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Appendix K

English translations of Japanese Citations

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Appendix L

Studies on the Mechanisms of Action of Green Tea Catechins

Studies Investigating the Mechanisms of Action for Green Tea Catechins

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>He, Z., et al. 2008. Fyn is a novel target of (-)-epigallocatechin gallate in the inhibition of JB6 and CI14 cell transformation. <i>Mol. Carcinogen.</i> 47(3):172-83.</p>	<p><i>In vitro</i> study of the role of Fyn in the regulation of cell transformation using knockout siRNA plasmid. siRNA was stably co-transfected into JB6 CI14 cells.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Cytotoxic effects of EGCG on JB6 Cells • Anchorage-independent transformation assay • <i>In vitro</i> pull-down assay • Purification of GST-Fyn-SH2 and GST-Fyn-SH3 fusion proteins • Determination of GST-Fyn binding affinity with EGCG • EGCG effects on Fyn kinase activity <i>in vitro</i> • Immunoprecipitation and kinase assays • Determination of AP-1, STAT1, and CREB DNA binding 	<ul style="list-style-type: none"> • EGCG inhibits EGF-induced JB6 CI14 cell transformation in a dose-dependent manner • EGCG inhibits EGF-induced Fyn phosphorylation in a dose-dependent manner • EGCG specifically binds with the Fyn SH2 domain • EGF-induced phosphorylation of p38 MAP kinase (Thr180/Tyr182), ATF-2 (Thr71), and STAT1 (Thr727) is inhibited in siRNA-Fyn-JB6 CI14 cells • siRNA-Fyn inhibits EGF-induced cell transformation • EGF-induced DNA binding ability of AP-1, CREB, and STAT1 is decreased in siRNA-Fyn-JB6 CI14 cells 	<p>This is a novel and important mechanism that may be involved in EGCG-induced inhibition of cell transformation</p>
<p>Devika, P.T., Stanley Mainzen Prince, P. 2008. (-) Epigallocatechin gallate (EGCG) prevents</p>	<p>Male albino Wistar rats weighing 140-160 g were pre-treated with EGCG (30 mg/kg) by gavage daily for a period of 21 days. ISO was then subcutaneously injected into rats at intervals of 24 hours for 2 days</p>	<ul style="list-style-type: none"> • Pretreatment with EGCG significantly decreased the levels of TBARS and lipid hydroperoxides and increased the activities of SOD and catalase in the heart mitochondria • Pretreatment with EGCG significantly 	<p>Study confirms preventative effects of EGCG on isoproterenol-induced mitochondrial damage in experimentally</p>

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<p>mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: A transmission electron microscope and <i>in vitro</i> study. <i>Pharmacol. Res.</i> 57(5):351-357.</p>	<p><u>Studies:</u></p> <ul style="list-style-type: none"> • Isolation of heart mitochondria • Estimation of lipid peroxidation products, antioxidants, mitochondrial enzymes, and protein content in heart mitochondria • TEM studies • <i>In vitro</i> studies on the effects of EGCG on scavenging, superoxide anion, and hydroxyl radicals. 	<p>increased the activities of GPx, GRx, GST, and GSH in the heart mitochondria</p> <ul style="list-style-type: none"> • Pretreatment with EGCG significantly increased activities of ICDH, SDH, MDH, α-KGDH, NADH-dehydrogenase and cytochrome-x-oxidase • Pretreatment with EGCG resulted in mild separation of cristae without swelling and vacuolation • EGCG scavenges radicals <i>in vitro</i> in a concentration-dependent manner 	<p>induced myocardial infarction in Wistar rats</p>
<p>Jia, Y., Alayash, A.I. 2008. Effects of (-)-epigallocatechin gallate on the redox reactions of human hemoglobin. <i>Free Radic. Biol. Med.</i> [epub ahead of print]</p>	<p><i>In vitro</i> study investigating the effects of EGCG on auto-oxidation, H₂O₂-induced oxidation and subsequent ferryl heme reduction kinetics in chemically cross-linked human Hb (DBBF), a diaspirin cross-linked human Hb that has been intensively investigated.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Hb auto-oxidation experiments • Ferryl hemoglobin formation and reduction • Sulf hemoglobin measurement • Kinetics of ferryl hemoglobin reduction 	<ul style="list-style-type: none"> • EGCG appears to exhibit strong pro-oxidant effects on DBBF and unmodified HbA₀ • Considerable reduction in the rates of oxidation of DBBF in the presence of EGCG and SOD were seen • In the presence of EGCG, rates of oxidation of DBBF were increased by approximately 56 times, showing an accelerated oxidation process triggered by EGCG • EGCG is a highly potent reductant of ferryl Hb when compared with some iron chelators 	<p>DBBF (ferrous) is rapidly oxidized to the ferric form in the presence of EGCG relative to normal spontaneous oxidation of Hb. A balance between the pro and antioxidant properties of EGCG should be taken into account if EGCG is used in combination therapy with redox active</p>

