ELLAGIC ACID
49 ABSTRACTS

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Ellagic acid Overview
Ellagic acid is a powerful antioxidant, polyphenol found in red raspberries, Arctic bramble, and cloudberries strawberries and walnuts. Human research reveals that the body readily absorbs ellagic acid from red raspberries, leading to inhibition of abnormal division of cells and promoting the normal death of healthy cells. Ellagic acid has been clinically shown to cause apoptosis (cell death) in certain cancer cells and has exhibited anti-carcinogenic effects against a wide range of carcinogens in several tissues. Ellagic acid contributes to significant inhibition of colon, esophageal, liver, lung, tongue, and skin cancers in studies with rats and mice, both in vitro and in vivo.

Research Overview
Research on ellagic acid shows the following effects:

1. Reduces the risk of esophageal cancer by collecting in the epithelial cells in the digestive tract
2. Reduces the development of esophageal cancer
3. Decreases lipid peroxidation
4. Is an effective free radical scavenger
5. Reduces tumor incidence and development
6. Protects from radiation-induced chromosome damage
7. Prevents liver cancer
8. Prevents lung cancer
9. Prevents skin cancer
10. Prevents embryo mutation and carcinogenesis in animal models given toxins
11. Works synergistically with quercetin as an anticarcinogenic

ELLEGIC ACID: 49 RESEARCH ABSTRACTS

HUMAN **

CANCER **

1. J Nutr. 2003 Aug;133(8):2669-74.
Low concentrations of quercetin and **ellagic** acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells.
Mertens-Talcott SU, Talcott ST, Percival SS.
Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611, USA.

Little information is available regarding possible synergistic or antagonistic biochemical interactions among polyphenols contained in fruits and vegetables. Identifying potential interactions among these compounds may help to define the efficiency of polyphenol-containing foods in **cancer** prevention as related to structure-function activity of the compounds. The objective of this study was to investigate interactions between quercetin and **ellagic** acid, two polyphenolics that are present predominantly in small fruits, on cell death and proliferation-related variables in the MOLT-4 human leukemia cell line. Assays were performed to determine cell cycle kinetics, proliferation, apoptotic DNA-fragmentation and caspase-3-activity after 12, 24 and 48 h. **Ellagic** acid significantly potentiated the effects of quercetin (at 5 and 10 micro mol/L each) in the reduction of proliferation and viability and the induction of apoptosis. Significant alterations in cell cycle kinetics were also observed. The synergy was confirmed by an isobolographic analysis of the cell proliferation data. **The interaction of ellagic acid and quercetin demonstrated an enhanced anticarcinogenic potential of polyphenol combinations, which was not based solely on the additive effect of individual compounds, but rather on synergistic biochemical interactions.**

2. Biochem Pharmacol. 2003 Sep 15;66(6):907-915.
Intestinal epithelial cell accumulation of the **cancer** preventive polyphenol **ellagic** acid-extensive binding to protein and DNA.
Whitley AC, Stoner GD, Darby MV, Walle T.
Department of Cellular and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 173 Ashley Avenue, PO Box 250505, 29425, Charleston, SC, USA

Ellagic acid (EA), a polyphenol present in many berries, has been demonstrated to be preventive of esophageal **cancer** in animals both at the initiation and promotion stages. To be able to extrapolate these findings to humans we have studied the transcellular absorption and epithelial cell accumulation of [14C]EA in the human intestinal Caco-2 cells. The apical (mucosal) to basolateral (serosal) transcellular transport of 10microM [14C]EA was minimal with a P(app) of only 0.13x10(-6)cm/s, which is less than for the paracellular transport marker mannitol. In spite of observations of basolateral to apical efflux, Caco-2 cell uptake studies showed high accumulation of EA in the cells (1054+/-136pmol/mg protein), indicating facile absorptive transport across the

apical membrane. Surprisingly, as much as 93% of the cellular EA was irreversibly bound to macromolecules (982+/-151pmol/mg protein). To confirm the irreversible nature of the binding to protein, Caco-2 cells treated with 10microM [14C]EA were subjected to SDS-PAGE analysis. This resulted in radiolabeled protein bands trapped in the stacking gel, consistent with [14C]EA-crosslinked proteins. Treatment of Caco-2 cells with 10microM [14C]EA also revealed irreversible binding of EA to cellular DNA as much as five times higher than for protein (5020+/-773pmol/mg DNA). Whereas the irreversible binding to protein required oxidation of EA by reactive oxygen species, this did not seem to be the case with the DNA binding. The avid irreversible binding to cellular DNA and protein may be the reason for its highly limited transcellular absorption. Thus, EA appears to accumulate selectively in the epithelial cells of the aerodigestive tract, where its **cancer** preventive actions may be displayed.

3. Anticancer Res. 2001 Jan-Feb;21(1A):359-64.
IGF-II down regulation associated cell cycle arrest in colon **cancer** cells exposed to phenolic antioxidant **ellagic** acid.
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Altered cell and tissue differentiation is characteristic of premalignant lesions long before they become invasive and metastatic. One approach to controlling preneoplastic lesions is to block their expansion with non-toxic agents that suppress cell proliferation and induce apoptosis. Here, we show that **ellagic** acid, a natural, dietary phenolic antioxidant when given at 10(-5) M for 48 hours to colon **cancer** cells (SW 480), induced down regulation of insulin like growth factor IGF-II, activated p21(waf1/Cip1), mediated a cumulative effect on G1/S transition phase and caused apoptotic cell death. SW480 colon **cancer** cells expressed significant mRNA levels for the mitogenic insulin like growth factor (IGF-II). **Collectively, these observations suggest that growth inhibition by ellagic acid is mediated by signaling pathways that mediate DNA damage, triggers p53, which in turn activates p21 and at the same time alters the growth factor expression, resulting in the down regulation of IGF-II.**

4. Urol Res. 2001 Dec;29(6):371-6.
Ellagic [correction of **ellagic**a] acid inhibits arylamine N-acetyltransferase activity and DNA adduct formation in human bladder tumor cell lines (T24 and TSGH 8301).
Lin SS, Hung CF, Tyan YS, Yang CC, Hsia TC, Yang MD, Chung JG.
Department of Radiological Technology, Chungtai Institute of Health Sciences and Technology, Taichung, Taiwan, Republic of China.

The fact that vitamin C (ascorbic acid) exhibits a protective effect in certain

types of **cancer** is well documented. Our previous studies demonstrated that human bladder tumor cell line (T24) has N-acetyltransferase (NAT) activity in cytosols and intact cells. The present studies examined the inhibition of arylamine NAT activity and carcinogen (2-aminofluorene)-DNA adduct formation by **ellagic** acid (EA) in human bladder tumor cell lines (T24 and TSGH 8301). Two assay systems were performed, one with cellular cytosols (9,000 g supernatant), the other with intact bladder tumor cell suspensions. NAT activity and 2-aminofluorene-DNA adduct formation in T24 and TSGH 8301 cells was inhibited by EA in a dose-dependent manner in both systems, i.e.. the greater the concentration of EA in the reaction the greater the inhibition of NAT activity (dose- and time-course dependent effects). The data also indicated that EA decreased the apparent Km and Vmax of NAT enzymes from T24 and TSGH 8301 cells in cytosols. NAT activity and 2-aminofluorene-DNA adducts in T24 is higher than in TSGH 8301. **This report is the first to demonstrate that EA affects human bladder tumor cell NAT activity.**

5. Effect of chemopreventive agents on DNA adduction induced by the potent mammary carcinogen dibenzo[a,l]pyrene in the human breast cells MCF-7.

