Mechanistic pathways of antioxidant cytoprotection by a novel IH636 grape seed proanthocyanidin extract

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To understand the bioavailability and mechanistic pathways of cytoprotection by IH636 grape seed proanthocyanidin extract (GSPE, commercially known as ActiVin) a series of in vitro and in vivo studies were conducted. Comparative protective abilities of GSPE, and vitamins C and E, singly and in combination, were assessed against smokeless tobacco extract (STE)-induced oxidative stress, DNA fragmentation and apoptotic cell death in a primary culture of normal human oral keratinocytes. GSPE protected against STE-induced oxidative stress, DNA damage and apoptotic cell death, and provided better protection as compared to vitamins C and E, singly and in combination. The bioavailability and protective ability of GSPE were examined against acetaminophen (AP)-induced hepato- and nephrotoxicity, amiodarone (AM)-induced lung toxicity, doxorubicin (DX)-induced cardiotoxicity and dimethylhydrazine (DM)-induced splenic toxicity in mice. GSPE-fed animals were compared with GSPE-untreated mice to evaluate the protective ability of GSPE against these structurally diverse drugs/chemicals. Serum chemistry changes, histopathology and DNA damage were evaluated. Results indicate that GSPE preexposure to the drugs/chemicals such as AP, AM, DX or DM treatment, provided near complete protection in terms of serum chemistry changes and inhibition of both forms of cell death, e.g., apoptosis and necrosis. DNA damage in various tissues triggered by these agents was significantly reduced in GSPE-fed animals. Histopathological examination of multiple target organs provided similar data. The results suggest that GSPE exposure is bioavailable and provides significant multiorgan protection against structurally diverse drug- and chemical-induced toxic assaults. Further, these studies exhibited a series of mechanistic information including free radical scavenging ability, anti-endonucleolytic activity, cytochrome P450 2E1 inhibitory activity, anti-necrotic, anti-apoptotic and anti-carcinogenic activities, modulatory effects on antioxidative and apoptotic regulatory genes such as Bcl2, c-myc and p53, which may be responsible for the novel chemoprotective properties exhibited by GSPE.

Free radicals have been implicated in more than one hundred disease conditions in humans, including cardiovascular, brain and ocular dysfunctions, arthritis, ischemia and reperfusion injury of many tissues, diabetes, tumor promotion and carcinogenesis, and AIDS. Antioxidants/free radical scavengers function as inhibitors at both initiation and promotion/proagation/transformation stages of tumor promotion/carcinogenesis and protect cells against oxidative damage. Antioxidants inhibit both initiation and promotion stages in carcinogenesis and counteract cell immortalization and transformation. There is considerable evidence for a role of the antioxidant nutrients including vitamins C, E and β-carotene, in the maintenance of human health. The consumption of edible plants, fresh fruits and green vegetables has been demonstrated to prevent the occurrence of a number of diseases in humans and animals. Fresh vegetables, fruits and their seeds are rich sources of vitamins C, E and β-carotene, and/or protease inhibitors, compounds which may protect the organism against free radical-induced injury and diseases.

It is important to consider the following points to evaluate the therapeutic potential of a given natural or synthetic 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
9. Tissue repair and regeneration (!)
10. DNA repair activity

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Proanthocyanidins are powerful naturally occurring polyphenolic antioxidants widely available in fruits, vegetables, seeds, nuts, flowers and bark. Proanthocyanidins are known to possess antibacterial, antiviral, antiinflammatory, antiallergic and vasodilatory actions. They have also been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility. Proanthocyanidins have been shown to modulate the activity of regulatory enzymes including cyclooxygenase, lipoxygenase, protein kinase C, angiotensin-converting enzyme, hyaluronidase enzyme and cytochrome P450 activities. Concentration- or dose-dependent free radical scavenging abilities of a novel IH636 grape seed proanthocyanidin extract (GSPE) were assessed in both in vitro and in vivo models, and compared with vitamins C, E and β-carotene. GSPE protected against hydrogen peroxide-induced oxidative damage to cultured macrophage J774A.1 and adrenal pheochromocytoma PC-12 cells in a dose-dependent manner as demonstrated by laser scanning confocal microscopy. GSPE exhibited selective cytotoxicity towards cultured human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal cells. Further, GSPE protected against chemotherapeutic drug(s) (4-hydroxyperoxycyclophosphamide or idarubicin)-induced cytotoxicity towards cultured normal human liver cells. In the in vivo studies, GSPE supplementation provided excellent protection against myocardial ischemia-reperfusion injury and myocardial infarction in rats, as well as against acute- and chronic stress-induced gastrointestinal mucosal lipid peroxidation, genomic DNA fragmentation and membrane microviscosity.