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		<ul style="list-style-type: none"> • The level of the sulHb, thus the level of detectable ferryl Hb in the reaction, was significantly reduced as EGCG medium increased • It is physiologically relevant to measure the ferryl Hb reduction by EGCG at low pH to evaluate its protective capability against the pro-oxidant activity of modified and unmodified Hbs, especially when used as cell free blood substitute. 	acellular Hbs.
<p>Beretta, G., et al. 2008. Quenching of α,β-unsaturated aldehydes by green tea polyphenols: HPLC-ESI-MS/MS studies. <i>J. Pharm. Biomed. Anal.</i> [epub ahead of print]</p>	<p><i>In vitro</i> study in human colon tumor cells exposed to butyrate, investigating whether EGCG was able to eliminate the formation of HNE (4-hydroxyl nonenol), an α,β-unsaturated aldehyde, by a quenching mechanism.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Consumption of HNE by catechins and green tea • Measurement of H_2O_2 • HPLC – DAD – ESI – MS Analysis • ESI – MS/MS • NMR analysis 	<ul style="list-style-type: none"> • Under the physiological conditions of the lower intestinal tract, EGCG dose- and time-dependently quenched 50 μM HNE • HNE-trapping activity of EGCG is strictly pH-dependent • The reaction mixture of HNE and EGCG after 24-hr incubation did not show epoHNE or the formation of an adduction product between EGCG and epo-HEN, but only a dehydrated adduct between EGCG and HNE • The formation of a EGCG-HNE covalent adduct was established for HPLC-ESI-MS • EGCG can quench different types of 	<p>These results suggest that EGCG and green tea extract, besides the proposed mechanisms of chemoprevention that target multiple cell-signaling pathways that control cell proliferation and apoptosis in cancer cells, can also prevent protein carbonylation in the tumor tissue environment, depending on the pH of the medium</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		<p>α,β-unsaturated aldehydes</p> <ul style="list-style-type: none"> • EGCG is the most active of the green tea catechins in HNE entrapping; only adducts between HNE and EGCG were detected in the green tea chromatogram, indicating that EGCG plays a primary role in HNE sequestering • EGCG's ability to quench HNE through formation of a compound with a highly stable C-C bond, a mechanism different from HNE quenching by histidine-containing dipeptides, which involves the C-N bond • In an aqueous environment, EGCG is present as a tautomeric pair. This equilibrium involves the carbanionic form of dissociated EGCG, with the negative charge equally distributed between positions C-6 and C-8 	<p>surrounding the tissue, the type of tumor, the stage of dysregulation of lipid peroxidation, and finally, the stage of carcinoma development.</p>
<p>Ehrhoefer, D.E., et al. 2008. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers.</p>	<p><i>In vitro</i> study in molecular chaperone His-tagged αS protein demonstrating the modulation of αS and Aβ fibril formation pathway by EGCG using biochemical, biophysical, and cell biological methods. <u>Studies:</u></p> <ul style="list-style-type: none"> • Analyzed the effect of EGCG on αS 	<ul style="list-style-type: none"> • EGCG stimulates αS oligomer assembly • EGCG binds directly to natively unfolded αS • EGCG directly binds to αS polypeptide main chains • EGCG prevents αS β-sheet formation 	<ul style="list-style-type: none"> • EGCG efficiently inhibits fibrillogenesis of both α-synuclein and amyloid-β by directly binding to the natively

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p><i>Nat. Struct. Mol. Biol.</i> 15(6): 558-566.</p>	<p>aggregation using a Thioflavin T (ThT) binding assay</p> <ul style="list-style-type: none"> • Investigated the effect of EGCG on αS fibrillogenesis by negative-stain EM • Analyzed the effect of EGCG on αS aggregation by size-exclusion chromatography • Determined whether EGCG directly associates with unfolded αS using a nitroblue tetrazolium (NBT) staining assay • Investigated whether EGCG also binds directly to both SDS-stable αS oligomers, examining time-resolved aggregation reactions using the NBT assays • Since αS and BSA proteins are both recognized by EGCG, determined individual binding using NMR techniques • Recorded two-dimensional ^1H and ^{15}N spectra of αS with different molar ratios of EGCG • Performed CD spectroscopy experiments with EGCG-treated and untreated αS protein • Carried out <i>in vitro</i> seeding experiments 	<ul style="list-style-type: none"> • EGCG stimulates assembly of non-toxic, off-pathway oligomers • EGCG prevents amyloid-β fibrillogenesis 	<p>unfolded polypeptides and preventing their conversion into toxic, on-pathway aggregating intermediates</p> <ul style="list-style-type: none"> • This suggests a generic effect on aggregation pathways in neurodegenerative diseases

