Cellular Protection with Proanthocyanidins Derived from Grape Seeds

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ABSTRACT: Grape seed proanthocyanidins have been reported to possess a broad spectrum of pharmacological and medicinal properties against oxidative stress. We have demonstrated that IH636 proanthocyanidin extract (GSPE) provides excellent protection against free radicals in both *in vitro* and *in vivo* models. GSPE had significantly better free radical scavenging ability than vitamins C, E and β-carotene and demonstrated significant cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal cells. GSPE protected against tobacco-induced apoptotic cell death in human oral keratinocytes and provided protection against cancer chemotherapeutic drug-induced cytotoxicity in human liver cells by modulating cell cycle/apoptosis regulatory genes such as bcl2, p53 and c-myc. Recently, the bioavailability and mechanistic pathways of cytoprotection by GSPE were examined on acetaminophen-induced hepatotoxicity and nephrotoxicity, amiodarone-induced pulmonary toxicity, doxorubicin-induced cardiotoxicity, DMN-induced immunotoxicity and MOCAP-induced neurotoxicity in mice. Serum chemistry changes, integrity of genomic DNA and histopathology were assessed. GSPE pre-exposure provided near complete protection in terms of serum chemistry changes and DNA damage, as well as abolished apoptotic and necrotic cell death in all tissues. Histopathological examination reconfirmed these findings. GSPE demonstrated concentration-/dose-dependent inhibitory effects on the drug metabolizing enzyme cytochrome P450 2E1, and this may be a major pathway for the anti-toxic potential exerted by GSPE. Furthermore, GSPE treatment significantly decreased TNFα-induced adherence of T-cells to HUVEC by inhibiting VCAM-1 expression. These results demonstrate that GSPE is highly bioavailable and may serve as a potential therapeutic tool in protecting multiple target organs from structurally diverse drug- and chemical-induced toxicity.

KEYWORDS: proanthocyanidins; free radicals; antioxidants

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INTRODUCTION

Free radicals have been implicated in more than one hundred disease conditions in humans, including arteriosclerosis, AIDS, arthritis, brain and cardiovascular dysfunctions, carcinogenesis, cataracts, diabetes, ischemia and reperfusion injury of many tissues, ocular dysfunction and tumor promotion.[1–3] 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.[1,2] Proanthocyanidins are powerful naturally occurring polyphenolic antioxidants widely available in fruits, vegetables, seeds, nuts, flowers and bark.[4,5] Proanthocyanidins are known to possess antibacterial, antiviral, antiinflammatory, antiallergic and vasodilatory actions.[4–6] They have also been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility.[4] Proanthocyanidins have been shown to modulate the activity of regulatory enzymes including cyclooxygenase, lipooxygenase, protein kinase C, angiotensin-converting enzyme, hyaluronidase enzyme and cytochrome P450 activities.[4–6] In our laboratory, concentration- and dose-dependent free radical scavenging abilities of a novel IH636 grape seed proanthocyanidin extract (GSPE) were assessed both *in vitro* and *in vivo* models, and compared with vitamins C, E and β-carotene. GSPE exhibited significantly better protection than vitamin C, E and β-carotene.[7-9] GSPE demonstrated selective cytotoxicity towards cultured human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal cells.[10]

To understand the mechanistic pathways of cytoprotection and organ-specific bioavailability of GSPE, a series of experiments were conducted. Protective ability of GSPE was assessed against smokeless tobacco–induced oxidative stress, genomic DNA fragmentation, and apoptotic cell death in a primary culture of normal human oral keratinocytes, and compared with vitamins C and E, singly and in combination. The protective ability of GSPE was also assessed against chemotherapeutic drug(s) (4-hydroxyperoxycyclophosphamide or idarubicin)–induced cytotoxicity towards cultured normal human liver cells. Furthermore, organ-specific bioavailability and protective ability of GSPE was investigated against a broad spectrum of drug- and chemical-induced multiorgan toxicity in mice. The chemoprotective ability of GSPE was assessed against acetaminophen (APAP)-induced hepatotoxicity, amiodarone (AMI)-induced pulmonary toxicity, doxorubicin (DOX)-induced cardiotoxicity, dimethylnitrosamine (DMN)-induced splenotoxicity and O-ethyl-S,S-dipropyl phosphorodithioate (MOCAP)-induced neurotoxicity. Serum chemistry changes, integrity of genomic DNA and histophathologic assessment were conducted. Furthermore, the regulatory effect of GSPE and drug metabolizing enzyme cytochrome P450 2E1 and cell adhesion molecules were determined.

