic cell death in human oral keratinocyte cells

The protective ability of GSPE was assessed against smokeless tobacco-induced oxidative damage and programmed cell death (apoptosis) in a primary culture of human oral keratinocytes. Approximately 9, 29 and 35% apoptotic cell death were observed in these cells following treatment with 100, 200 and 300 μg/ml of tobacco extract, respectively. Pretreatment of the 300 μg/ml tobacco- treated cells with 100 mg GSPE/ml reduced tobacco-induced apoptopic cell death by $\approx 85\%$ in oral cells, while a combination of vitamins E and C (75 μM each) reduced tobacco-induced apoptotic cell death by only 46% (Fig. 3) (Bagchi et al., 1999).

2.3.4. Differential cytotoxicity towards human normal and malignant cells

The cytotoxicity of GSPE was assessed towards selected human cancer cells, including cultured MCF-7 breast cancer, CRL 1739 gastric adenocarcinoma and A-427 lung cancer cells, by cytomorphology and MTT cytotoxicity assay, and compared these effects with two normal cells, including normal human gastric mucosal and J774.1 murine macrophage cells. GSPE demonstrated selective cytotoxicity towards MCF-7 breast, CRL-1739 gastric adenocarcinoma and A-427 lung cancer cells at 25 and 50 mg/l concentrations, while GSPE enhances the growth and viability of the normal cells at these concentrations (Ye et al., 1999).

2.3.5. Protection against chemotherapeutic agent-induced cytotoxicity towards human normal liver cells

Anticancer chemotherapeutic agents are very effective in inhibiting the growth of cancer cells in vitro and in vivo, however, toxicity to normal cells is a major problem. In a recent study, the effect of GSPE was assessed to ameliorate the chemotherapy-induced toxic effects in cultured Chang liver cells, established from non-malignant human tissue. Liver cells were treated in vitro with Idarubicin (30 nM) or 4-hydroxyperoxycyclophosphamide (4-HC) (1 μg/ml) with or without GSPE (25 μg/ml). The cells were grown in vitro and the growth rate was determined using MTT assay. GSPE significantly decreased the growth inhibitory and cytotoxic effects of Idarubicin and 4-HC on these liver cells. Since these chemotherapeutic agents are known to induce apoptosis in target cells, the human liver cells were analyzed for apoptopic cell population by flow cytometry. There was a significant decrease ($>50\%$) in the number of cells undergoing apoptosis following treatment with GSPE. An increased expression of apoptosis related gene bcl_2 was also observed in GSPE treated cells as demonstrated by Western blot analysis. Furthermore, GSPE downregulates the oncogene c-myc and modulate the apoptosis related gene p53 in these cells. Thus, GSPE ameliorates the toxic effects associated with these chemotherapeutic agents towards normal healthy cells (Joshi et al., 1999).

2.3.6. Comparative protective abilities of GSPE, vitamins C and E, and β-carotene against 12-O-tetradeca-noylphorbol-13-acetate (TPA)-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice

The protective abilities of GSPE, vitamin E, vitamin C, β-carotene and a combination of vitamins E and C against TPA-induced lipid peroxidation and DNA fragmentation in the brain and liver tissues of mice, as well as against production of reactive oxygen species (ROS) in the peritoneal