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	<p>with performed αS fibrils and EGCG-stabilized oligomers</p> <ul style="list-style-type: none"> • Tested whether amyloidogenic αS oligomers can be specifically detected with the conformation-specific antibody using time-resolved dot blot assays • Investigated EGCG-generated oligomers for mammalian cell toxicity using standardized 3-2,5-diphenyltetrazolium bromide reduction assays with PC12 cells • Incubated different [EGCG] with unpolymerized $A\beta_{42}$ polypeptide and monitored the formation of amyloid using the ThT assay 		
<p>Bandeled, O.J., Osherooff, N. 2008. (-)-Epigallocatechin gallate, a major constituent of green tea, poisons human type II topoisomerases. <i>Chem. Res. Toxicol.</i> 21(4): 936-943.</p>	<p><i>In vitro</i> study in recombinant wt- human topoisomerase IIα and β expressed in <i>Saccharomyces cerevisiae</i> and purified to determine the effects of green tea extract and EGCG on DNA cleavage and ligation mediated by human topoisomerase IIα and β.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Ligation of cleaved plasmid DNA by human Topoisomerase II • DNA cleavage site utilization 	<ul style="list-style-type: none"> • Enhancement of Topoisomerase II-mediated DNA cleavage by green tea extract • Enhancement of Topoisomerase II-mediated DNA cleavage by EGCG • EGCG is a redox-dependent Topoisomerase II poison 	<p>Results provide strong evidence that EGCG is a redox-dependent Topoisomerase II poison and utilizes a mechanism similar to that of 1,4-benzoquinone</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>Siu, A.W., et al. 2008. Glutamate-induced retinal lipid and protein damage: the protective effects of catechin. <i>Neurosci. Lett.</i> 432(3): 193-197.</p>	<p><i>In vitro</i> study investigating the effects of catechin on the glutamate-treated retina. Porcine retinal homogenates were incubated with glutamate (20 nmol) at 37° for 60 min. Catechin was co-incubated with the glutamate-treated retina in the same condition.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> ● MDA and protein levels were determined using the LPO586 and Bradford assays ● Various amounts of catechin and the effect of trolox were compared ● Retinal homogenates were incubated with glutamate or TRIS ● After glutamate treatment, protein spots with significant changes were marked and excised from the two-dimensional gels 	<ul style="list-style-type: none"> ● Mean MDA increased significantly after the glutamate incubation in a dose-dependent manner ● A significant difference exists between catechin and trolox ● Bonferroni's multiple comparisons showed that all catechin concentrations reduced MDA significantly ● Glutamate modifies the lipid and expressions of seven proteins in a homogenized receptor-free retinal system ● Catechin protects the retinal tissue from glutamate-induced LPO and protein damage ● Catechin significantly reverses the glutamate-mediated LPO and changes of pyruvate dehydrogenasem, 5HT receptor, thioredoxin peroxidase, and peroxiredoxin 6 expressions 	<p>Study shows that (a) retinal glutamate toxicity is mediated by LPO and protein modification, and (b) catechin ameliorates the toxicity</p>
<p>Kawai, Y., et al. 2008. Galloylated catechins as potent inhibitors of hypochlorous acid-induced DNA</p>	<p><i>In vitro</i> study screening HCL-60 cells with various phenolic compounds for their scavenging activity for chlorinating intermediates including HOCl and chloramines to determine if tea catechins are good inhibitors of DNA base</p>	<ul style="list-style-type: none"> ● Natural phenolic antioxidants for scavenging and/or reducing chlorinating intermediates <i>in vitro</i> - flavonoids including tea catechins and quercetin exhibit a strong inhibitory effect, especially ECg and EGCg 	<p>Results showed that green tea catechins, especially 3-galloylated catechins, may be the plausible candidates for the</p>