Smith WA, Freeman JW, Gupta RC. Graduate Center for Toxicology, 354 Health Sciences Research Building, University of Kentucky Medical Center, Lexington, KY 40536-0305, USA.

Mutat. Res. 2001 Sep 1; 480-481: 97-108.

Over 1500 structurally diverse chemicals have been identified which have potential cancer chemopreventive properties. The efficacy and mechanisms of this growing list of chemoprotective agents may be studied using short-term bioassays that employ relevant end-points of the carcinogenic process. In this study, we have examined the effects of eight potential chemopreventive agents, N-acetylcysteine (NAC), benzylisocyanate (BIC), chlorophyllin, curcumin, 1,2-dithiole-3-thione (D3T), **ellagic acid**, genistein, and oltipraz, on DNA adduction of the potent mammary carcinogen dibenzo[a,l]pyrene (DBP) using the human breast cell line MCF-7. Bioactivation of DBP by MCF-7 cells resulted in the formation of one predominant (55%) dA-derived and several other dA- or dG-derived DNA adducts. Three test agents, oltipraz, D3T, and chlorophyllin substantially (>65%) inhibited DBP-DNA adduction at the highest dose tested (30 microM). These agents also significantly inhibited DBP adduct levels at a lower dose of 15 microM, while oltipraz was effective even at the lowest dose of 5 microM. **Two other agents, genistein and ellagic acid were moderate (45%) DBP-DNA adduct inhibitors at the highest dose tested, while NAC, curcumin, and BIC were ineffective.** These studies indicate that the MCF-7 cell line is an applicable model to study the efficacy of cancer chemopreventive agents in a human setting. Moreover, this model may also provide information regarding the effect of the test agents on carcinogen bioactivation and detoxification enzymes.

6. p53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells
Narayanan B.A.; Geoffroy O.; Willingham M.C.; Re G.G.; Nixon D.W.
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Cancer Letters (CANCER LETT.) (Ireland) 1999, 136/2 (215-221)

Ellagic acid is a phenolic compound present in fruits and nuts including raspberries, strawberries and walnuts. It is known to inhibit certain carcinogen-induced cancers and may have other chemopreventive properties. The effects of ellagic acid on cell cycle events and apoptosis were studied in cervical carcinoma (CaSki) cells. We found that ellagic acid at a concentration of 10sup -sup 5 M induced G1 arrest within 48 h, inhibited overall cell growth and induced apoptosis in CaSki cells after 72 h of treatment. **Activation of the cdk inhibitory protein p21 by ellagic acid suggests a role for ellagic acid in cell cycle regulation of cancer cells.**

7. Mutat Res 1998 Feb 26;398(1-2):183-7
Inhibitory effects of ellagic acid on the direct-acting mutagenicity of aflatoxin B1 in the Salmonella microsuspension assay.
Loarca-Pina G, Kuzmicky PA, de Mejia EG, Kado NY
Departamento de Investigacion y Posgrado, Facultad de Quimica, Universidad Autonoma de Queretaro, Qro., Mexico.

Ellagic acid (EA) is a phenolic compound that exhibits both antimutagenic and anticarcinogenic activity in a wide range of assays in vitro and in vivo. It occurs naturally in some foods such as strawberries, raspberries, and grapes. In the previous work, we used the Salmonella microsuspension assay to examine the antimutagenicity of EA against the potent mutagen aflatoxin B1 (AFB1) using tester strains TA98 and TA100. Briefly, the microsuspension assay was approximately 10 times more sensitive than the standard Salmonella/microsome (Ames) test in detecting AFB1 mutagenicity, and EA significantly inhibited mutagenicity of all AFB1 doses in both tester strains with the addition of S9. The greatest inhibitory effect of EA on AFB1 mutagenicity occurred when EA and AFB1 were incubated together (with metabolic enzymes). Lower inhibition was apparent when the cells were first incubated with EA followed by a second incubation with AFB1, or when the cells were first incubated with AFB1 followed by a second incubation with EA alone, all with metabolic enzymes. The result of these sequential incubation studies indicates that one mechanism of inhibition could involve the formation of an AFB1-EA chemical complex. In the present study, we further examine the effect of EA on AFB1 mutagenicity, but without the addition of exogenous metabolic enzymes. We report the mutagenicity of AFB1 in the microsuspension assay using TA98 and TA100 without the addition of S9.

Neither the concentrations of AFB1 (0.6, 1.2, and 2.4 microg/tube) nor the concentrations of EA (0.3, 1.5, 3, 10, and 20 microg/tube) were toxic to the bacteria. The results indicate that AFB1 is a direct-acting mutagen, and that EA inhibits AFB1 direct-acting mutagenicity.

8. Structure-function relationships of the dietary anticarcinogen ellagic acid
Barch D.H.; Rundhaugen L.M.; Stoner G.D.; Pillay N.S.; Rosche W.A.
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Carcinogenesis (CARCINOGENESIS) (United Kingdom) 1996, 17/2 (265-269)

Ellagic acid is a complex planar molecule which demonstrates a variety of anticarcinogenic activities. Ellagic acid has been shown to inhibit the CYP1A1-dependent activation of benzo(a)pyrene; to bind to and detoxify the diol-epoxide of benzo(a)pyrene; to bind to DNA and reduce the formation of 0sup 6-methylguanine by methylating carcinogens; and to induce the phase II detoxification enzymes glutathione S-transferase Ya and NAD(P)H:quinone reductase. Chemical analogs of ellagic acid were synthesized to examine the relationship between the hydroxyl and lactone groups of the ellagic acid molecule and its different anticarcinogenic activities. These studies demonstrated that both the 3-hydroxyl and the 4-hydroxyl groups were required for ellagic acid to directly detoxify the diol-epoxide of benzo(a)pyrene, while only the 4-hydroxyl groups were necessary for ellagic acid to inhibit CYP1A1-dependent benzo(a)pyrene hydroxylase activity. Induction of glutathione S-transferase Ya and NAD(P):quinone reductase required the lactone groups of ellagic acid, but the hydroxyl groups were not required for the induction of these phase II enzymes. In addition, the lactone groups, but not the hydroxyl groups, were required for the analogs to reduce the carcinogen-induced formation of 0sup 6-methylguanine. **Thus, different portions of the ellagic acid molecule are responsible for its different putative anticarcinogenic activities.**

The dietary anticancer agent ellagic acid is a potent inhibitor of DNA topoisomerases in vitro
Constantinou A.; Stoner G.D.; Mehta R.; Rao K.; Runyan C.; Moon R.
Surgical Oncology, University of Illinois, College of Medicine, 840 South Wood St.,Chicago, IL 60612 United States
Nutrition and Cancer (United States) 1995, 23/2 (121-130)