To understand the organ-specific bioavailability and mechanistic pathways of cytoprotection by 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. 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 (AP)-induced hepato- and nephrotoxicity, amiodarone (AM)-induced pulmonary toxicity, doxorubicin (DX)-induced cardiotoxicity and dimethylnitrosamine (DM)-induced spleenotoxicity.

Materials and Methods

Chemicals — IH636 Grape seed proanthocyanidin extract (GSPE, commercially known as ActiVin) was obtained from InterHealth Nutraceuticals, Inc. (Benicia, CA). This is a standardized water-ethanol extract from red grape seeds. High-performance liquid chromatographic and gas chromatography-mass spectrometric analyses demonstrated that GSPE contains oligomeric proanthocyanidins (OPC) including 54% dimeric-, 13% trimeric and 7% tetrameric OPCs, and a small amount of catechin derivatives and other flavonoids. Chemicals and solvents used in this study were obtained from Sigma Chemical Company (St. Louis, MO) and were of analytical grade or highest grade available.

Cell culture and treatment — Human oral keratinocytes were cultured from retromolar tissue obtained from human volunteers as described earlier. The cells were used for all studies after they had reached >60% confluence. Smokeless tobacco extract (STE) was prepared as described earlier. Cells were treated with either 0 or 300 μg/ml concentrations of STE, and STE-induced oxidative stress and fragmentation of genomic DNA were determined by lipid peroxidation and DNA ladder analysis by gel electrophoresis, while apoptotic cell death was determined by flow cytometry. Furthermore, comparative protective abilities of vitamin C (75 μM), vitamin E (75 μM), a combination of vitamins C and E (75 μM each) and GSPE (100 μg/ml) were assessed.

Animals and treatment — All animal treatment and protocols received prior approval by the Institutional Laboratory Animal Care and Use Committee, and met or exceeded any local, state, or federal standards. Male ICR (CD-1) mice weighing approximately 35 g (3 months old) were obtained from Harlan-Sprague-Dawley, Inc. (Indianapolis, IN) and given access to lab chow (Purina Laboratory Rodent Chow, St Louis, MO) and tap water ad libitum. Animals were allowed to acclimate in an environment of controlled temperature (22°C-25°C), humidity, and 12 hr light/12 hr dark cycle for at least seven days prior to study. Treated animals were starved for only 1 hr immediately following drug/toxicant treatment and returned to normal access to food and water.

Acetaminophen (AP) treatment: GSPE and AP were dissolved in saline (pH 7.4). Four groups of animals were maintained. Control animals received saline only, GSPE alone group received 100 mg GSPE/kg/day, po for 7 days, AP alone group was
given 500 mg AP/kg dose, ip for a total period of 24 hr and GSPE+AP-treated group was administered 100 mg GSPE/kg/day, po for 7 days and AP was administered 500 mg/kg, ip for a total period of 24 hr on day 7 following GSPE exposure. This hepato- and nephrotoxic dose of AP was selected from our previous studies\(^\text{16}\).

Amiodarone (AM) treatment: AM was dissolved in warm saline (pH 7.4). Four groups were maintained. Control group received saline, GSPE alone group received 100 mg GSPE/kg/day for 10 days, AM-treated group received four ip injections of 50 mg AM/kg for four days and GSPE+AM-treated group (received a total 10 days of GSPE 100 mg/kg/day po, and four 50 mg/kg, po doses of AM from day 7 to day 10. The AM dose used in this study was shown to cause pulmonary toxicity in previous studies\(^\text{17,18}\).

Doxorubicin (DX) treatment: DX was dissolved in normal saline (pH 7.4). Four experimental groups were maintained. Control group received saline, GSPE alone group received 100 mg GSPE/kg/day, po for 9 days, DX-treated group received a single ip injection of 20 mg DX/kg for 48 hr and GSPE+DX-treated group received 100 mg GSPE/kg/day po for 9 days and a 20 mg/kg, po dose of DX was administered on day 7, one hr after regular GSPE exposure, and the animals were sacrificed 48 hr after DX exposure on day 9. The cardiotoxic dose of DX was selected from previous studies\(^\text{19,20}\).