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<p>damage. <i>Chem. Res. Toxicol.</i> 21(7):1407-1414.</p>	<p>chlorination. <u>Studies:</u></p> <ul style="list-style-type: none"> ● Performed inhibition assay for the formation of chlorinated dC residues ● Performed analysis of chlorinated catechin derivatives ● Performed analysis of ECg and chlorinated ECg in HL-60 cells ● Performed HPLC-MS/MS analysis ● Immunocytometric analysis of HOCl-modified DNA 	<ul style="list-style-type: none"> ● Four catechins inhibited the formation of 5-Cl₂dC in DNA in a dose-dependent manner. The inhibitory effect of ECg and EGCg was much stronger than that of the non-galloylated catechins ● ECg and EGCg more efficiently inhibit the formation of the Cl₂dC residues in DNA ● Catechins dose-dependently reduced N-Cl₂dC with the appearance of unmodified dC ● ECg inhibits HOCl-derived cellular DNA damage ● Pretreatment with different concentrations of ECg for 2 hours dependently inhibit the DNA chlorination in HL-60 cells 	<p>prevention of inflammation-derived DNA damage and perhaps carcinogenesis</p>
<p>Sukthankar, M., et al. 2008. A green tea component suppresses posttranslational expression of basic fibroblast growth factor in colorectal cancer.</p>	<p><i>In vitro</i> study examining posttranslational regulation of bFGF by EGCG in human colorectal cancer cells. bFGF in intestinal tumor formation of APC^{Min/+} mice with and without catechin treatment was also examined. <u>Studies:</u></p> <ul style="list-style-type: none"> ● RT-PCR and Western blot analysis on cell cultures 	<ul style="list-style-type: none"> ● Both EGCG and ECG completely suppressed bFGF protein expression ● EGCG suppresses bFGF expression in LoVo cells in a time-dependent manner ● All cells showed a response to EGCG, except MCF-7 breast cancer cells, suggesting that bFGF expression occurred in most of the cancer cells 	<p>Catechin compounds have fewer adverse effects than chemotherapeutic agents and hence can be used in cancer therapeutics to suppress growth and metastasis by</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p><i>Gastroenterology</i>. 134(7): 1972-1980.</p>	<ul style="list-style-type: none"> • vFGF expression vector cloned, transfected, performed histidine pull-down experiment • Performed enzyme-linked immunosorbent assay for bFGF • Performed ubiquitin and 20S proteasome assay • Performed animal study (mice receive EGCG or ECG in drinking water for 2 months) • Immunostain for factor VIII 	<p>and that EGCG may reduce bFGF expression</p> <ul style="list-style-type: none"> • Proteasome inhibition restores the suppression of bFGF by EGCG • Only lactacystin containing trypsin-like inhibitory property affects bFGF suppression by EGCG • Exogenous bFGF proteins respond to cycloheximide • EGCG may suppress intestinal tumorigenesis <i>in vivo</i> by reducing bFGF expression and angiogenesis 	<p>targeting proteins such as bFGF</p>
<p>Spina, M., et al. 2008. Mechanism of inhibition of wt-dihydrofolate reductase from <i>E. coli</i> by tea epigallocatechin-gallate. <i>Proteins</i>. 72(1):240-251.</p>	<p><i>In vitro</i> study using wild-type DHFR from AG-1 strain of <i>E. coli</i>. This article reports a detailed kinetic and equilibrium study on the inhibition of DHFR by green tea EGCG, taking into account inhibitory effects.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • DHFR assays and kinetic data analysis • Inhibition of dihydrofolate reductase by tea catechin • Biocensor studies • Molecular modeling of the DHFR/EGCG complex 	<ul style="list-style-type: none"> • EGCG strongly inhibits the activity of wt-DHFR from <i>E. coli</i> • EGCG acts as a bisubstrate inhibitor: in fact, according to one of the tested mechanisms, it is possible for inhibitor (I) to bind to free enzyme, E-DHF complex (NADPH site) and/or E-NADPH complex (DHF site) • Biphasic association and dissociation time courses of EGCG binding to immobilized DHFR are reported: this behavior is ascribed to the possible ability of EGCG to bind both to folate and NADPH sites • EGCG association rate to DHFR at 	<p>Data confirmed the selectivity of antifolate compounds with respect to the different source of enzyme (bacterial or mammalian DHFR) and the possible role of tea catechins as chemopreventative agents.</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
		DHF site was about 30-fold higher in comparison with the association at NADPH site	
Coyle, C.H., et al. 2008. Antioxidant effects of green tea and its polyphenols on bladder cells. <i>Life Sci.</i> 83 (1-2):12-8.	<p><i>In vitro</i> study utilizing normal/malignant (high/low grade) human bladder uroepithelial cells to investigate the antioxidant properties of green tea extract (GTE), polyphenon-60 (PP-60; 60% pure catechins), and two GTE components (EGC and EGCG) following oxidative stress with H₂O₂.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> ● Assessment of cell viability using the Sigma <i>In Vitro</i> toxicology assay kit ● Measure apoptosis using the Annexin V detection kit ● Measure intracellular reactive oxygen species (ROS) 	<ul style="list-style-type: none"> ● PP-60, ECG and EGCG catechins protect human bladder cells from H₂O₂-induced oxidative stress ● PP-60, EGC, and EGCG significantly improved the viability of UROtsa cells compared to H₂O₂-treated cells ● ECG and EGCG catechins protect human bladder cells from H₂O₂-induced apoptosis ● Catechins modulate apoptosis in normal human bladder cells via alteration of reactive oxygen species (ROS) signaling ● H₂O₂ stimulates ROS production in normal human bladder cells 	These findings demonstrate that urothelium cell death via H ₂ O ₂ -induced oxidative stress is mediated, in part, through superoxide (O ₂ ⁻), and potentially, direct H ₂ O ₂ mechanisms, suggesting that green tea polyphenols can protect against oxidative stress/damage and bladder cell death
Nishikawa, H., Kitani S. 2008. Tea catechins have dual effect on mast cell degranulation induced by compound 48/80. <i>Int.</i>	<p><i>In vitro</i> study to investigate catechin's effect on intracellular ROS generation and degranulation in mast cell activation.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> ● Measure the intracellular free Ca²⁺ concentration ● Measure degranulation by monitoring release of β-hexosaminidase from cells 	<ul style="list-style-type: none"> ● The compound 48/80 activates CM-MC mast cell ● C48/80 increased intracellular DCF oxidation dose and time dependently ● The intracellular ROS generation by NADPH oxidase is related with C48/80-induced degranulation from CM-MC. 	There is an optimal level of ROS for degranulation, and catechins have a dual function by controlling the ROS levels.