9. Ellagic acid and 12 related agents have been tested for their ability to inhibit the activities of human DNA topoisomerase (topo) I and II. Using specific in vitro assays, we found ellagic acid and flavellagic acid to be potent inhibitors of the catalytic activities of the two topoisomerases. The minimum concentration required to inhibit >= 50% of catalytic activity (IC$D5inf 0) of ellagic acid was determined at 0.6 and 0.7 mug/ml for topo I and topo II, respectively. Flavellagic acid's IC50 was determined

at 3.0 and 3.6 mug/ml for topo I and topo II, respectively. Unlike topoisomerase poisons, these two plant phenols did not trap the enzyme-DNA reaction intermediate, known as the cleavable complex. In contrast, ellagic acid prevented other topo I and topo II poisons from stabilizing the cleavable complex, suggesting that the mode of its action is that of an antagonist. Structure-activity studies identified the 3,3'-hydroxyl groups and the lactone groups as the most essential elements for the topoisomerase inhibitory actions of plant phenols. **On the basis of these findings and other properties of ellagic acid, a mechanistic model for the documented anticarcinogenic effects of the agent is proposed.**

10. Antimutagenic effects of polyphenolic compounds
Teel R.W.; Castonguay A.
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Cancer Letters (CANCER LETT.) (Ireland) 1992, 66/2 (107-113)

Smokers expose themselves to potent carcinogens daily. One of them is the nicotine-derived nitrosamine 4-(methylnitrosamino)1-(3-pyridyl)-1-butanone (NNK). Since estimates are that humans consume 1 g of phenolic compounds/day, we investigated the inhibitory effects of five structurally related polyphenolic compounds on the mutagenicity of NNK in Salmonella typhimurium TA1535. NNK at a concent**rat**ion of 80 mM was activated by hamster liver microsomes. **The antimutagenic effilcacies were dose-related between the non-toxic concentrations of 0.1 and 0.5 mmol/dish in the following order: esculetin > ellagic acid > (+)-catechin > propyl gallate > (-)esculin. At the highest non-toxic dose tested (0.5 mmol/dish), these polyphenolics inhibited mutagenesis in TA1535 by 77%, 67%, 62%, 59% and 53%, respectively. The results of this study demonstrated that polyphenolic compounds may inhibit the activation of NNK.**

Protective effects of antioxidants on experimental liver injuries
11. Suzuki M.; Kumazawa N.; Ohta S.; Kamogawa A.; Shinoda M.
Faculty of Pharmaceutical Science, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142 Japan
Yakugaku Zasshi (YAKUGAKU ZASSHI) (Japan) 1990, 110/9 (697-701)

Protective effects of 14 kinds of antioxidant on liver injury induced by carbon tetrachloride (CCl4) were investigated in terms of serum enzyme activities and bilirubin concent**rat**ion. Consequently, the significant protective effects were found in sesamol, **ellagic acid,** cysteamine and cysteine. These antioxidants clearly decreased the lipid peroxide in the liver tissue. The protective effects on CCl4-induced liver injury in vivo were independent of the inhibitory activities on lipid peroxidation in hepatic mitochondria fraction in vitro.

12. ANIMAL **

CANCER **

Cancer Res. 2001 Aug 15;61(16):6112-9.
Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries.
Kresty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, Blackwood M, Stoner GD.
Division of Environmental Health Sciences, School of Public Health, Comprehensive **Cancer** Center, The Ohio State University, Columbus, Ohio 43210, USA.

Fruit and vegetable consumption has consistently been associated with decreased risk of a number of aerodigestive tract **cancer**s, including esophageal **cancer**. We have taken a "food-based" chemopreventive approach to evaluate the inhibitory potential of lyophilized black raspberries (LBRs) against N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumorigenesis in the F344 rat, during initiation and postinitiation phases of carcinogenesis. Anti-initiation studies included a 30-week tumorigenicity bioassay, quantification of DNA adducts, and NMBA metabolism study. Feeding 5 and 10% LBRs, for 2 weeks prior to NMBA treatment (0.25 mg/kg, weekly for 15 weeks) and throughout a 30-week bioassay, significantly reduced tumor multiplicity (39 and 49%, respectively). In a short-term bioassay, 5 and 10% LBRs inhibited formation of the promutagenic adduct O(6)-methylguanine (O(6)-meGua) by 73 and 80%, respectively, after a single dose of NMBA at 0.25 mg/kg. Feeding 5% LBRs also significantly inhibited adduct formation (64%) after NMBA administration at 0.50 mg/kg. The postinitiation inhibitory potential of berries was evaluated in a second bioassay with sacrifices at 15, 25, and 35 weeks. Administration of LBRs began after NMBA treatment (0.25 mg/kg, three times per week for 5 weeks). LBRs inhibited tumor progression as evidenced by significant reductions in the formation of preneoplastic esophageal lesions, decreased tumor incidence and multiplicity, and reduced cellular proliferation. At 25 weeks, both 5 and 10% LBRs significantly reduced tumor incidence (54 and 46%, respectively), tumor multiplicity (62 and 43%, respectively), proliferation rates, and preneoplastic lesion development. Yet, at 35 weeks, only 5% LBRs significantly reduced tumor incidence and multiplicity, proliferation indices and preneoplastic lesion formation. In conclusion, dietary administration of LBRs inhibited events associated with both the initiation and promotion/progression stages of carcinogenesis, which is promising considering the limited number of chemopreventives with this potential.

13, Anti**cancer** Res. 2001 Nov-Dec;21(6A):3903-8.
Strong antioxidant activity of **ellagic** acid in mammalian cells in vitro revealed by the comet assay.
Festa F, Aglitti T, Duranti G, Ricordy R, Perticone P, Cozzi R.
Dipartimento di Biologia, Universita degli Studi Roma TRE, Italy.

Oxidative stress due to oxygen and various radical species is associated with the induction of DNA single- and double-strand breaks and is considered to be a first step in several human degenerative diseases, **cancer** and ageing. Naturally occurring antioxidants are being extensively analysed for their ability to protect DNA against such injury. We studied three naturally occuring compounds, Ascorbic Acid, Melatonin and **Ellagic** acid, for their ability to modulate DNA damage produced by two strong radical oxygen inducers (H2O2 and Bleomycin) in cultured CHO cells. The alkaline Comet assay was used to measure DNA damage and a cytofluorimetric analysis was performed to reveal the intracellular oxidative species. **The data showed a marked reduction of H2O2- and Bleomycin-induced DNA damage exerted by Ellagic Acid**. On the contrary Ascorbic acid and Melatonin appeared to induce a slight increase in DNA damage per se. In combined treatments, they caused a slight reduction of H2O2-induced damage, but they did not efficiently modulate the Bleomycin-induced one. **The Dichlorofluorescein diacetate (DCFH-DA) cytofluorimetric test confirmed the strong scavenging action exerted by Ellagic Acid.**

14. Neurochem Res. 2000 Nov;25(11):1503-8.
Effects of **ellagic** acid by oral administration on N-acetylation and metabolism of 2-aminofluorene in rat brain tissues.
Lin SS, Hung CF, Ho CC, Liu YH, Ho HC, Chung JG.
Department of Radiological Technology, Chungtai Institute of Health Sciences and Technology, Taichung, Taiwan, Republic of China.