IN VITRO FREE RADICAL SCAVENGING ABILITY

The free radical scavenging abilities 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. A concentration-dependent inhibition was demonstrated by GSPE. At a

100 mg/L concentration, GSPE exhibited 78–81% inhibition of superoxide anions and hydroxyl radicals. Under identical conditions, vitamin C inhibited these two free radicals by approximately 12–19%, while vitamin E inhibited these two oxygen radicals by 36–44%.[7] GSPE also demonstrated superior RSA against peroxyl radicals as compared to Trolox.[9]

Concentration-dependent protective ability of GSPE was assessed against hydrogen peroxide–induced oxidative stress in cultured J774A.1 macrophage and neuroactive PC-12 adrenal pheochromocytoma cells using a laser scanning confocal microscopy. The overall intracellular oxidized states of these cells following incubation with hydrogen peroxide was assessed at an excitation wavelength of 513 nm using 2,7-dichlorofluorescein diacetate as fluorescent probe. Approximately 5.8- and 4.5-fold increases in fluorescence intensity were observed following incubation of J774A.1 and PC-12 cells with 0.50 mM hydrogen peroxide for 24 h, respectively. Pretreatment of the J774A.1 cells with 50 and 100 mg/L GSPE decreased hydrogen peroxide-induced fluorescent intensity by 36% and 70%, while under these same conditions approximately 50% and 70% in fluorescence intensities were observed in PC-12 cells, respectively. Thus, GSPE provided significant protection against oxidative stress induced by hydrogen peroxide in these cells.[11]

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, A-427 lung cancer, and CRL-1739 gastric adenocarcinoma cells at 25 and 50 mg/L concentrations for 0–72 h using cytomorphology and MTT cytotoxicity assay, and these effects were compared with those on two normal cells, including normal human gastric mucosal and J774A.1 macrophage cells. Concentration- and time-dependent cytotoxic effects were induced by GSPE in human breast, lung, and gastric adenocarcinoma cells, while under these same conditions GSPE enhanced the growth and viability of the normal cells.[10]

SMOKELESS TOBACCO–INDUCED OXIDATIVE STRESS AND APOPTOTIC CELL DEATH AND PROTECTION BY ANTIOXIDANTS

The protective ability of GSPE was assessed against smokeless tobacco–induced oxidative damage and programmed cell death (apoptosis) in a primary culture of human keratinocytes. Approximately 9, 29, and 25% apoptotic cell death were observed in these cells following treatment with 100, 200, and 300 µg/mL tobacco-treated cells, respectively. Pretreatment of the 300 µg/mL tobacco-treated cells with 100 mg GSPE/L reduced tobacco-induced apoptotic cell death by 85% in oral cells, while a combination of vitamins E and C (75 µM each) reduced tobacco-induced apoptotic cell death by 46%.[12]

PROTECTION AGAINST CANCER CHEMOTHERAPY DRUG-INDUCED TOXICITY IN HUMAN LIVER CELLS

Anticancer chemotherapeutic agents are effective in inhibiting growth of cancer cells *in vitro* and *in vivo*, however, toxicity to normal cells is a major problem in this therapeutic intervention. The effect of GSPE was assessed to ameliorate chemotherapy drug–induced toxic effects in cultured Chang epithelial cells, established from nonmalignant human liver tissue. These cells were treated *in vitro* with idarubicin (Ida) (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 of the cells was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue] assay. Results showed that GSPE decreased the growth inhibitory and cytotoxic effects of Ida as well as 4-HC on Chang epithelial cells. Because these chemotherapeutic agents are known to induce apoptosis in the target cells, the Chang epithelial cells were analyzed for apoptotic cell population by flow cytometry. There was a significant decrease in the number of cells undergoing apoptosis following treatment with GSPE. An increased expression of the anti-apoptotic protein bcl2 was observed in GSPE-treated cells. These results indicate that GSPE can be a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents and one of the mechanisms of action of GSPE includes upregulation of bcl2 expression.[13]