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<i>Immunopharmacol.</i> 8(9):1207-1215.	<p>into the supernatant</p> <ul style="list-style-type: none"> Measure intracellular ROS generation using a fluorometric assay 	<ul style="list-style-type: none"> Catalase above 10 units/ml removed inhibitory effects by low ROS or the degranulation by high exogenous ROS and that there is a presence of optimal amount of ROS for mast cell activation 	
<p>Lee, S.I., et al. 2008. Effect of green tea and (-)-epigallocatechin gallate on ethanol-induced toxicity in HepG2 cells. <i>Phytother. Res.</i> 22(5):669-674.</p>	<p><i>In vitro</i> study in HepG2 cells to compare EGCG, L-theanine, and caffeine for their protective effects against ethanol-induced cytotoxicity, using GGT as a marker.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> GGT activity was assayed calorimetrically Cell viability was assayed using MMT Determined intracellular GSH content 	<ul style="list-style-type: none"> EGCG was found to inhibit the ethanol-induced cell death more efficiently than did green tea extract itself GTE and EGCG inhibited the GGT increase dose-dependently while L-theanine had no significant effect and caffeine enhanced GGT release even more EGCG, 4-MP, GSH, NAC, SAM, and acivicin failed to preserve intracellular GSH upon exposure to ethanol EGCG and 4-MP lowered the GSH significantly, even in the absence of ethanol treatments 	<ul style="list-style-type: none"> The cyto-protective effects of green tea could be attributed to the inhibition of GGT activity by EGCG. Study suggests that GGT inhibitors including EGCG may provide a novel strategy for attenuating ethanol-induced liver damage.
<p>Oh, C.J., et al. 2008. Epigallocatechin gallate, a constituent of green tea, regulates high glucose-induced</p>	<p><i>In vitro</i> study with U9 37 cells to investigate the effect of EGCG on high glucose-induced apoptosis. Cells were in two separate groups; pre-treated with 1 μM EGCG for 2 hours or not. They were then exposed to 35 mM glucose for 2 days</p>	<ul style="list-style-type: none"> ROS plays a pivotal role in apoptosis and reductants can block or delay this process Cells pre-treated with 1 μM EGCG for 2 hours were significantly more resistant than un-treated cells to high 	<p>EGCG pre-treated cells showed significant suppression of apoptotic features such as DNA</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>apoptosis. <i>Arch. Pharm. Res.</i> 31(1):31-40.</p>	<p><u>Studies:</u></p> <ul style="list-style-type: none"> • Immunoblot analysis • DAPI staining • DNA fragmentation using the diphenylamine assay • PI staining to determine the portion of apoptotic cells • Determine cellular reduction status using hydrogen peroxide • Oxidative DNA damage estimated using a fluorescent binding assay • Mitochondrial damage determined by mitochondrial membrane potential transition (MPT) 	<p>glucose-induced apoptosis</p> <ul style="list-style-type: none"> • When cells were exposed to glucose, apoptotic cells were increased markedly in untreated cells as compared to EGCG-treated cells • The pre-treatment of EGCG resulted in a significantly lower intracellular level of H₂O₂ as compared to control • The decrease in the efficiency of GSH recycling may be responsible for the higher concentration of intracellular peroxides in control cells treated with high glucose • EGCG appears to protect cells from oxidative DNA damage caused by high glucose • High glucose-induced cleavage of procaspase-3 into the active form of caspase-3 and caspase-3 induces degradation of PARP or lamin B. The results indicate that EGCG exhibits a protective effect on the high glucose-induced apoptosis 	<p>fragmentation, damage to mitochondrial function, and modulation of apoptotic marker proteins upon exposure to high glucose. Study indicates that EGCG may play an important role in regulating the apoptosis induced by high glucose presumably through scavenging of reactive oxygen species</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>Erguder, I.B., et al. 2008. Effects of aqueous green tea extract on activities of DNA turn-over enzymes in cancerous and non-cancerous human gastric and colon tissues. <i>Altern. Ther. Health Med.</i> 14(3):30-33.</p>	<p><i>In vitro</i> study to investigate possible effects of green tea extract on the activities of DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in gastric and colon tissues from patients with stomach and colon cancer. <u>Studies:</u></p> <ul style="list-style-type: none"> • Six cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues obtained surgically were treated with aqueous green tea extract at three different concentrations (0.05%, 0.5%, and 1.25%) for 1 hour and then ADA and XO levels were measured 	<ul style="list-style-type: none"> • In both cancerous and non-cancerous tissue, XO activities were found to elevate in correlation with increased extract concentrations in both cancer types. ADA activity was found to decrease in the cancerous part of the stomach tissue and to increase in the non-cancerous part. 	<p>Green tea may support the medical treatment of stomach and colon cancer</p>
<p>Mukai, D., et al. 2008. Potential anthelmintics: polyphenols from the tea plant <i>Camellia sinensis</i> L. are lethally toxic to <i>Caenorhabditis elegans</i>. <i>Nat. Med. (Tokyo)</i>. 62(2):155-159.</p>	<p><i>In vitro</i> study to report the isolation and identification of a new tannin gallate, with known polyphenols, from the tea plant <i>Camellia sinensis</i> L. and their toxicities to <i>C. elegans</i>, with reference to novel anthelmintic relevancies. <u>Studies:</u></p> <ul style="list-style-type: none"> • Identify plant material, extraction, and isolation • Enzymatic hydrolysis of compound 8 • Prepare egg-bearing adults of <i>C. elegans</i> and determine LC₅₀ 	<ul style="list-style-type: none"> • Compound 8 consists of two flavan-3-ol units and one galloyl group • A diagnostic HMBC correlation between H-3' and carbonylic C-7'' was indicative of the presence of a C-3'-O-C-7'' linkage in 8 • Green tea polyphenols are toxic to <i>C. elegans</i> 	<p>These data shows that many green tea polyphenols may be potential anthelmintics</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>Hisamura, F., et al. 2008. Synergistic effect of green tea polyphenols on their protection against FK-506 induced cytotoxicity in renal cells. <i>Am. J. Chin. Med.</i> 36(3): 615-624.</p>	<p><i>In vitro</i> study to evaluate the synergistic interactions of tea polyphenols on FK506-induced cell death and ROS levels in LLC-PK1 renal cells</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> ● Cytotoxicity of FK506 measured by the MTT assay ● Measured ROS formation using hydrogen peroxide ● Measured caspase activity using CPP32/Caspase-3 colorimetric protease assay kit 	<ul style="list-style-type: none"> ● The effect of tea polyphenols on FK 506-induced cytotoxicity in LLC-PK1 cells shows a dose-dependent protective effect for EGCG and EGC ● CO-treatments with EGCG and EGC, EGCG or EGC, and EGC and EGC have strong synergistic effects on the protection of FK 506-induced cell death ● EGCG, EGC, and the combined treatment of EGCG and EGC show significant suppression of the increased levels of intracellular ROS. Time-dependent effects ● EGCG, EGC, and combined treatment of EGCG and EGC suppressed the level of caspase-3 activity in cells treated with FK 506 	<p>Synergistic effects of the constituents of green tea extract suggest that its protective effects may reside in more than just one of its constituents</p>
<p>Devika, P.T., Mainzen Prince, P.S. 2008. (-)-Epigallocatechin gallate EGCG prevents isoprenaline-induced cardiac marker enzymes and</p>	<p><i>In vivo</i> study using male albino Wistar rats weighing 140-160 g. Different doses of EGCG (5, 10, 20, and 30 mg/kg) were dissolved in DMSO and administered by gavage at 7, 14, 21, and 28 days before isoprenaline administration to determine dose-dependence and duration of treatment against ISO-induced myocardial infarction.</p>	<ul style="list-style-type: none"> ● Pretreatment with EGCG significantly reduced the increase in heart weight ● Pretreatment with EGCG significantly decreased the activities of enzymes in serum CK, CK-MB, LDH, AST, and ALT ● Pretreatment of EGCG significantly increased activities of enzymes (CK, LDH, AST, and ALT) in the heart 	<p>EGCG exhibits beneficial effects on enzymes and electrolytes in the heart due to antioxidant and membrane-stabilizing effects</p>