Numerous studies have demonstrated that the Acetyl Coenzyme A-dependent arylamine NAT enzyme exist in many tissues of experimental animals including humans, and that NAT has been shown to be exist in mouse brain tissue. Increased NAT activity levels are associated with increased sensitivity to the mutagenic effects of arylamine carcinogens. Attenuation of liver NAT activity is related to breast and bladder **cancer** processes. Therefore, the effects of **ellagic** acid (EA) on the in vitro and in vivo N-acetylation of 2-aminofluorene (AF) were investigated in cerebrum, cerebellum and pineal gland tissues from male Sprague-Dawley rats. For in vitro examination, cytosols with or without EA (0.5-500 microM) co-treatment decreased 7-72%, 15-63% and 10-78% of AF acetylation for cerebrum, cerebellum and pineal gland tissues, respectively. For in vivo examination, EA and AF at the same time treated groups with all 3 examined tissues did show significant differences (the changes of total amounts of AF and AF metabolites based on the Anova analysis) when compared to the ones without EA cotreatment rats. **The pretreatment of male rats with EA (10 mg/kg) 24 hr prior to the administration of AF (50 mg/kg) (one day of EA administration suffice to induce large changes in phase II enzyme activity) resulted in a 76% decrease in total AF and metabolites in pineal gland** but did not show significant differences in cerebrum and cerebellum tissues. **This is the first demonstration to show that EA decreases the N-acetylation of carcinogens in rat brain tissues.**

15. Food Chem Toxicol. 1999 Apr;37(4):313-8.
Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by **ellagic** acid and quercetin in mice.
Khanduja KL, Gandhi RK, Pathania V, Syal N.
Department of Biophysics, Postgraduate Institute of Medical Education & Research, Chandigarh, India.

The polyphenolic antioxidants, consumed as an integral part of vegetables, fruits and beverages, are suggested as possessing anticarcinogenic properties. In the present study we have looked into the anticarcinogenic potential of plant polyphenols **ellagic** acid (EA) and quercetin against N-nitrosodiethylamine-induced lung tumorigenesis in mice. **Ellagic** acid was able to significantly reduce tumour incidence to 20% from the control value of 72.2%. Similarly, tumour burden was also decreased, although not significantly, from 3.15 to 2.5. Quercetin (QR) caused the tumour incidence to decrease from 76.4% to 44.4% when fed until the third dose of carcinogen. Both of the polyphenols suppressed the tumour incidence mainly by acting at the initiation phase of the carcinogenesis, since continuing the feeding of polyphenols until the termination of the experiment did not cause any apparent change in tumour incidence or tumour burden. Besides this, **ellagic** acid was found to be a better chemopreventor than quercetin. In order to search for their mechanism of action, the effect of feeding of these compounds on reduced glutathione (GSH), an important endogenous antioxidant, and on lipid peroxidation was investigated. Both **ellagic** acid and QR caused a significant increase in GSH and decrease in NADPH- and ascorbate-dependent lipid peroxidation. **Ellagic acid was found to be more effective in decreasing the lipid peroxidation and increasing the GSH. This may be one of the reasons for its observed better anticarcinogenic property as compared to quercetin.**

16. Anti-tumor promoting activity of polyphenols from Cowania mexicana and Coleogyne ramosissima
Ito H.; Miyake M.; Nishitani E.; Mori K.; Hatano T.; Okuda T.; Konoshima T.; Takasaki M.; Kozuka M.; Mukainaka T.; Tokuda H.; Nishino H.; Yoshida T.
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Cancer Letters (Ireland) 1999, 143/1 (5-13)

Chemical investigation on polyphenol-rich fractions of Cowania mexicana and Coleogyne ramosissima (Rosaceae) which showed significant inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), has led to the characterization of 10 compounds including C-glucosidic ellagitannin monomers and dimers from the former plant, and 17 polyphenols including flavonoid glycosides from the latter. The effects of individual components and their analogues

with related structures on the TPA-induced EBV-EA activation were then evaluated. Among the compounds isolated from C. mexicana, two C-glucosidic ellagitannins, alienanin B and stenophyllanin A and a nitrile glucoside (lithospermoside), and among the constituents from C. ramosissima, two flavonoid glycosides, isorhamnetin 3-O-beta-D-glucoside and narcissin were revealed to possess strong inhibitory effects on EVB-EA activation, the potencies of which were either comparable to or stronger than that of a green tea polyphenol, (-)-epigallocatechin gallate. These polyphenols except for nitrile glucoside, which was not tested owing to an insufficient amount, were also found to exhibit anti-tumor promoting activity in two-stage mouse skin carcinogenesis using 7,12-dimethylbenz[a]anthracene (DMBA) and TPA.

17. Mutat Res. 1999 Mar 10;425(1):143-52.
Determining efficacy of **cancer** chemopreventive agents using a cell-free system concomitant with DNA adduction.
Smith WA, Gupta RC.
Graduate Center for Toxicology, Room 354, Health Sciences Research Building, University of Kentucky Medical Center, Lexington, KY 40536-0305, USA.

The large (>2000) and expanding number of natural and synthetic agents with potential **cancer** chemopreventive properties renders it economically and physically impossible to test each of these agents for their efficacy in the widely accepted 2-year animal bioassay and clinical trials. Therefore, there is a growing need for relevant short-term screening tests to study these compounds such that only the most efficacious ones undergo extensive long-term studies. We have previously reported in a pilot study that the use of a microsome-mediated test system concomitant with DNA adduction is a pertinent and relevant model for rapidly studying the efficacy and mechanisms of **cancer** chemopreventive agents. We have extended this study to investigate 26 additional agents for their potential chemopreventive abilities by studying their effects on microsome-mediated benzo[a]pyrene (BP)-DNA adduction. These agents had differential effects on the two major adducts of BP-DNA, i.e., BP-7,8-diol-9,10-epoxide (BPDE)-deoxyguanosine (dG) and 9-OH-BP-dG-derived adducts. These agents were therefore categorized into five classes. **Three test agents (ellagic acid, genistein and oltipraz) were strong inhibitors of both adducts.** These agents diminished BP-DNA adduction by 65-95% and were categorized as Class I agents. Six other agents (benzyl isocyanate, R(+)-1-phenylethyl isocyanate, linoleic acid ethyl ester, (+)-biotin, indole-3-carboxylic acid and beta-carotene) moderately inhibited both BP-DNA adducts (25-64%); these compounds were identified as Class II agents. Six additional test agents inhibited only one adduct selectively and nine others were ineffective; these agents were categorized as Class III and Class IV, respectively. Interestingly, seven test agents enhanced BPDE-dG or 9-OH-BP-dG or both adducts and were categorized as Class V agents. Four of these Class V agents concomitantly inhibited BPDE-dG while enhancing 9-OH-BP-dG. This emphasizes the importance of

studying individual DNA adducts in contrast to total DNA binding. In conclusion, Class I and Class II agents may be good candidates for further chemoprevention studies. Copyright 1999 Elsevier Science B.V.

18. Toxicol Sci. 1999 Dec;52(2 Suppl):95-100.
Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal **cancer**.
Stoner GD, Kresty LA, Carlton PS, Siglin JC, Morse MA.
Division of Environmental Health Sciences, The Ohio State University School of Public Health, and The Ohio State University Comprehensive **Cancer** Center, The Ohio State University, Columbus 43210, USA. stoner.21@osu.edu

A group of arylalkyl isothiocyanates were tested for their abilities to inhibit tumorigenicity and DNA methylation induced by the esophageal-specific carcinogen, N-nitrosomethylbenzylamine (NMBA) in the F344 rat esophagus. Phenylpropyl isothiocyanate (PPITC) was more potent than either phenylethyl isothiocyanate (PEITC) or benzyl isothiocyanate (BITC). Phenylbutyl isothiocyanate (PBITC), however, had a lesser inhibitory effect on esophageal tumorigenesis, and phenylhexyl isothiocyanate (PHITC) actually enhanced esophageal tumorigenesis. Thus, the two- and three-carbon isothiocyanates were more effective inhibitors of NMBA-esophageal carcinogenesis than the longer chain isothiocyanates. The effects of the isothiocyanates on tumorigenesis were well correlated as to their effects on DNA adduct formation. The most likely mechanism of inhibition of tumorigenesis by these isothiocyanates is via inhibition of the cytochrome P450 enzymes responsible for the metabolic activation of NMBA in rat esophagus. A freeze-dried strawberry preparation was also evaluated for its ability to inhibit NMBA-esophageal tumorigenesis. It proved to be an effective inhibitor, although not as potent as either PEITC or PPITC. The inhibitory effect of the berries could not be attributed solely to the content of the chemopreventive agent, **ellagic** acid, in the berries.

19. Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity
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Journal of Experimental and Clinical Cancer Research (J. EXP. CLIN. CANCER RES.) (Italy) 1998, 17/4 (431-434)

Induction of micronuclei and chromosomal aberrations produced by whole body exposure of r-radiation (1.5-3.0 Gy) in **mice** was found to be significantly inhibited by oral administration of natural antioxidants, curcumin (400 mu moles), ellagic acid (200 mu moles) and bixin (200 mu moles) per kilogram body weight. These antioxidants induced inhibition of micronucleated polychromatic and normochromatic erythrocytes, was

comparable with alpha-tocopherol (200 mu moles) administration. Curcumin and ellagic acid were also found to significantly reduce the number of bone marrow cells with chromosomal aberrations and chromosomal fragments as effectively as alpha- tocopherol. Moreover, administration of antioxidants inhibited the DNA strand breaks produced in **rat** lymphocytes upon radiation as seen from the DNA unwinding studies. **These results indicated that antioxidant curcumin, ellagic acid and bixin provide protection against chromosome damage produced by radiation.**

20. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity
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Cancer Letters (CANCER LETT.) (Ireland) 1997, 115/2 (129-133)

Food additives such as turmeric (Curcuma longa), and active ingredient curcumin (diferuloyl methane), asafoetida (flavouring agent), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ellagic acid were found to inhibit the mutagenesis induced by aflatoxin Binf 1 (AFBinf 1) (0.5 mug/plate) in Salmonella tester strains TA 98 and TA 100. Turmeric and curcumin, which were the most active, inhibited mutation frequency by more than 80% at concentrations of 2 mug/plate. Other food additives were also significantly effective. Dietary administration of turmeric (0.05%), garlic (0.25%), **curcumin and ellagic acid (0.005% each**) to **rats** significantly reduced the number of gammaglutamyl transpeptidase-positive foci induced by AFBinf 1 which is considered as the precursor of hepatocellular neoplasm. These results indicate the usefulness of antioxidant food additives in ameliorating aflatoxin-induced mutagenicity and carcinogenicity.

21. Inhibition of liver fibrosis by ellagic acid
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Indian Journal of Physiology and Pharmacology (INDIAN J. PHYSIOL. PHARMACOL.) (India) 1996, 40/4 (363-366)

Chronic administration of carbon tetrachloride in liquid paraffin (1:7) ip; 0.15 ml, (20 doses) has been found to produce severe hepatotoxicity, as seen from the elevated levels of serum and liver glutamate-pyruvate transaminase, alkaline phosphatase and lipid peroxides. The chronic administration of carbon tetrachloride was also found to produce liver fibrosis as seen from pathological analysis as well as elevated liver-hydroxy proline. Oral administration of ellagic acid was found to significantly reduce the elevated levels of enzymes, lipid peroxide and liver hydroxy proline in these animals and rectified liver pathology. **These results indicate that ellagic acid administration orally can circumvent the**

carbon tetrachloride toxicity and subsequent fibrosis.

22. Antitumorigenic and antipromoting activities of **ellagic acid**, ellagitannins and oligomeric anthocyanin and procyanidin
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International Journal of Oncology (INT. J. ONCOL.) (Greece) 1997, 10/2 (367-373)

We previously showed that **ellagic acid** (EA) was inhibiting lung tumorigenesis induced by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanone (NNK) in A/J **mice**. In the present study, we observed that the inhibition of lung tumorigenesis was independent of the solvent used to purified EA. Pomegranate peels extract containing punicalagin (alpha and beta anomers) (10 g/kg diet) and oligomeric anthocyanins (6 g/kg diet) did not inhibit lung tumorigenesis. Raspberry extract (2 x 15 mg) containing sanguiin H6 and lambertianin D as well as oligomeric procyanidins (2 x 15 mg) inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity by about 30%. The same treatments inhibit TPA-stimulated hydroperoxide (HPx) production by about 30 and 70%. Raspberry ellagitannins and oligomeric procyanidins respectively inhibit TPA stimulated DNA synthesis by 42 and 26%. Our results suggest that hydrolyzable and condensed tannins from various sources, which can inhibit the ODC, HPx, and DNA responses to TPA, might also inhibit the tumor-promoting activity of this agent. **The results of this study show that EA and ellagitannins have different antitumorigenic and antipromoting activities.**

23. Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer
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Cancer Letters (CANCER LETT.) (Ireland) 1997, 114/1-2 (113-119)

A group of arylalkyl isothiocyanates were tested for their abilities to inhibit tumorigenicity and DNA methylation induced by both the tobacco-specific nitrosamine, NNK, in A/J mouse lung and the esophageal-specific carcinogen, NMBA, in F344 **rat** esophagus. In addition, **ellagic acid** was tested for its ability to inhibit NMBA-induced esophageal tumorigenesis. In the strain A lung tumor model, PEITC effectively inhibited NNK-induced lung tumors at a dose of 5 mumol, but was not

inhibitory at lower doses. PPITC, PBITC, PPeITC, and PHITC were all considerably more potent inhibitors of NNK lung tumorigenesis than PEITC, and PHITC was the most potent inhibitor of all. Thus, in the strain A lung tumor model, there was a trend of increased inhibitory efficacy among arylalkyl isothiocyanates with increased alkyl chain length. In the F344 **rat** esophageal tumor model, PPITC was clearly more potent-than PEITC, BITC and PBITC had little inhibitory effect on esophageal tumorigenesis, and in a separate experiment, PHITC actually enhanced esophageal tumorigenesis. Thus, the structure-activity relationships for inhibition of tumorigenesis by arylalkyl isothiocyanates were considerably different in the two animal models. However, the effects of the isothiocyanates on tumorigenesis were well-correlated to their effects on DNA adduct formation in either model. The most likely mechanism of inhibition of tumorigenesis by these isothiocyanates is via inhibition of the cytochrome p450 enzymes responsible for activation of NNK in mouse lung or NMBA in **rat** esophagus. **Ellagic acid** was an effective inhibitor of esophageal tumorigenesis, although not as potent as PEITC or PPITC. Like the isothiocyanates, ellagic acid inhibits cytochrome p450-mediated activation of NMBA.