Dimethyl nitrosamine (DM) treatment group: DM was dissolved in normal saline (pH 7.4). Four experimental groups were maintained. Control group received saline, GSPE alone group received 100 mg GSPE/kg/day, po for 8 days, DM-treated group received a single ip injection of DM which was given at a dose of 10 mg/kg for a total period of 48 hr and GSPE+DM-treated group was given 100 mg GSPE/kg/day, po for 8 days and a single dose of 10 mg DM/kg was administered ip on day 7, one hr after regular GSPE exposure, and the animals were sacrificed 48 hr following DM administration. The toxic dose of DM was selected from previous studies\(^\text{21,22}\).

Serum chemistry—Serum ALT (alanine aminotransferase, EC 2.6.1.2) level, a marker of hepatoxicity, blood urea nitrogen (BUN) level, a marker of kidney damage, and creatine kinase (CK) activity, a marker enzyme for cardiac damage, were measured using Sigma Diagnostic Kits 52-UV, 67-UV and 47-UV, respectively.

Histology—Section (2-3 mm thick) of the respective tissue was collected at the time of sacrifice and preserved in 10% buffered formalin. Normal, apoptotic and necrotic cells were identified from 5- to 7.5 μm PAS-stained tissue sections. Our previously published guidelines were followed to characterize normal apoptotic and necrotic cells\(^\text{16}\).

Lipid peroxidation—Production of thiobarbituric acid reactive substances (TBARS), a commonly used technique to assess lipid peroxidation, was assessed according to the method previously described by us\(^\text{23}\). Malondialdehyde was used as the standard. Absorbance values were measured at 535 nm and an extinction coefficient of 1.56 x 10^5 M^-1 cm^-1 was used.

DNA fragmentation and DNA ladder analysis by gel electrophoresis—Frozen tissue samples were homogenized in lysis buffer (5 mM Tris HCl, 20 mM EDTA, 0.5% Triton X-100, pH 8). Homogenates were centrifuged at 27,000 g for 20 min to separate intact chromatin in the pellets from fragmented DNA in the supernatant fractions. Pellets were resuspended in 0.5 N perchloric acid and 5.5 N perchloric acid was added to supernatant samples to reach a concentration of 0.5 N. Samples were heated at 90°C for 15 min and centrifuged at 1,500 g for 10 min to remove protein. Resulting supernatant fractions were reacted with diphenylamine reagent for 16-20 hr at room temperature, and absorbances were measured at 600 nm. DNA fragmentation in control samples is expressed as percent of total DNA appearing in the supernatant fraction. Treatment effects are reported as percent of control fragmentation\(^\text{9}\).

Calcium activated endonuclease-dependent ladder-like DNA fragmentation of STE/drug/chemical- and/or antioxidant-treated cells/tissues were assessed by gel electrophoresis as described earlier\(^\text{23}\). A 1-kb Hind III digest of DNA served as a molecular marker.

Cell cycle analysis—Smokeless tobacco- and/or antioxidant-treated cells were analyzed using a Becton Dickinson FACS STAR Plus Flow Cytometer (San Jose, CA). Following data collection, cell cycle distribution and apoptosis (Ap) were modelled using ModFit cell cycle analysis software (Verity Software House, Topsham, ME). The correlation between the flow cytometric data measuring the Ap peak and DNA laddering has been described by Telford et al.\(^\text{24}\) and Joshi et al\(^\text{15}\).

Statistics—The data were analyzed using standard statistical analysis, i.e., ANOVA and Scheffe’s post-hoc test. All values are reported as mean ± SE from 4-9 samples. Statistical significance was set at P < 0.05.
Results

The objective of this study was to determine organ-specific bioavailability, protective ability against structurally diverse drugs and chemicals, and mechanistic pathways of cytoprotection by GSPE. Accordingly, a series of in vitro and in vivo studies were conducted and the results are as follows:

Comparative protective ability of GSPE and selected antioxidants against STE-induced oxidative stress, DNA damage and apoptotic cell death in a primary culture of human oral keratinocytes—Approximately 7.6- and 2.9-fold increases in lipid peroxidation and DNA fragmentation were observed following incubation of oral keratinocytes with 300 μg/ml of STE, respectively (Table 1). STE-induced a ladder-like pattern of endonucleolytic DNA degradation, and partial reversal of STE-induced oligonucleosome length DNA laddering was observed in cells treated with various antioxidants. Near complete protection was provided by GSPE (Figure not shown). Flow cytometric analyses were conducted to determine STE-induced apoptotic cell death in human oral keratinocytes and protective abilities of antioxidants. Approximately 35% apoptotic cell death were observed in keratinocytes following treatment with 300 μg/ml STE. Pretreatment of the 300 μg/ml STE-treated cells with 100 μg GSPE/ml reduced apoptotic cell death by approximately 85% in human oral keratinocyte cells, while pretreatment with a combination of vitamins E and C (75 μM each) reduced STE-induced apoptotic cell death by only 46% (Table 1).