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membrane-bound ATPases. <i>J. Pharm. Pharmacol.</i> 60(1):125-133.	<u>Studies:</u> <ul style="list-style-type: none"> • Assay of marker enzymes • Assay of Na⁺/K⁺ATPase • Assay of Ca²⁺ ATPase • Assay of Mg²⁺ ATPase • Estimation of Na⁺, K⁺, Ca²⁺ and protein concentration • Separation and quantification of LDH isoenzymes 	<ul style="list-style-type: none"> • EGCG pretreatment decreased the intensity of LDH 1 and LDH 2 • Pretreatment with EGCG significantly increased the activity of Ca²⁺ and Mg²⁺-ATPases • Pretreatment with EGCG significantly decreased the levels of sodium and calcium and increased the levels of potassium in the heart 	
Renno, W.M., et al. 2008. Effect of green tea on kidney tubules of diabetic rats. <i>Br. J. Nutr.</i> 6:1-8.	<p><i>In vivo</i> study examining the long-term effect of green tea extract on streptozotocin-induced diabetic nephropathy and on the glycogen accumulation in the kidney tubules. Male Sprague-Dawley rats were randomly assigned to normal control groups (2, 6, 8, and 12 weeks) and five diabetic groups (N=10) of comparable age. A GT diabetic group received 16% concentration of GT for 12 weeks post-diabetes induction as their sole source of drinking water.</p> <u>Studies:</u> <ul style="list-style-type: none"> • Blood glucose levels determined • Blood samples taken by cardiac puncture and used for determination of blood urea nitrogen • Serum glycosylated protein level measured using method of McFarland et 	<ul style="list-style-type: none"> • Body weights of diabetic rats treated with GT were significantly lower than the normal control group • Diabetic rats treated with GT showed significant reduction in both serum glucose and glycosylated protein • Blood urea nitrogen level was significantly lower • GT-treated group showed significant higher Ccr • Urinary urea nitrogen excretion was significantly lower • Creatine, glucose. and protein excretion declined • The number of glycogen-filled tubules decreased almost completely in the GT-treated kidneys 	Results indicate that in STZ diabetes, kidney function appears to be improved with GT consumption which also prevents glycogen accumulation in the renal tubules, probably by lowering blood levels of glucose. Therefore, GT could be beneficial therapy in the management of diabetic nephropathy

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	<p>al.</p> <ul style="list-style-type: none"> • Performed all experimental protocols and group treatments • Performed specimen collection and histological staining • Quantification of glycogen-filled proximal tubules 		
<p>Call, J.A., et al. 2008. Endurance capacity in maturing mdx mice is markedly enhanced by combined voluntary wheel running and green tea extract. <i>J. Appl. Physiol.</i> [epub ahead of print].</p>	<p><i>In vivo</i> study using male mdx mice testing endurance exercise and GTE (0.5% added into diet) separately and in combination to increase endurance and antioxidant activities. Three hypothesis were tested: 3 weeks of endurance exercise initiated immediately post-weaning age (21 days) will improve skeletal function and serum antioxidant capacity, and decrease muscle lipid peroxidation; GTE supplementation without running will demonstrate similar effects; GTE supplementation in combination with endurance exercise will be more beneficial than either alone.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Conducted voluntary wheel running experiment • Tested excised muscles for isometric contractile and mechanical properties • Determine contractile protein contents 	<ul style="list-style-type: none"> • GTE mice ran a total distance of 128% greater than controls • Absolute myosin content, total muscle protein relative to muscle mass, and total contractile protein relative to total muscle protein were all increased in GTE mice • 22% greater antioxidant capacity • Demonstrated 64% less lipid peroxidation in the gastrocnemius, and 29% less lipid peroxidation in the heart • Had 95% greater CS activity in the quadriceps muscles • 35% great CS activity in the heart muscle • β-oxidation activity was 36% greater in the quadriceps and 35% greater in the heart muscle 	<p>Data suggest that both endurance exercise and GTE may be beneficial therapeutic strategies to improve muscle function</p>