24. The effects of dietary **ellagic acid** on **rat** hepatic and esophageal mucosal cytochromes P450 and phase II enzymes
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Carcinogenesis (CARCINOGENESIS) (United Kingdom) 1996, 17/4 (821-828)

Ellagic acid (EA), a naturally occurring plant polyphenol possesses broad chemoprotective properties. Dietary EA has been shown to reduce the incidence of N-2-fluorenylacetamide-induced hepatocarcinogenesis in **rats** and N-nitrosomethylbenzylamine (NMBA)-induced **rat** esophageal tumors. In this study changes in the expression and activities of specific **rat** hepatic and esophageal mucosal cytochromes P450 (P450) and phase II enzymes following dietary EA treatment were investigated. Liver and esophageal mucosal microsomes and cytosol were prepared from three groups of Fisher 344 **rats** which were fed an AIN-76 diet containing no EA or 0.4 or 4.0 g/kg EA for 23 days. In the liver total P450 content decreased by up to 25% and P450 2E1-catalyzed p-nitrophenol hydroxylation decreased by 15%. No changes were observed in P450 1A1, 2B1 or 3A1/2 expression or activities or cytochrome h$D5 activity, P450 reductase activity decreased by up to 28%. Microsomal epoxide hydrolase (mEH) expression decreased by up to 85% after EA treatment, but mEH activities did not change. The hepatic phase II enzymes glutathione transferase (GST), NAD(P)H:quinone reductase (NAD(P)H:QR) and UDP glucuronosyltransferase (UDPGT) activities increased by up to 26, 17 and 75% respectively. Assays for specific forms of GST indicated marked increases in the activities of isozymes 2-2 (190%), 4-4 (150%) and 5-5 (82%). In the **rat** esophageal mucosa only P450 1A1 could be

detected by Western blot analysis and androstendione was the only P450 metabolite of testosterone detectable. However, there were no differences in the expression of P450 1A1, the formation of androstendione or NAD(P)H:QR activities between control and EA-fed **rats** in the esophagus. Although there was no significant decrease in overall GST activity, as measured with 1-chloro-2,4-,dinitrobenzene (CDNB), there was a significant decrease in the activity of the 2-2 isozyme (66% of control). In vitro incubations showed that EA at a concentration of 100 muM inhibited P450 2E1, 1A1 and 2B1 activities by 87, 55 and 18% respectively, but did not affect 3A1/2 activity. Using standard steady-state kinetic analyses, EA was shown to be a potent non-competitive inhibitor of both liver microsomal ethoxyresorufin O-deethylase and p-nitrophenol hydroxylase activities, with apparent K(i) values of ~55 and 14 muM respectively. In conclusion, these results demonstrate that EA causes a decrease in total hepatic P450 with a significant effect on hepatic P450 2E1, increases some hepatic phase II enzyme activities (GST, NAD(P)H:QR and UDPGT) and decreases hepatic mEH expression. It also inhibits the catalytic activity of some P450 isozymes in vitro. Thus the chemoprotective effect of EA against various chemically induced cancers may involve decreases in the rates of metabolism of these carcinogens by phase I enzymes, due to both direct inhibition of catalytic activity and modulation of gene expression, in addition to effects on the expression of phase II enzymes, thereby enhancing the ability of the target tissues to detoxify the reactive intermediates.

25. Antimutagenicity of ellagic acid against aflatoxin Binf 1 in the Salmonella microsuspension assay
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Mutation Research - Environmental Mutagenesis and Related Subjects (Netherlands) 1996, 360/1 (15-21)

Ellagic acid (EA) is a phenolic compound with antimutagenic and anticarcinogenic properties. It occurs naturally in some foods such as strawberries, raspberries, grapes, black currants and walnuts. In the present study, we used the Salmonella microsuspension assay to examine the antimutagenicity of EA against the potent mutagen aflatoxin Binf 1 (AFBinf 1) using tester strains TA98 and TA100, Further, we used a two-stage incubation procedure that incorporates washing the bacterial cells free of the incubation mixture after the first incubation to investigate EA and AFBinf 1 interaction. Three different concentrations of AFBinf 1 (2.5, 5 and 10 ng/tube) were tested against five different concentrations of EA for TA98 and TA100**. EA significantly inhibited mutagenicity of all doses of AFBinf 1 in both tester strains with the addition of S9.** EA alone was not mutagenic at the concentrations tested. The greatest inhibitory effect of

EA on AFBinf 1 mutagenicity occurred when EA and AFBinf 1 were incubated together. Lower inhibition was apparent when the cells were first incubated with EA followed by a second incubation with AFBinf 1, and also when the cells were first incubated with AFBinf 1 followed by a second incubation with EA alone. The results of the sequential incubation studies support the hypothesis that one mechanism of inhibition could involve the formation of a chemical complex between EA and AFBinf 1.

26. Organ specific, protocol dependent modulation of 7,12-dimethylbenz[a]anthracene carcinogenesis in rainbow trout (Oncorhynchus mykiss) by dietary **ellagic acid**
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Carcinogenesis "CARCINOGENESIS", vol. 17, no. 11, p. 2403-2409, Nov 1996

This study investigated pre-initiation and post-initiation effects of dietary **ellagic acid** (EA) on 7,12-dimethylbenz[a]anthracene (DMBA) multi-organ carcinogenesis in rainbow trout (Oncorhynchus mykiss). EA at 100, 250 (study 2), 1000 and 2000 (study 1) p.p.m. suppressed stomach adenopapilloma incidence by 33, 60, 70 and 78% (P less than or equal to 0.001), respectively, as well as tumor multiplicity ($P < 0.01$) and size ($P < 0.001$) when fed continuously following DMBA initiation. However, continuous EA feeding also produced modest (250 p.p.m.) to extensive (1000, 2000 p.p.m.) growth rate suppression in these studies. Retrospective logistic regression modeling of the data allowed separation of growth-related from non-growth-related inhibitory effects. By this approach: (i) tumor development showed a similarly strong dependence (same regression slope) on animal growth rate in all treatment groups; (ii) EA-mediated reduction in mean population growth contributed to suppressed stomach tumor response above 250 p.p.m. EA; and (iii) even at high, toxic doses EA displayed inhibitory mechanisms additional to, and distinct from, growth suppression effect. The effects of post-initiation EA were organ specific. Chronic EA treatment significantly suppressed swim-bladder as well as stomach tumor incidence at doses greater than or equal to 1000 p.p.m., but increased liver tumor incidence at doses greater than or equal to 250 p.p.m. Three protocols examined EA effects on the initiation process. EA fed at 1000 p.p.m. concurrently with 750 p.p.m. dietary DMBA for 7 weeks modestly reduced stomach tumor incidence (from 85 to 78%, $P < 0.05$) and multiplicity (from 6.3 plus or minus 4.3 to 4.9 plus or minus 2.9, $P < 0.01$), but did not alter swim-bladder or liver response. The effect of EA pretreatment prior to DMBA single-dose initiation by gill uptake was also examined. When fed for 1 week prior to initiation, 2000 p.p.m. EA again imposed a small reduction in stomach adenoma incidence (from 88 to 78%; $P < 0.05$) and multiplicity (from 5.5 plus or minus 3.2 to 4.4 plus or minus 3.2; $P < 0.01$). However, when EA was pre-fed for 3 weeks

instead of 1 week, protection in the stomach was lost and response in liver and swim-bladder significantly increased. In sum, these studies demonst**rat**e that EA influence on DMBA tumorigenesis in this multi-organ model is highly protocol dependent and organ specific. Post-initiation dietary EA consistently suppressed stomach tumor development in trout, at EA doses far lower than those required for protection in rodents. At higher doses, however, EA also displayed toxicity and a potential in some protocols to enhance tumor response in other organs.