Acetaminophen (AP)-induced hepatotoxicity and protection by GSPE—A single ip dose of AP (500 mg/kg) caused significant hepatotoxicity and nephrotoxicity as demonstrated by a dramatic increase in the serum alanine aminotransferase (ALT) activity—a biomarker of hepatotoxicity, which exceeded control (45±2 U/L) by approximately 663-fold (29,813±463 U/L), and caused a significant increase in serum blood urea nitrogen (BUN) level—a marker of nephrotoxicity, which exceeded control (21±3 mg/dl)

![Fig. 1 — Induction of internucleosomal DNA fragmentation by AP and its reversal by GSPE. Electrophoretogram demonstrating GSPE-mediated protection from AP-induced genomic DNA fragmentation of mouse kidney (lane 1, control; lane 2, 7-day GSPE; lane 3, 24 hr 500 mg/kg AP; lane 4, 7-day GSPE + 24 hr AP 500 mg/kg). See Materials and Methods section for details.](image-url)
by 3.2-fold (67±7 mg/dl). No significant change in ALT and BUN levels were observed following oral administration of GSPE (100 mg/kg/day) alone for 7 days (ALT: 27±2 U/L; BUN: 19±2 mg/dl), while 7 days of GSPE pretreatment followed by AP exposure provided excellent hepatoprotection (approximately 91% protection) as demonstrated by the ALT activity (2,792±78 U/L), as well as significant nephroprotection (approximately 53%) as exhibited by the BUN level (32±4 mg/dl). GSPE treatment alone for 7 days did not alter the integrity of genomic DNA of liver and kidney tissues. Approximately 4.8-and 2.6-fold increases in DNA fragmentation were observed in the liver and kidney tissues following treatment with AP alone, respectively, as compared to the control animals. GSPE preexposure exhibited a significant impact on AAP-induced fragmentation of genomic DNA. GSPE preexposure provided 67 and 76% protection to genomic DNA of the liver and kidney tissues, respectively. GSPE preexposure did not fully reverse AAP-induced DNA fragmentation reflecting the residual damage in the heavily injured cells associated with ongoing recovery, repair, and renovation process. Further, AP-induced a ladder-like pattern of

![Fig. 2](image-url)

Light photomicrographs of paraffin-embedded PAS-stained mouse kidney sections. See Materials and Methods section for details. (A) representative mouse kidney section (X 400) treated with vehicle, no pathologic changes present; (B) representative kidney section (X 400) from mouse treated with GSPE (100 mg/kg, po) for 7 days, no pathologic features present; (C) representative mouse kidney section (X 400) treated with AP for 24 hr, and (D) representative kidney section (X 400) from mouse treated with GSPE (100 mg/kg, po) for 7 days followed by AP for 24 hr. See near complete protection afforded by GSPE on AP-induced changes.
endonucleolytic DNA degradation, and GSPE provided significant protection. Figure 1 demonstrates GSPE-mediated protection against AP-induced genomic DNA fragmentation of mouse kidney. To evaluate the degree of protection by GSPE, formalin-fixed paraffin-embedded PAS stained mouse liver and kidney sections from variously treated groups were examined using a brightfield microscope. Control and GSPE treated liver and kidney sections showed normal architecture identical to each other in the total absence of apoptotic and necrotic cells. AAP alone showed all the typical characteristics of unprogrammed consequences of necrosis in presence of many apoptotic cells with diverse morphology, while GSPE pretreatment provided significant protection. Figure 2 demonstrates GSPE-mediated protection against AP-induced kidney injury histopathologically. Thus, GSPE provided excellent protection against AP-induced hepat- and nephrotoxicity.