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	<p>and myosin heavy chain (MyHC) isoform distribution</p> <ul style="list-style-type: none"> • Determine creatine kinase activity and antioxidant capacity • Determine lipid peroxidation • Determine citrate synthase activity • Determine beta-hydroxyl acyl-CoA dehydrogenase (BHAD) activity 		
<p>Leong, H., et al. 2008. Inhibition of mammary tumorigenesis in the C3(1)/SV40 mouse model by green tea. <i>Breast Cancer Res. Treat.</i> 107 (3): 359-369.</p>	<p><i>In vivo</i> study using C3(1)/SV40 mice in 3 groups receiving either 0.1%, 0.3%, or 0.5% Poly E in tap water every Monday, Wednesday, and Friday beginning at 6 weeks of age as sole source of drinking water (equivalent to daily consumption of six cups of green tea by the average adult human). Animals from each group were killed at 8, 12, 15, and 20 weeks of age.</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Measured chemoprevention with administration of green tea polyphenols • Analyzed mammary glands • Performed an enzyme-linked immunosorbant assay 	<ul style="list-style-type: none"> • Green tea alters the natural history and histopathology of mammary tumor function • Green tea inhibits mammary ductal growth • Green tea has anti-angiogenic effects 	<p>Data strongly supports potential use of green tea as a breast cancer chemopreventative agent</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
<p>Hsu, C.H., et al. 2008. Effect of green tea extract on obese women: a randomized double-blind, placebo-controlled clinical trial. <i>Clin. Nutr.</i> 27(3): 363-370.</p>	<p>A randomized, double-blind, placebo-controlled, clinical trial from July 2006 to June 2007 in Taipei Hospital, Taiwan. Seventy-eight of 100 obese women aged between 16 and 60 years with BMI > 27 kg/m² and who had not received any other weight control agent within the previous 3 months of the study. Subjects were randomly divided into Groups A and B.</p> <ul style="list-style-type: none"> • Group A (n=41) received GTE • Group B (n= 37) took cellulose as a placebo • Took one capsule (400 mg) three times a day for 12 weeks • BW, BMI, and WC were measured at the beginning of the study and after 12 weeks of treatment • A hormone peptides analysis was also conducted 	<ul style="list-style-type: none"> • There was no statistical difference in percent reduction in BW, BMI, and WC between the GTE and placebo groups after 12 weeks of treatment • After treatment, the GTE group revealed significant reduction in WC, and levels of LDL-cholesterol and triglyceride, and marked increase in the levels of HDL-cholesterol, adiponectin and ghrelin • The placebo group showed significant reduction in HC and triglyceride only, and marked increase in the level of ghrelin alone 	<p>This study shows no statistical difference in percent reduction in body weight, BMI, or waist circumference between the GTE and placebo groups after 12 weeks of treatment.</p>
<p>Gawande, S., et al. 2008. Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)-epigallocatechin-3-galate from green</p>	<p><i>In vivo</i> study to determine bioavailability of EGCG. Five human volunteers, were each given a single oral dose of GTE (A), nutrient mixture (NM) containing GTE (B), and formulation B along with black grapes 250 mg C0. Blood samples were drawn at 0, 2, 4, 6, and 8 hours.</p>	<ul style="list-style-type: none"> • EGCG mean plasma levels at hour 2 show significant differences between Group A and Groups B & C. C_{max} for EGCG was reached in hour 4 of each treatment. At hour 6, EGCG levels were only significant for Group C • Mean values for AUC at 8 hours show significantly higher values for C 	<p>Supplementation of nutrient mixture normally prescribed to cancer patients containing ascorbic acid, selenium, N-acetyl cysteine and other nutrients</p>

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tea extract: a human study. <i>Phytother. Res.</i> 22 (6):802-808.	<p><u>Studies:</u></p> <ul style="list-style-type: none"> • Samples collected for initial processing • Extraction and separation of EGCG from plasma • Extraction and analysis of quercetin equivalents in plasma 	<p>compared to groups A and B</p> <ul style="list-style-type: none"> • The percent increase in the availability of EGCG for B and C groups was 14% and 27% respectively • For plasma levels of quercetin equivalents, the AUC of group C was significantly higher than the AUC values of groups B and A. The percent increase in the availability of quercetin for groups B and C was 9% and 12% respectively. 	<p>(formulation B) resulted in an increase of the systemic availability of EGCG by 14%, with C giving an additional 13%.</p>
Mnich, C.D., et al. 2008. Green tea extract reduces induction of p53 and apoptosis in UVB-irradiated human skin independent of posttranscriptional controls. <i>Exp. Dermatol.</i> [epub ahead of print].	<p><i>In vivo</i> study to investigate the photochemopreventative effect of low-dose GTE under everyday conditions, addressing questions like GTP stability and tachyphylaxis. The study irradiated up to 100 ml/cm² of UVB light skin patches that were pretreated with either OM24 – containing lotion or a placebo lotion (application was applied 3 x daily for 24 days; irradiated on days 5 and 33 from 10 mJ/cm² to 100 mJ/cm²). Biopsies taken from both irradiated and un-irradiated skin for both immunohistochemistry and DNA microarray analysis</p>	<ul style="list-style-type: none"> • The number of UV-induced p53-positive keratinocytes was significantly reduced in OM24 compared to placebo-pretreated samples by 31.9% on day 6, and 36.3% on day 34 • The level of UV-induced apoptosis was also reduced by OM24 pretreatment on days 6 and 34 • 213 were identified genes that undergo at least a 2-fold expression change upon UV-exposure after either OM24 or placebo-pretreatment • Both analytical approaches show that OM24 pretreatment does not result in any gene expression changes that are 	<p>Study suggests GTE as a suitable everyday photochemopreventative agent.</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	<u>Studies:</u> <ul style="list-style-type: none"> • Erythema quantification • Immunohistochemistry and quantification of biopsies • Histochemical quantification • Gene expression analysis 	significantly different from placebo-pretreatment	
<p>Lee, H.J., et al. 2008. Modification of gamma-radiation response in mice by green tea polyphenols. <i>Phytother Res.</i> [epub ahead of print].</p>	<p><i>In vivo</i> study using 7-week old female ICR mice for the jejunal crypt survival and apoptosis assays; 7-week male ICR mice for the endogenous spleen colony formation experiment. Animals were irradiated with 12 Gy for the jejunal crypt survival assay, 6.5 Gy for endogenous spleen colony, and 2 Gy for experiment on apoptosis</p> <p><u>Studies:</u></p> <ul style="list-style-type: none"> • Jejunal crypt: 66 female mice (6 per group) divided into a) untreated controls b) irradiated control c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Animals were sacrificed 3.5 days after irradiation • Endogenous spleen: 81 male mice (9 per group) divided into a) irradiated control, b) GT/ GTPs/ catechins / DDC administration in combination with radiation. Mice were sacrificed 9 days after irradiation. Splens were then 	<ul style="list-style-type: none"> • Only the pretreatment with GT, DDC, and ECG resulted in a significant increase in the number of surviving crypts • The number of spleen colonies was higher in the mice that received the GT, GTPs, and EC • Pretreatment of GT, DDC, or polyphenols was associated with decreases in the number of cells with nuclei positively stained for apoptosis compared with control group • GT administration prior to irradiation protected the jejunal crypt, increased the formation of endogenous colonies, and reduced the frequency of radiation-induced apoptosis 	<p>Each of the catechins was a much less effective radioprotector, suggesting that total extract or a mixture of GTPs may be more effective than individual catechins</p>