ACETAMINOPHEN (APAP)-INDUCED HEPATO- AND NEPHROTOXICITY, AMIODARONE (AMI)-INDUCED PULMONARY TOXICITY AND DOXORUBICIN (DOX)-INDUCED CARDIOTOXICITY *IN VIVO*, AND PROTECTION BY GSPE

The short and long term protective effects of GSPE were examined on APAP overdose-induced lethality and liver toxicity. Mice were administered nontoxic doses of GSPE (three or seven days, 100 mg/kg, p.o.) followed by hepatotoxic doses of APAP (400 or 500 mg/kg, i.p.). GSPE dramatically decreased APAP-induced mortality, serum alanine aminotransferase (ALT) activity, a biomarker of hepatotoxicity, and hepatic DNA damage. APAP caused a massive elevation in ALT activity, which exceeded control value (45 ± 2 U/L) by approximately 663-fold (29,813 ± 463 U/L). In contrast, ALT activity did not change following oral administration of GSPE alone for seven days (27 ± 2 U/L). However, this mode of GSPE preexposure followed by APAP administration showed excellent hepatoprotection. GSPE+APAP combination also showed a dramatic decrease in ALT activity (2,792 ± 78 U/L). Histopathological evaluation of liver and kidney sections showed a remarkable interference of GSPE against APAP toxicity and substantial inhibition of apoptotic and necrotic liver cell death. APAP was also shown to phosphorylate (deactivate) the bcl-X_L gene, a death inhibitor gene and a positive regulator of the bcl2 family of genes. In contrast, GSPE alone enhanced the expression of the bcl-X_L gene and significantly reduced APAP-induced phosphorylation of bcl-X_L gene. Thus, GSPE significantly

attenuates APAP-induced lethality, liver toxicity, hepatic DNA damage, ALT activity, apoptotic cell death and positively influences gene expression.[14–16]

This high dose of APAP (500 mg/kg, p.o.) also caused a significant increase in serum BUN level (3.2-fold increase). Vehicle alone (control) or GSPE alone (100 mg/kg/day) did not alter kidney function at all, rather BUN levels remained the same.[16,17] However, GSPE preexposure for seven days significantly reduced APAP-induced BUN elevation. A 3.2-fold BUN increase by APAP alone was reduced to 1.5-fold by GSPE+APAP. GSPE preexposure was very effective in minimizing injury inflicted by APAP in both liver and kidneys. DNA damage was also assessed in the variously treated tissues. GSPE treatment alone for seven days did not alter the integrity of genomic DNA in the kidneys. Approximately 4.8- and 2.6-fold increases in DNA fragmentation in the liver and kidney tissues were observed following APAP treatment (500 mg/kg, p.o.). However, GSPE preexposure exhibited a significant effect on APAP-induced DNA fragmentation in the liver (157% of control) and kidney (106% of control) tissues. Similar to serum chemistry parameters, GSPE preexposure did not fully reverse APAP-induced DNA fragmentation, reflecting the residual damage in the heavily injured cells associated with ongoing recovery, repair, and healing processes.[16,17]

The potential of GSPE to defend the lungs from AMI-induced changes were evaluated biochemically and histopathologically. Four consecutive doses of AMI (a total of 200 mg/kg in four days) caused approximately a 9.0-fold increase in serum CK (creatine kinase) activity, and a 5-fold increase in serum ALT activity. Control and GSPE alone sera showed normal ranges of BUN and CK indicating AMI's failure to influence these two organs. However, GSPE preexposure prior to and during AMI treatment considerably reduced pulmonary injury, and brought down the serum chemistry changes close to normal. The integrity of lung DNA was determined quantitatively by sedimentation assay and qualitatively by agarose gel electrophoresis. AMI alone induced moderate DNA fragmentation (152%), and the GSPE+AMI group showed near total countering of AMI-induced pulmonary genomic DNA damage. This effect was due to bioavailability of GSPE in the lung to defend against AMI-related toxic effects. Agarose gel electrophoresis of genomic DNA isolated from lung tissue mirrored the quantitative assay. Histopathological evaluation supported biochemical data.[16,17]