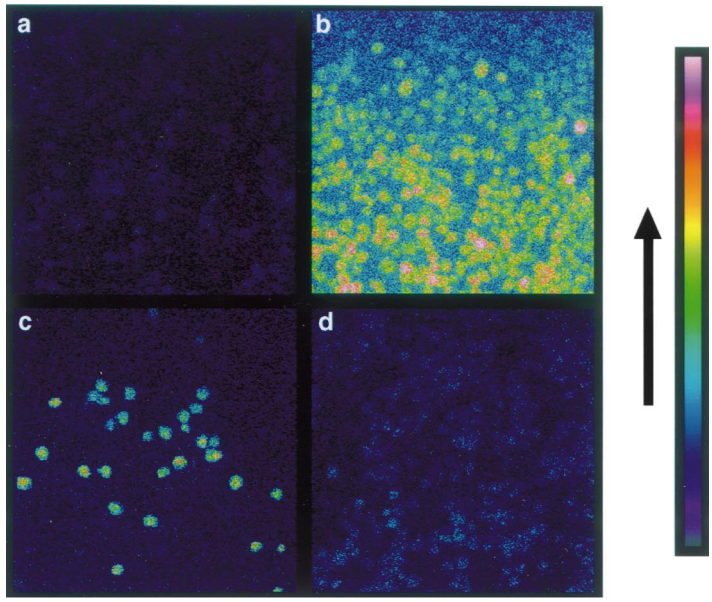

Fig. 1

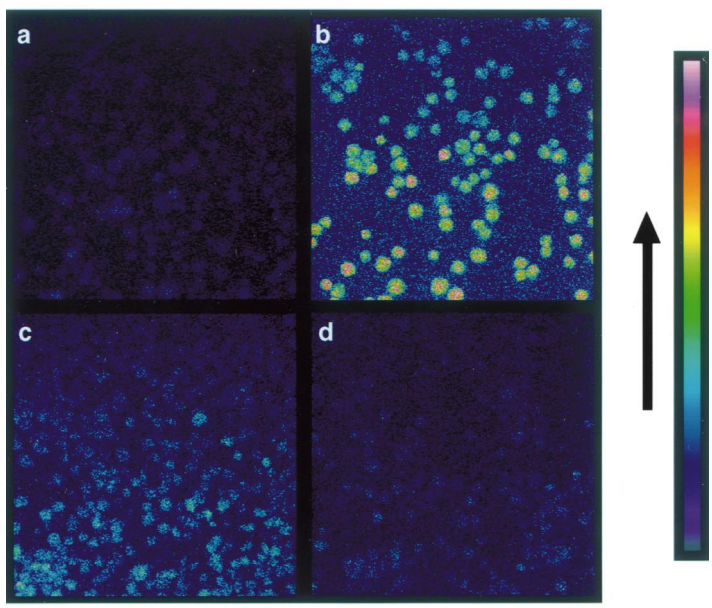

Fig. 2

Fig. 1. Hydrogen peroxide-induced modulation of intracellular oxidized states of cultured macrophage J774A.1 cells, and concentration-dependent protection by GSPE. (a) control; (b) 0.50 mM H_2O_2; (c) GSPE (50 mg/l) + 0.50 mM H_2O_2; (d) GSPE (100 mg/l) ± 0.50 mM H_2O_2.

Fig. 2. Hydrogen peroxide-induced modulation of intracellular oxidized states of cultured adrenal pheochromocytoma PC-12 cells, and concentration-dependent protection by GSPE. (a) control; (b) 0.50 mM H_2O_2; (c) GSPE (50 mg/l) + 0.50 mM H_2O_2; (d) GSPE (100 mg/l) ± 0.50 mM H_2O_2.