Citation	Study Design	Results	Relevance to Kao GRAS Determination
	<p>removed</p> <ul style="list-style-type: none"> Apoptosis experiment: 44 females (4 per group) divided into a) untreated control, b) irradiated control, c) GT/ GTPs/ catechins/ DDC administration in combination with radiation. Mice were sacrificed 6 hours after irradiation 		

Legend:

5-CldC: Cationic lipid-DNA (non-coding) complexes are activators of the innate immune response that increase survival of rodents with some acute viral infections and cancers

α -KDGH: α -ketoglutarate dehydrogenase is a multienzymatic complex made up of three different types of enzymes, responsible for the conversion of α -ketoglutarate in to succinyl CoA. This is an important step in the citric acid cycle as it can be used to regulate energy production

ALT: Alanine Aminotransferase is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT is measured to determine if the liver is damaged or diseased

AP-1: Activator protein 1; is a transcription factor which is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families. AP-1 upregulates transcription of genes containing the TPA response element

AST: Aspartate Aminotransferase is an enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream

BSA: Bovine serum albumin (BSA) is one of the most commonly used carriers for conjugation in antibody production. It belongs to the class of serum proteins called albumins, which make up about half of the protein in plasma and are the most stable and soluble proteins in plasma

Ccr: Cinnamoyl-coa reductase

CD: Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry

CK: Creatine kinase catalyses the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP)

CK-MB: the CK enzyme consists of two subunits, either brain or muscle type. CK-MB is one of the three isoenzymes. The expression of genes for these subunits are located on different chromosomes: *B* on 14q32 and *M* on 19q13

CREB: cAMP response element-binding: proteins are transcription factors which bind to certain DNA sequences called *cAMP response elements* (CRE) and thereby increase or decrease the transcription of certain genes

CS: Chitin synthesis

DAPI: 4',6-diamidino-2-phenylindole is a fluorescent stain that binds strongly to DNA

DCF: dichlorofluorescein oxidation is used to measure the production of reactive oxygen species (ROS) within cells

DHFR: Dihydrofolate reductase reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor

DMSO: Dimethyl sulfoxide is a colorless liquid that is an important polar aprotic solvent that dissolves both polar and nonpolar compounds

EGF: Epidermal growth factor

EM: Electron microscope

ESI – MS/MS: Electrospray ionization tandem mass spectrometry

GGT: Gamma-glutamyl transferase

GST: Glutathione S-transferase gene

Gy: the SI unit of absorbed radiation dose- One gray is the absorption of one joule of radiation energy by one kilogram of matter

HPLC – DAD – ESI – MS: High-performance liquid chromatography-diode array detector (HPLC-DAD) coupled with electron-spray mass spectrometry (ESI-MS) is used to detect flavonoid profiles

ICDH: Iso-Citrate DeHydrogenase; enzyme which participates in the citric acid cycle by catalyzing the third step

ICR: Imprinting Control Region mouse

LDH: Lactate dehydrogenase is an enzyme that catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺

LPO: Lipid peroxidation refers to the oxidative degradation of lipids; free radicals remove electrons from the lipids in cell membranes, resulting in cell damage

MDA: Malondialdehyde is a reactive species occurs naturally and is a marker for oxidative stress

MDH: Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (using NAD⁺) and vice versa (this is a reversible reaction)

MMT: Methylcyclopentadienyl manganese tricarbonyl

NAC: N-acetylcysteine

OM24: a Swiss phytochemical extract; is a powerful Anti-Oxidant

RT-PCR: Reverse transcriptase Polymerase Chain Reaction

SAM: S-Adenosyl-L-homocysteine is an amino acid derivative used in several metabolic pathways in the organism *E. coli*.

SDH: Succinate-coenzyme Q reductase is an enzyme complex bound to the inner mitochondrial membrane. It is the only enzyme that participates in both the citric acid cycle and the mitochondrial electron transport chain

SDS: Sodium Dodecyl Sulfate

SOD: Enzyme superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide

STAT1: Member of the Signal Transducers and Activators of Transcription family of transcription factors; STAT1 is involved in upregulating genes due to a signal by either type I or type II interferons

TBARS: Thiobarbituric Acid Reactive Substances Assay

TEM: Transmission electron microscope

TRIS: Tris (hydroxymethyl) aminomethane- used as a buffer solution

PARP: Poly (ADP-ribose) polymerase is a protein involved in a number of cellular processes involving mainly DNA repair and programmed cell death

HMBC: heteronuclear multiple bond coherence experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity

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SUBMISSION END

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