Protective effect of GSPE against amiodarone (AM)-induced lung toxicity—Following administration of AM alone, serum CK, a marker enzyme of tissue necrosis, activity exceeded control (1,113 ± 40 U/L) by approximately 8.0-fold (8,891 ± 332 U/L), while serum ALT activity exceeded control (29±2 U/L) by approximately 5-fold (147±24 U/L). No significant increase was observed in the BUN level. Further, serum from control and GSPE-exposed mice showed normal levels. GSPE preexposure provided 79% and 50% protection against AM-induced CK activity (1,850±241 U/L) and ALT activity (74±3 U/L), respectively, as compared to AM-treated samples. DNA isolated from control and GSPE preexposed animals did not change and were intact. AM alone caused 1.5-fold increase in the fragmentation of genomic DNA in the lung tissue, while GSPE preexposure provided near complete protection. AM-induced a ladder-like pattern of endonucleolytic DNA degradation, and GSPE provided significant protection (Fig. 3). Formalin-fixed H&E stained lung sections were observed using a brightfield microscope. Control and GSPE treated tissues showed normal architecture of the lung, whereas AM alone induced moderate changes in some areas of the lung. Morphologically speaking, AM presumably induced phospholipidosis in the alveolar and bronchiolar epithelial cells. Lung sections from GSPE+AM exposed animals (Fig. 1D) closely resembled either control or GSPE alone treated tissues (Fig. 4). Thus, GSPE demonstrated excellent protective ability against AM-induced lung toxicity.

Protective ability of GSPE against Doxorubicin (DX)-induced cardiotoxicity—Compared to the control and GSPE exposure alone, DX alone induced a 6-fold increase in both serum CK activity, a marker enzyme for cardiac damage, and serum ALT activity, a marker enzyme of liver damage (Table 2). A 6.0-fold increase in CK activity is toxicologically significant and may threaten animal survival, while a 6-fold increase in ALT activity is not that detrimental. No significant damage was observed on the BUN level. The integrity of cardiac genomic DNA isolated from control or GSPE exposed animals did not change and were intact. DX alone caused approximately 1.8-fold increase in genomic DNA fragmentation in the cardiac tissue, while DX-induced changes in the integrity of the genomic DNA were completely abolished by 7 day GSPE preexposure. DX-induced a ladder-like pattern of endonucleolytic DNA degradation, and GSPE provided significant protection (figure not shown). Formalin-fixed H&E stained heart sections were observed using a brightfield microscope. In both control and GSPE-treated tissues, normal architecture of the heart was intact. DX alone induced elongation
of cardiac myocytes, myocyte nuclear swelling and/or nucleolysis, and numerous intercellular spaces. Tissue sections of GSPE+DX closely resembled either control or GSPE alone treated tissues. Residual necrosis was minimal (figure not shown). Thus, DX-induced cardiotoxicity was significantly ameliorated by GSPE.

Protective ability of GSPE against dimethylnitrosamine (DM)-induced spleenotoxicity—DM is a well-known immunotoxin as well as a classic hepatotoxin and spleen is the primary target of DM. Unfortunately, there is no known serum chemistry marker for spleenotoxicity. A 10 mg/kg ip dose of DM increased serum ALT activity, a marker of hepatotoxicity, which exceeded control (26±3 U/L) by 52.3-fold (1,361±45 U/L) increase in the ALT activity, while GSPE preexposure provided approximately 94% protection against DM-induced hepatic injury as demonstrated by the ALT activity (88±9 U/L). Compared to control and GSPE exposed animals, DM alone caused a massive fragmentation of genomic DNA in the spleen. DM-induced a 2.5-fold increase in DNA fragmentation in the spleen tissue, which was com-

Fig. 4—Light photomicrographs of paraffin-embedded PAS-stained mouse lung tissue sections. See Materials and Methods section for details. (A) representative mouse lung section (X 400) treated with vehicle, no pathologic changes present; (B) representative section (X 400) from mouse treated with GSPE (100 mg/kg, po) for 10 days, no pathologic features present; (C) representative mouse lung section (X 400) treated with four 50 mg/kg ip doses of AM, and (D) representative section (X 400) from mouse treated with GSPE (100 mg/kg, po) for 10 days and four 50 mg/kg/day ip doses of AM from day 7 through day 10. See near complete protection afforded by GSPE on AM-induced changes.
and DNA fragmentation in mice (TPA)-induced hepatic and brain lipid peroxidation treated against 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced hepatic and brain lipid peroxidation and DNA fragmentation in mice.