27. Inhibitory effects of vitamin E and ellagic acid on 8-hydroxydeoxyguanosine formation in liver nuclear DNA of **rats** treated with 2-nitropropane
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Cancer Letters (CANCER LETT.) (Ireland) 1995, 91/1 (139-144)

The effects of five naturally occurring antioxidants, beta-carotene (BC), vitamin C (VC), vitamin E (VE), ellagic acid (EA) and epigallocatechin gallate (EGCG) on 8-hydroxydeoxyguanosine (8-OH-dG) formation by 2-nitropropane (2-NP), a hepatocarcinogen in **rats**, were studied. **Four days oral administration of VE (100 mg/kg BW/day) or EA (100 mg/kg BW/day) significantly inhibited 8-OH-dG formation in the liver nuclear DNA of male F-344 rats injected with 2-NP (100 mg/kg BW, i.p., killed 6 h later).** The same treatment with EGCG (100 mg/kg BW/day) showed slight, but not significant, inhibition. In contrast, 4 days' oral administration of BC (100 mg/kg BW/day) or VC (300 mg/kg BW/day) and 3 weeks' feeding of the two (either at 0.5% in the diet) did not produce any inhibitory effects on 8-OH-dG formation. **Thus, it is expected that VE and EA may have anticarcinogenic effects towards 2-NP.**

28. Taurine and ellagic acid: Two differently-acting natural antioxidants
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Environmental and Molecular Mutagenesis (ENVIRON. MOL. MUTAGEN.) (United States) 1995, 26/3 (248-254)

Naturally occurring antimutagenic compounds are extensively analyzed for their capacity to protect cells from induced damage. We selected two agents, taurine and ellagic acid, treated in the literature as antioxidants, but whose activity is insufficiently known. This paper reports on the ability of these agents to act against damage induced by mitomycin-C and hydrogen peroxide in Chinese hamster ovary cells cultivated in vitro. Cytogenetic and cytofluorimetric analyses were performed. **Ellagic acid proved to have more than one mechanism of action, probably as a**

scavenger of oxygen species produced by Hinf 2Oinf 2 treatment, and as a protector of the DNA double helix from alkylating agent injury. In our experimental conditions, taurine seems able to scavenge oxygen species.

29. J Cell Biochem Suppl 1995;22:169-80
Polyphenols as cancer chemopreventive agents.
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This article summarizes available data on the chemopreventive efficacies of tea polyphenols, curcumin and ellagic acid in various model systems. Emphasis is placed upon the anticarcinogenic activity of these polyphenols and their proposed mechanism(s) of action. Tea is grown in about 30 countries and, next to water, is the most widely consumed beverage in the world. Tea is manufactured as either green, black, or oolong; black tea represents approximately 80% of tea products. Epidemiological studies, though inconclusive, suggest a protective effect of tea consumption on human cancer. Experimental studies of the antimutagenic and anticarcinogenic effects of tea have been conducted principally with green tea polyphenols (GTPs). GTPs exhibit antimutagenic activity in vitro, and they inhibit carcinogen-induced skin, lung, forestomach, esophagus, duodenum and colon tumors in rodents. In addition, GTPs inhibit TPA-induced skin tumor promotion in **mice**. Although several GTPs possess anticarcinogenic activity, the most active is (-)-epigallocatechin-3-gallate (EGCG), the major constituent in the GTP fraction. Several mechanisms appear to be responsible for the tumor-inhibitory properties of GTPs, including enhancement of antioxidant (glutathione peroxidase, catalase and quinone reductase) and phase II (glutathione-S-transferase) enzyme activities; inhibition of chemically induced lipid peroxidation; inhibition of irradiation- and TPA-induced epidermal ornithine decarboxylase (ODC) and cyclooxygenase activities; inhibition of protein kinase C and cellular proliferation; antiinflammatory activity; and enhancement of gap junction intercellular
communication. Curcumin is the yellow coloring agent in the spice tumeric. It exhibits antimutagenic activity in the Ames Salmonella test and has anticarcinogenic activity, inhibiting chemically induced preneoplastic lesions in the breast and colon and neoplastic lesions in the skin, forestomach, duodenum and colon of rodents. In addition, curcumin inhibits TPA-induced skin tumor promotion in **mice**. The mechanisms for the anticarcinogenic effects of curcumin are similar to those of the GTPs. Curcumin enhances glutathione content and glutathione-S-transferase activity in liver; and it inhibits lipid peroxidation and arachidonic acid metabolism in mouse skin, protein kinase C activity in TPA-treated NIH 3T3 cells, chemically induced ODC and tyrosine protein kinase activities in **rat** colon, and 8-hydroxyguanosine formation in mouse fibroblasts. Ellagic acid is a polyphenol found abundantly in various fruits, nuts and vegetables. Ellagic acid is active in antimutagenesis assays, and has been shown to inhibit chemically induced cancer in the lung, liver, skin
and esophagus of rodents, and TPA-induced tumor promotion in mouse skin.

30. Ellagic acid induces transcription of the rat glutathione S-transferase-Ya gene.

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Carcinogenesis 1995 Mar;16(3):665-8

Induction of glutathione S-transferase (GST) enzymes can increase detoxification of carcinogens and reduce carcinogen-induced mutagenesis and tumorigenesis. To determine if the anticarcinogen **ellagic acid** induces cellular enzymes which detoxify carcinogens, we examined the effect of **ellagic acid** on the expression of glutathione S-transferase-Ya. Rats fed ellagic acid demonstrated significant increases in total hepatic GST activity, hepatic GST-Ya activity and hepatic GST-Ya mRNA. To determine if the observed increase in GST-Ya mRNA was due to **ellagic acid** inducing transcription of the GST-Ya gene, transfection studies were performed with plasmid constructs containing various portions of the 5' regulatory region of the **rat** GST-Ya gene. The transfection studies demonstrated that **ellagic acid** increased GST-Ya mRNA by inducing transcription of the GST-Ya gene and demonstrated that this induction is mediated through the antioxidant responsive element of the GST-Ya gene.

31. Ann N Y Acad Sci 1993 May 28;686:177-85
Pulmonary carcinogenesis and its prevention by dietary polyphenolic compounds.
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The aims of this study were to define the cumulative exposure of Canadian smokers to NNK and to characterize the efficacy of ellagic acid to inhibit lung tumorigenesis induced by NNK. The sales-weighted average of NNK deliveries from Canadian cigarettes was 73.2 ng/cigarette. NNK deliveries were highly correlated to declared tar values and were linear with puff volumes between 20 and 50 ml. Ellagic acid inhibited lung tumorigenesis induced by NNK in A/J **mice**. This inhibition was related to the logarithm of the dose of ellagic acid added to the diet. The biodistribution of ellagic acid was studied in **mice** gavaged with ellagic acid. Pulmonary levels of ellagic acid were directly proportional to the dose of ellagic acid between 0.2 and 2.0 mmol/kg b.w.