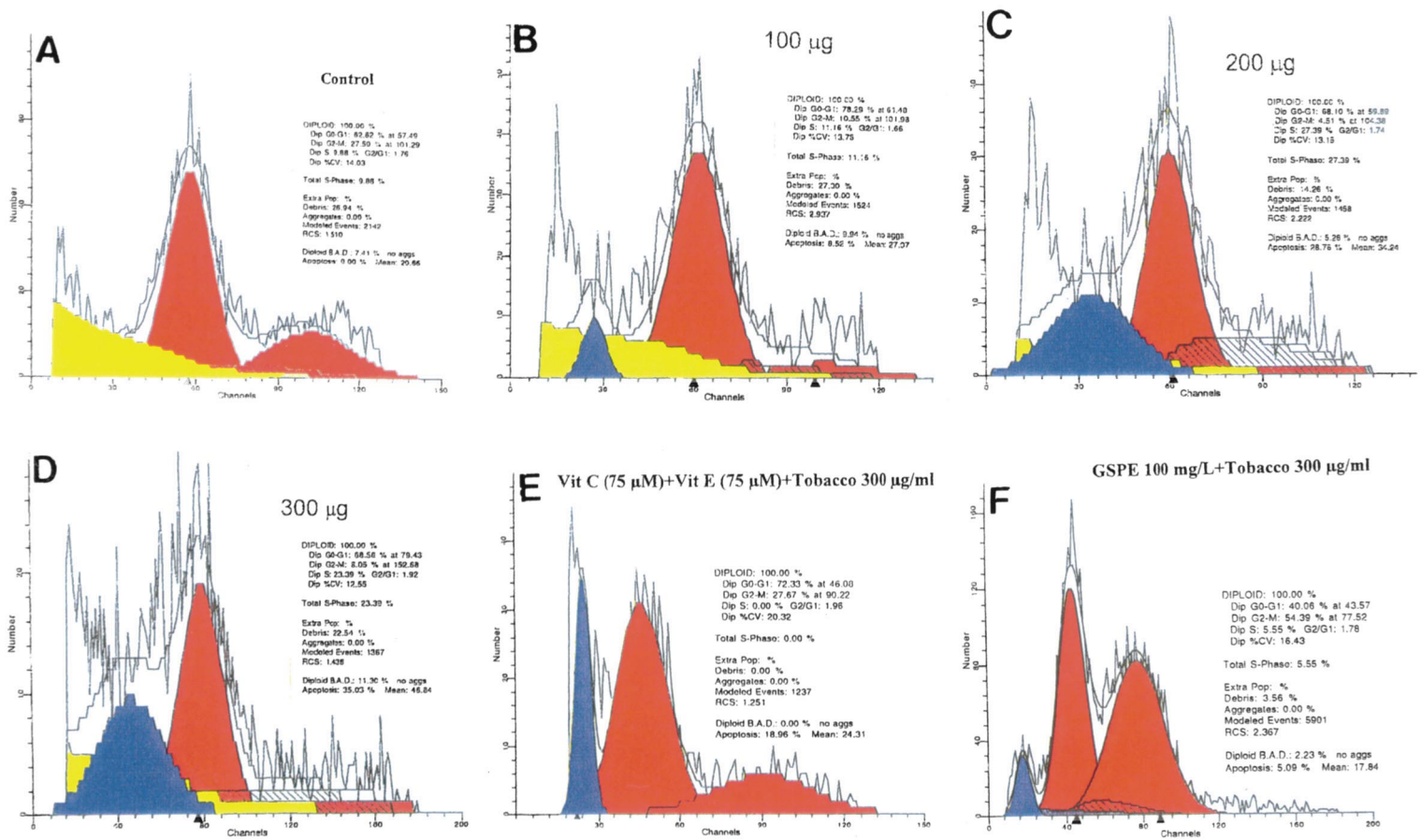

Fig. 3. Flow cytometric analysis of cell cycle distribution and programmed cell death (apoptosis) in human oral keratinocytes in response to increasing concentrations of smokeless tobacco extracts (STE), and protection by selected antioxidants. (A) control; (B) STE (100 µg/ml); (C) STE (200 µg/ml); (D) STE (300 µg/ml); (E) STE (300 µg/ml) plus vitamins C plus E (75 µM each); (F) STE (300 µg/ml) plus GSPE (100 µg/ml).

macrophages of mice, were assessed in vivo. TPA is a well-known inducer of ROS and tumor promotion in living organisms. Pretreatment of mice with GSPE (100 mg/kg body weight), vitamin E (100 mg/kg body weight), vitamin C (100 mg/kg body weight), β-carotene (50 mg/kg body weight) and a combination of vitamins E plus C (100 mg/kg body weight each) decreased TPA-induced production of ROS in peritoneal macrophages by 71, 43, 16, 17 and 51%, respectively, via chemiluminescence response, and 69, 32, 15, 18 and 47%, respectively, via cytochrome *c* reduction as compared to controls. Pretreatment of mice with the same dosages of GSPE, vitamin E, vitamin C, β-carotene and a combination of vitamins E plus C decreased TPA-induced DNA fragmentation by 50, 31, 14, 11 and 40% respectively, in brain tissue, and 47, 30, 10, 11 and 38%, respectively, in liver tissue, while lipid peroxidation was reduced by 61, 45, 13, 8 and 48%, respectively, in brain tissue, 46, 36, 12, 7 and 39%, respectively, in liver mitochondria and 59, 47, 14, 12 and 53%, respectively, in liver microsomes compared to controls. Pretreatment of mice with GSPE (25, 50 and 100 mg/kg body weight) resulted in a significant dose-dependent inhibition of TPA-induced production of ROS in peritoneal macrophage cells, and lipid peroxidation and DNA fragmentation in brain and liver tissues compared to controls. These results demonstrate that GSPE is bioavailable to the target organs, and provides significantly greater protection against ROS and free radical-induced lipid peroxidation and DNA damage than vitamins E, C and β-carotene, as well as a combination of vitamins E plus C (Bagchi et al., 1998a).

2.3.7. Age-related hypertension and glycosylated hemoglobin study

The pathogenesis of the aging phenomenon and chronic diseases associated with aging is attributed, in part, to glycosylation of proteins and nucleic acids and augmented free radical formation causing increased tissue damage. In normotensive rats, it was demonstrated that chronic supplementation of GSPE (250 ppm) in conjunction with a niacin-bound chromium (5 ppm Cr) and methionine-bound zinc (18 ppm Zn) significantly reduced systolic blood pressure and glycosylated hemoglobin (HbA1C), as well as decreased lipid peroxidation and free radical formation. These results demonstrate potential long-term health benefits of GSPE used in combination with these chromium and zinc supplements (Pruess et al., 1997).