The cytoprotective efficacy of GSPE was tested on DOX-induced cardiotoxicity in mice. DOX alone induced a 6.0-fold increase in serum CK activity. Coupled with CK activity, a 6.0-fold increase in serum ALT activity in the absence of any increase in BUN was observed in DOX-exposed animals. In this context, a 6.0-fold increase in CK activity is considered toxicologically significant and may threaten animal survival, whereas a 6.0-fold increase in ALT activity is totally a reversible change and does not pose any danger to animal health. Despite significant alterations induced by DOX, 7-day GSPE preexposure totally abolished DOX-effects and brought down the CK/ALT activities close to normal. These data also suggests that GSPE was bioavailable in both the heart and the liver in order to counteract DOX-effects.[16,17]

12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA), DIMETHYLNITROSAMINE (DMN) AND MOCAP-INDUCED MULTIORGAN TOXICITY, AND PROTECTION BY GSPE

The protective abilities of GSPE, vitamin E, vitamin C, β-carotene and a combination of vitamins E plus 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 macrophages of mice, were assessed *in vivo*.[8] TPA is a well-known inducer of ROS and tumor promotion *in vivo*. Pretreatment of mice with GSPE (100 mg/kg), vitamin E (100 mg/kg), vitamin C (100 mg/kg), β-carotene (50 mg/kg) and a combination of vitamins E plus C (100 mg/kg 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% in brain tissues, and 47, 30, 10, 11 and 38% in liver tissues, respectively, while lipid peroxidation was reduced by 61, 45, 13, 8, and 48% in brain tissues, and 46, 36, 12, 7, and 39% in liver mitochondria and 59, 47, 14, 12, and 53% in liver microsomes, respectively, as compared to controls. Pretreatment of mice with GSPE (25, 50 and 100 mg/kg) 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.[8]

DMN is a potent immunotoxin as well as a carcinogen. The spleen is one of the primary targets of DMN besides many other organs in the body. Unfortunately, splenotoxicity lacks a serum chemistry marker. A 10 mg/kg i.p. dose of DMN induced a 52.3-fold increase in ALT activity, while GSPE preexposure provided a 94% protection against DMN-induced ALT leakage. Compared with the control and GSPE-exposed animals, DMN alone caused a massive fragmentation of genomic DNA in the spleen and induced massive cell death by apoptosis in addition to necrosis. On a quantitative basis DMN caused a 2.5-fold increase in DNA fragmentation over control tissues. However, the damage induced by DMN was completely abolished by GSPE preexposure. A near total recovery from the damage was evident in GSPE+DMN exposed mice. Qualitatively, an identical pattern of changes in the integrity of DNA, was observed. GSPE provided dramatic protection against DMN-induced splenotoxicity in hispathological examination.[16,18] The dramatic protective effects of GSPE on DMN-induced splenotoxicity, raises several possibilities:

- *(i)* interference with DMN metabolism;
- *(ii)* detoxification of DMN metabolites including biological reactive intermediates;
- *(iii)* interference with endonuclease activity, and
- *(iv)* interference with DNA methylation.

Whether GSPE influenced DMN-induced changes in bcl-X_L expression in the spleen remains open to future investigation.