Discussion

A broad spectrum of toxicity studies including acute oral, acute dermal, dermal irritation, eye irritation and long-term chronic toxicity, were conducted on GSPE, and these studies demonstrated that GSPE is safe. GSPE demonstrated excellent free radical scavenging abilities in both in vitro and in vivo models. GSPE exhibited excellent scavenging abilities against biochemically generated superoxide anion, hydroxyl radicals and peroxyl radicals. Further, GSPE exhibited concentration-dependent protective ability against hydrogen peroxide-induced modulation of intracellular oxidized states in cultured J774A.1 macrophage and PC-12 adrenal pheochromocytoma cells. GSPE also protected against chemotherapeutic drugs-induced cytotoxicity on normal human Chang liver cells. In a series of in vivo models, GSPE exhibited bioefficacy and protective ability protected against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced hepatic and brain lipid peroxidation and DNA fragmentation in mice, acute and chronic stress-induced oxidative gastrointestinal mucosal injury in rats, and myocardial ischemia-reperfusion injury and myocardial infarction in rats. GSPE exhibited selective cytotoxicity towards human MCF-7 breast cancer, A-427 lung cancer and CRL-1739 human gastric cancer cells, while enhancing the growth and viability of normal human gastric mucosal cells and murine macrophage J774A.1 cells. A large number of in vitro and in vivo studies have been conducted on proanthocyanidins. However, organ-specific bioavailability of GSPE and mechanistic pathways of cytoprotection by GSPE are not clearly understood. This study was focused to determine organ-specific bioavailability, protective ability of GSPE against structurally diverse drugs and chemicals, and mechanistic pathways of cytoprotection by GSPE.

Previous studies have demonstrated the toxic effects of smokeless tobacco extract (STE) on human oral keratinocytes as demonstrated by concentration-dependent increases in the release of lactate dehydrogenase, oxidative stress, DNA damage and enhanced production of nitric oxide. STE dosage was

<table>
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<tr>
<th>Sample</th>
<th>Serum alanine aminotransferase (ALT) activity (U/L)</th>
<th>Creatine kinase (CK) activity (U/L)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>45 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,009 ± 24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSPE</td>
<td>28 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>975 ± 83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dxorubicin (DX)</td>
<td>284 ± 68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5,949 ± 612&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSPE + DX</td>
<td>181 ± 43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,457 ± 193&lt;sup&gt;d&lt;/sup&gt;</td>
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See Materials and Methods section for details. Values with non-identical superscripts are significantly different (P < 0.05).
selected from our previous studies, while the antioxidant doses were selected from our previous studies and extrapolation from human equivalency doses. Toxic doses of acetaminophen (AP), amiodarone (AM), doxorubicin (DX) and dimethyl nitrosamine (DM) were selected from previous studies. AM-induced lung toxicity is one of the major complications in patients receiving administration of AM, the widely used antiarrhythmic drug. The indications for AM included activity of interstitial pneumonitis including sustained ventricular tachycardia and atrial fibrillation. Free radical scavenging and antiendonucleolytic activity may play a role, at least in part, in inducing these novel chemoprotective properties of GSPE. These studies demonstrate that GSPE is bioavailable and can protect vital target organs from structurally diverse drug and chemical-induced organ toxicity. GSPE protected genomic DNA fragmentation and exhibited antiendonucleolytic activity against STE-, AP-, AM-, DX- and DM-induced cell and tissue injury. Free radical scavenging and antiendonucleolytic activity may play a role, at least in part, in inducing these novel chemoprotective properties of GSPE. Also, GSPE may induce significant detoxification by preventing/scavenging the formation of toxic metabolites from these structurally diverse drugs/chemicals. Further, GSPE exhibited significant protective ability against STE-induced apoptotic cell death in human oral keratinocytes, and structurally diverse drug- and chemical-induced necrotic cell death in multiple target organs. We demonstrated that GSPE diminishes cytotoxicity and growth inhibitory effects induced by chemotherapeutic drugs including idarubicin and 4-hydroxyperoxy-cyclophosphamide, on normal human Chang liver cells in vitro. These chemotherapeutic drugs induced apoptotic cell death in Chang liver cells, and we analyzed these liver cells for apoptotic cell population by flow cytometry. There was a significant decrease in the number of cells undergoing apoptosis following treatment with GSPE. Further, an increased expression of anti-apoptotic gene Bcl-

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