2.3.8. Protection against acetaminophen overdose-induced hepatoxicity and DNA damage

The short and long term protective effects of GSPE were examined on acetaminophen overdose-induced lethality and liver toxicity. Mice were administered nontoxic doses of GSPE (3 or 7 days, 100 mg/kg, p.o.) followed by hepatotoxic doses of acetaminophen (400 or 500 mg/kg, i.p.).GSPE dramatically decreased acetaminophen-induced mortality, serum alanine aminotransferase (ALT) activity, a marker of liver toxicity, and hepatic DNA damage. Histopathological evaluation of liver sections showed a remarkable interference of GSPE against acetaminophen toxicity and substantial inhibition of apoptotic and necrotic liver cell death. Acetaminophen was also shown to phosphorylate (deactivate) the *bcl*-X_L gene, a death inhibitor gene and a positive regulator of the bcl_2 family of genes. In contrast, ActiVin alone enhanced the expression of *bcl*-X_L gene and significantly reduced acetaminophen-induced phosphorylation of *bcl*-X_L gene. Thus, GSPE significantly attenuates acetaminophen-induced lethality, liver toxicity, hepatic DNA damage, ALT activity, apoptotic cell death and positively influence gene expression (Ray et al., 1999).

2.3.9. Protection against myocardial ischemia-reperfusion injury in rats

Free radicals play a crucial role in the pathogenesis of myocardial ischemia-reperfusion injury. The protective ability of GSPE was assessed during post-ischemic reperfusion injury and ischemic arrest in the heart. Sprague-Dawley rats were divided into two groups: the experimental group was fed GSPE 100 mg/kg body weight for 3 weeks, while the control group was fed water alone. After 3 weeks, rats hearts were made globally ischemic for 30 min followed by 2 h of reperfusion. Left ventricular functions were continuously monitored and release of creatine kinase (CK, a marker for tissue necrosis and inflammation) and malondialdehyde (MDA, a marker for

oxidative stress) were estimated. Myocardial infarct size was measured by TTC staining. GSPE-supplemented group provided elevated cardioprotection as evidenced by improved post-ischemic left ventricular functions (dp, dp/dt_{max}) and aortic flow, as well as by reduced CK release and MDA formation in the coronary effluent as compared to control groups. Myocardial infarct size was also reduced by approximately 25% in the GSPE-fed group. The results show that GSPE can provide cardioprotection presumably by virtue of its potent in vivo free radical scavenging ability (Sato et al., 1999).

2.3.10. Renal protection in vivo study

The protective effect of GSPE was assessed on acetaminophen- induced nephrotoxicity and genomic DNA damage in kidneys. Male ICR mice (3 months old) were fed 100 mg GSPE/kg body weight p.o. for 7 days followed by administration of acetaminophen 500 mg/kg i.p. for 24 h. Blood was collected for determination of BUN (blood urea nitrogen), and the kidneys examined for histopathology and DNA damage. Exposure to acetaminophen alone caused greater than 3-fold increase in BUN (acetaminophen: 67 mg BUN/dl, control: 21 mg BUN/dl). While GSPE alone did not cause any damage to the kidneys (19 mg BUN/dl), GSPE pre-exposure to acetaminophen-treated animals significantly reduced damage to the kidneys (32 mg BUN/dl). Histopathological evaluation of kidney sections mirrored the serum chemistry findings. GSPE also protected against acetaminophen-induced genomic DNA damage. Thus, GSPE can protect kidney and renal function in mice from acetaminophen-induced toxicity (Ray et al., 1998).

2.3.11. Protection against acute and chronic stress-induced oxidative gastrointestinal injury in rats

The protective ability of GSPE against stress-induced gastrointestinal mucosal lipid peroxidation, DNA fragmentation and membrane microviscosity were determined in rats, and correlated with increased production of ROS. Acute stress was induced by water-immersion for 90 min, while chronic stress was induced by water-immersion for 15 min/day for 15 consecutive days. Half of the animals exposed to acute and chronic stress were pretreated orally with 100 mg GSPE/kg body weight/day for 15 consecutive days. Acute stress produced maximal injury to both gastric and intestinal mucosa as compared to chronic stress. Acute stress increased lipid peroxidation, DNA fragmentation and membrane microviscosity by 3.3-, 4.1- and 11.6-fold, respectively, in the gastric mucosa, and 4.4-, 5.2- and 16.6-fold in intestinal mucosa. GSPE decreased acute stress-induced lipid peroxidation, DNA fragmentation and membrane microviscosity by 15, 12 and 13%, respectively, in the gastric mucosa, and by 13, 14 and 16%, respectively, in the intestinal mucosa. Chronic stress increased lipid peroxidation, DNA fragmentation and membrane microviscosity by 2.9-, 3.3- and 6.3- fold, respectively, in the gastric mucosa, and 3.3-, 4.2- and 9.3-fold, respectively, in the intestinal mucosa. GSPE decreased chronic stress-induced lipid peroxidation, DNA fragmentation and membrane microviscosity by 23, 21 and 25%, respectively, in the gastric mucosa, and by 26, 26 and 25%, respectively, in the intestinal mucosa. Thus, stress induces oxidative gastrointestinal injury through enhanced production of ROS, and GSPE can provide significant protection against oxidative gastrointestinal injury by scavenging these ROS (Bagchi et al., 1999a).