O-Ethyl-S,S-dipropyl phosphorodithioate (MOCAP) induced a 3.5-fold increase in serum CK levels along with marginally altering serum ALT and BUN levels. GSPE+MOCAP treated animals showed a serum chemistry profile, which closely resembled control animals or those treated with GSPE alone. Following treatment with MOCAP, most animals were lethargic and showed loss of control in their activities. Some animals died because of non-specific neurotoxicity. MOCAP did not alter the integrity of genomic DNA significantly. Similarly, GSPE had no adverse effects on the brain DNA. In histopathological evaluation, MOCAP did not induce any overt histopathological changes except mild periventricular leukomalacia, mineralization and apoptotic cell death. These effects were partially reversed in GSPE+MOCAP exposed brains. Apoptotic cell death was observed microscopically in the absence of massive genomic DNA fragmentation.[16,18]

HUMAN CLINICAL TRIALS

Hypercholesterolemia, a significant cardiovascular risk factor, is prevalent in the American population. Many drugs lower circulating cholesterol levels, but they are not infrequently associated with severe side effects. We examined 40 hypercholesterolemic subjects (total cholesterol 210–300 mg/dL) in a randomized, double-blind, placebo-controlled study.[19] The four groups of 10 subjects received either placebo b.i.d., chromium polynicotinate (CM) 200 μg b.i.d., GSPE 100 mg b.i.d., or a combination of CM and GSPE at the same dosage b.i.d. Over two months, the average percent change ± SEM in total cholesterol from baseline among groups was: placebo −3.5% ± 4, GSPE −2.5% ± 2, CM −10% ± 5, and combination −16.5% ± 3. The decrease in the last group was significantly different from placebo ($p < 0.01$). The major decrease in cholesterol concentration was in the LDL levels: placebo −3.0% ± 4, GSPE −1.0% ± 2.0, CM −14% ± 4.0, and combination −20% ± 6.0. Again, the combination of CM and GSPE significantly decreased LDL when compared to placebo ($p < 0.01$). HDL levels did not change among the groups. Also, there was no significant difference in the triglyceride concentrations among the groups; and no statistically significant differences were seen in the levels of autoantibodies to oxidized LDL. However, the trend was for the two groups receiving GSPE to have greater decreases in the latter parameter, i.e., –30.7% and –44.0% in the GSPE and combined groups in contrast to −17.3% and −10.4% in the placebo and chromium groups. We determined the number of subjects in each group who decreased autoantibodies to oxidized LDL greater than 50% over 8 weeks and found these ratios among groups: placebo, 2/9; CM, 1/10; GSPE, 6/10; and combined, 3/8. Thus, 50% of subjects (9/18) receiving GSPE had a greater than 50% decrease in autoantibodies compared to 16% (3/19) in the two groups not receiving GSPE. No significant changes occurred in the levels of circulating homocysteine and blood pressure among the four groups. These results demonstrate that a combination of CM and GSPE can decrease total cholesterol and LDL levels significantly. Furthermore, there was a trend toward decreased circulating autoantibodies to oxidized LDL in the two groups receiving GSPE.[19]

In another human clinical study, GSPE supplementation ameliorated symptoms of chronic pancreatitis in patients after traditional therapy had failed. GSPE 100 mg t.i.d. provided effective symptom control by reducing both pain index and incidence of vomiting in these patients significantly.[20]

MECHANISMS OF CYTOPROTECTION BY GSPE

GSPE preexposure significantly demonstrated profound antiendonucleolytic, antilipoperoxidative, antiapoptogenic and antinecrogenic potential. A series of *in vivo* studies demonstrated the bioavailability of GSPE to multiple target organs including liver, kidney, heart, spleen and the brain. Orally administered GSPE for 7–10 consecutive days followed by exposure to toxic doses of diverse organotropic chemicals protected select organ toxicities. Furthermore, these studies addressed antinephrotoxic, anti-immunotoxic and antineurotoxic potential of GSPE. Data presented here show that GSPE administration for several days is clearly bioavailable in multiple target organs.[14–18]

Previous studies from our laboratories have linked the protective abilities of GSPE with inactivation of antiapoptotic gene bcl-X_L, and modification of several other critical molecular targets such as DNA-damage/DNA-repair, lipid peroxidation and intracellular Ca^{2+} homeostasis.[14] Especially, GSPE provided dramatic protection against APAP-induced hepato- and nephrotoxicity, significantly increased bcl-X_L expression in the liver tissue and antagonized both necrotic and apoptotic deaths of liver cells *in vivo*. However, it was not clear from this study whether the antiapoptotic and antinecrotic effects of GSPE were:

- *(i)* due to its interference with endonuclease activity,
- *(ii)* due to its antioxidant effect, or,
- *(iii)* due to its ability to inhibit microsomal drug metabolizing enzyme(s), such as cytochrome P450 2E1.