2.3.12. Protection against multiple target organ toxicities induced by amiodarone (lung), dimethylnitrosamine (spleen), cadmium chloride (kidney) and MOCAP (brain) in vivo

Drug or chemically induced degradation of DNA in cells has serious biological consequences such as apoptotic and necrotic cell death, mutation, and/or carcinogenic transformation. The protective ability of GSPE pre-exposure was examined on amiodarone-induced pulmonary toxicity, dimethylnitrosamine-induced splenotoxicity, cadmium chloride-induced nephrotoxicity, and MOCAP-induced neurotoxicity. Parameters of analysis included changes in serum chemistry, histopathology and integrity of genomic DNA. Results indicate that 7-day GSPE pre-exposure prior to the toxicants, such as amiodarone, cad-

mium chloride, and dimethylnitrosamine provided near complete protection in terms of serum chemistry changes (alanine aminotransferase, blood urea nitrogen and creatine kinase), and abolished both forms of cell death, e.g. apoptosis and necrosis. In addition, it also significantly protected the DNA damage triggered by these toxicants to various degrees. Evaluation of H&E stained lung, kidney and spleen tissues mirrored the serum chemistry and DNA changes. Surprisingly, MOCAP-exposed animals showed symptoms of severe neurotoxicity coupled with serum chemistry changes in the absence of DNA damage or brain pathology. GSPE demonstrated some protection against MOCAP-induced tissue injury. This study suggests that in vivo GSPE-exposure may protect multiple organs from a variety of toxic assaults (Ray et al., 1999a).

2.3.13. ActiVin against chronic pancreatitis in humans

This study reports two cases in which patients suffering from chronic pancreatitis symptoms were ameliorated with GSPE after traditional therapy had failed. (1) A 39 year old man with a 3 year history of idiopathic relapsing pancreatitis had abdominal pain four to five times a month, each lasting 3–7 days and requiring narcotic analgesics. Pain was assessed as 5/10 on the Numerical Rating Scale (NRS). Symptoms and pancreatic inflammation persisted despite medical therapy. After starting GSPE 100 mg po tid, only a single episode of pain (5/10, NRS) occurred in 7 months. (2) A 59 year old woman with chronic pancreatitis for 5 years had daily abdominal pain (7/10, NRS) and vomiting despite pancreatic enzyme supplements and pancreatic stent placement. Her pain improved (3/10, NRS) on GSPE 100 mg tid with no more vomiting in the last 4 months. GSPE, a potent scavenger of free radicals, appears to provide effective symptom control in chronic pancreatitis. GSPE was found to reduce both pain index and incidence of vomiting in these patients (Banerjee et al., 1998).

2.3.14. Sun protection factor and protection against UVA and UVB radiation in humans

Human volunteers were exposed to a 150 W Xenon Arc Solar Stimulator to produce a stimulation of the solar UVA-UVB spectrum. Proactive application of 1% GSPE creme 30 min prior to UVA-UVB radiation provided a 9% increase in SPF (sun protection factor) protection. Thus, topical application of GSPE enhanced the SPF through tissue conditioning and presumably by scavenging oxygen free radicals (Raysor and Bagchi, unpublished data).

3. Conclusions

Epidemiological evidence links high antioxidant status with low risk of degenerative disease including tumor promotion and cancer in humans. The increased consumption of fresh vegetables and fruits is usually associated with the decreased use of fish, meats and fats. Furthermore, supplementation of bioavailable and safe antioxidants are essential because we do not get enough antioxidant vitamins and minerals from foods and beverages we consume daily. These research studies demonstrate GSPE as a safe, novel, highly potent and bioavailable free radical scavenger and antioxidant possessing a broad spectrum of health benefits. GSPE functions at the genetic level and promotes therapeutic efficacy. Further mechanistic and clinical studies are in progress to unveil the mechanism of this novel natural antioxidant.

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