Since cytochrome P450 2E1 primarily metabolizes acetaminophen and other drugs/chemicals in mice and rats, we assessed cytochrome P450 2E1's catalytic activity in both *in vitro* and *in vivo* models. Overall this investigation compared the *in vitro* aniline hydroxylation patterns of:

- *(i)* *in vivo* GSPE-exposed and unexposed (control) mouse liver microsomes,
- *(ii)* induced (1% acetone in drinking water for 3 days) and uninduced rat liver microsomes in the presence and absence of GSPE *in vitro*, and
- *(iii)* control rat liver microsomes in the presence of an anti-APAP agent 4-AB *in vitro*.

For the *in vivo* assessment, male B6C3F1 mice were fed GSPE diet (ADI 100 mg/kg) for four weeks, and liver microsomes were isolated from both control and GSPE-fed mice for aniline hydroxylation, a specific marker of cytochrome P450 2E1 activity. Data showed that hydroxylation was 40% less in microsomes from GSPE-exposed livers compared to control microsomes. Similarly, when rat liver microsomes were incubated with various concentrations of GSPE *in vitro* (100 µg/mL), aniline hydroxylation

was inhibited to various degrees (uninduced: 40 and 60% and induced: 25 and 50%, respectively, with 100 and 250 µg/mL). Influence of GSPE on hydroxylation patterns were compared with another hepatoprotective agent 4-AB, a well-known modulator of nuclear enzyme poly(ADP-ribose)polymerase, and the data shows that 4-AB did not alter aniline hydroxylation at all. Collectively, these results may suggest that GSPE has the ability to inhibit cytochrome P450 2E1, and this additional cytoprotective attribute is in conjunction with its novel antioxidant and/or antiendonucleolytic potential.[21]

Altered expression of cell adhesion molecule expression has been implicated in a variety of chronic inflammatory conditions. Regulation of adhesion molecule expression by specific redox sensitive mechanisms has been reported. The effects of GSPE was evaluated on the expression of TNFα-induced ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells (HUVEC). GSPE at low concentrations (1–5 µg/ml), down-regulated TNFα-induced VCAM-1 expression but not ICAM-1 expression in HUVEC. Such regulation of inducible VCAM-1 by GSPE was also observed at the mRNA expression level. A cell-cell co-culture assay was performed to verify whether the inhibitory effect of GSPE on the expression of VCAM-1 was also effective in down-regulating actual endothelial cell/leukocyte interaction. GSPE treatment significantly decreased TNFα-induced adherence of T-cells to HUVEC.[22] The potent inhibitory effect of low concentrations of GSPE on agonist-induced VCAM-1 expression suggests therapeutic potential of this extract in inflammatory conditions and other pathologies involving altered expression of VCAM-1.[22]

CONCLUSION

A number of epidemiological studies have exhibited that high antioxidant status is directly linked to low risk of degenerative disease. Increased consumption of fresh fruits and green vegetables can promote antioxidant status in the human body. On the other hand, cigarette smoking, physical stress, and over-processed foods, laden with hydrogenated fat, sugar, refined flours and chemicals, can potentiate enhanced free radical production and oxidative stress in the human body leading to a broad spectrum of degenerative diseases. Unfortunately, while the physical, emotional, environmental and chemical stressors are growing, the essential nutrition in our increasingly processed and pre-packaged food supply is decreasing. Furthermore, while coping with our hectic lifestyle we have great difficulty finding time to consume a good amount of pesticide-free fresh fruits and green vegetables. Thus, a novel, natural and bioavailable antioxidant may improve our lifestyle by enhancing our antioxidant status. Taken together, our research studies demonstrate that GSPE is highly bioavailable and may serve as a potential therapeutic tool in protecting multiple target organs from structurally diverse drug-, environmental-, and chemical-induced toxic assaults. Further, mechanistic and clinical studies are in progress to unveil the benefits of this novel, natural antioxidant.

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