32. The effect of **ellagic acid** on xenobiotic metabolism by cytochrome P-450IIE1 and nitrosodimethylamine mutagenicity
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Cancer Letters (CANCER LETT.) (Ireland) 1992, 61/2 (129-134)

Ellagic acid (EA) is an inhibitor of the in vitro mutagenicity of N-nitrosodimethylamine (NDMA) in Salmonella typhimurium strain TA100 using

pyrazole-induced **rat** liver 9000 x g supernatant (S-9). In order to understand this activity, the effect of EA on the metabolic hydroxylation of 4-nitrophenol, a substrate, as is NDMA, for cytochrome P-450IIE1 was studied using pyrazole induced **rat** S-9 and microsomal protein. **It is shown that EA has an inhibitory effect on 4-nitrophenol hydroxylase with both enzyme preparations. This effect on cytochrome P-450IIE1 may be responsible, at least in part, for the inhibition of NDMA mutagenicity by EA.**

33. Ellagic acid protects **rat** embryos in culture from the embryotoxic effects of N-methyl-N-nitrosourea
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Te**rat**ology (TE**RAT**OLOGY) (United States) 1992, 46/2 (109-115)

Ellagic acid is a naturally occurring plant phenol that has demonstrated anticarcinogenic and antimutagenic activity in several test systems. Given the common proposed etiopathogenic processes of mutagenesis, carcinogenesis, and teratogenesis induced by genotoxic chemicals, the present study was initiated to determine whether ellagic acid would protect **rat** embryos in culture from the teratogenic effects of N-methyl-N-nitrosourea (MNU). Ellagic acid alone (as used in these experiments; 50 muM in DMSO) was not embryotoxic. **Ellagic acid (50 muM) significantly (P < 0.01) prevented MNU (75 muM)-induced effects including mortality (absence of heart beat), abnormal formation of the cephalic neural tube derivatives, and delayed differentiation as assessed by a morphological scoring system. These embryoprotective effects were dose responsive.** Sequential treatment of embryos with ellagic acid followed by MNU in fresh media also was embryoprotective with no diminution of effect. **The site at which ellagic acid interrupts the critical teratogenic events induced by MNU is apparently within the embryo and/or placenta.** This model of chemical embryoprotection may be useful in determining the role of cell death and/or mutation in the teratogenic mechanism of action of methylating agents.

34. Ellagic acid, an anticarcinogen in fruits, especially in strawberries: a review.
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HortScience vol. 26 (1): p.10-14
Publication Year: 1991

The various roles of **ellagic acid** as an anticarcinogenic plant phenol, including its inhibitory effects on chemically induced cancer, its effect on the human body, occurrence in plants and biosynthesis, allelopathic properties, activity in regulation of plant growth regulators, formation

of metal complexes, function as an antioxidant, insect growth and feeding inhibitor, and the inheritance of **ellagic acid** synthetic mechanisms are reviewed and discussed. 87 ref.

Modification of the mutagenicity of aflatoxin Binf 1 and N-methyl-N'-nitro-N-nitrosoguanidine by certain phenolic compounds
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Cancer Letters (CANCER LETT.) (Ireland) 1989, 45/3 (177-182)

Five natural and two synthetic phenolic compounds were tested for their ability to suppress mutagenicity of aflatoxin Binf 1 (AFBinf 1) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Salmonella typhimurium tester strain TA100. Caffeic acid and eugenol were observed to inhibit mutagenicity of both the carcinogens, while chlorogenic acid was effective in the case of AFBinf 1 alone and **ellagic acid** and butylated hydroxytoluene were found to be antimutagenic only for MNNG. These differential activities of the phenolic compounds appeared to be due to their different modes of action towards direct and indirect acting carcinogens.

35. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by hamster respiratory tissues cultured with **ellagic acid**
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Cancer Letters (CANCER LETT.) (Ireland) 1989, 46/2 (93-105)

Previous studies have shown that the nicotine-derived N-nitrosamine-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces tracheal papillomas and lung carcinomas in **Syrian golden hamsters**. In this study, we showed that hamster tracheal and lung explants metabolize NNK by alpha-carbon hydroxylation, pyridine N-oxidation and carbonyl reduction. alpha-Methylene hydroxylation and methyl hydroxylation yield methylating and pyridyloxobutylating intermediates, respectively. Levels of binding of the pyridyloxobutyl moiety to explant proteins was 200 times lower than the total amount of metabolites formed by alpha-carbon hydroxylation and released in the culture medium. Viable and heat-treated lung explants were cultured with (CHinf 3-sup 3H)NNK or (5-sup 3H)NNK. In viable explants, the rate of binding of the methyl group was 2-fold higher than the rate of binding of the pyridyloxobutyl moiety of NNK. Heat treatment reduced 54-fold the binding of (CHinf 3-sup 3H)NNK but only 5-fold the binding of (5-sup 3H)NNK. Tracheal explants were cultured with (5-sup 3H)NNK (5.6 muM) and **ellagic acid** (EA, 10 muM), a naturally-occurring plant phenol. EA did not inhibit any of the three metabolic pathways nor the binding of the

pyridyloxobutyl moiety to explant proteins. Lung explants were cultured with NNK (3.7 muM) and with or without EA (100 muM). EA inhibits alpha-carbon hydroxylation by 19% and the overall metabolism of NNK by 6%. Formation of 7-methylguanine and Osup 6-methylguanine was observed in lung explants and the levels of both adducts were reduced by EA (100 muM). These results suggest that high concentrations of EA modulate the metabolism of NNK and that NNK does not necessarily require enzymatic activation to bind to protein.

36. Ellagic acid metabolism and binding to DNA in organ explant cultures of the **rat**
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Cancer Letters (CANCER LETT.) (Ireland) 1987, 36/2 (203-211)

Ellagic acid (EA) is a plant phenolic compound with postulated antimutagenic and anticarcinogenic activity. In this study, explants of esophagus, forestomach, colon, bladder, trachea, lung and liver from male Sprague-Dawley **rats** (130-140 g) were incubated in cultured medium containing (sup 3H)EA (20 muM, 4.5 muCi/ml) for 24 h at 37degree C. After extraction, purification and quantitation of explant DNA significant differences in the binding of EA to the DNA was observed. The most binding occurred in esophagus and the least in lung. Analysis of the organosoluble fraction of the culture medium by high performance liquid chromatography yielded 3 metabolites of EA. None of the metabolites were identified. Elution of water-soluble metabolites from an alumina column showed that there were sulfate ester, glucuronide and glutathione conjugates of EA in the explant culture medium of all the organs. The profile of water-soluble conjugates was very similar between colon and forestomach and between trachea and lung. These results indicate that EA binds to DNA in different tissues and that tissues metabolize EA to both organosoluble and water-soluble products.

37. Ellagic acid binding to DNA as a possible mechanism for its antimutagnic and anticarcinogenic action
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Cancer Letters (CANCER LETT.) (Ireland) 1986, 30/3 (329-336)

Ellagic acid (EA), a plant phenol, is reported to possess antimutagenic and anticarcinogenic activity. In the present study, explants of esophagus, trachea, colon, forestomach and bladder from young male Sprague-Dawley **rats** were incubated in medium containing (sup 3H)EA (4.5 muCi/ml) for 24 h at

37degreeC. DNA from these explants was extracted, purified and quantitated to determine (sup 3H)EA binding to the DNA. Significant covalent binding of (sup 3H)EA to DNA occurred in all the explants. Calf thymus DNA incubated in 0.05 M sodium phosphate buffer containing (sup 3H)EA covalently bound (sup 3H)EA in a concentration dependent manner. Furthermore covalent binding of (sup 3H)EA to calf thymus DNA was inhibited by the addition of unlabeled EA that was concentration dependent over a range of 50-150 muM and by the addition of unlabeled adenosine, cytidine, guanosine or thymidine at a concentration of 1.0 mM. These results suggest that one of the mechanisms by which EA inhibits mutagenesis and carcinogenesis is by forming adducts with DNA, thus masking binding sites to be occupied by the mutagen or carcinogen.

38. Ellagic acid binding to DNA as a possible mechanism for its antimutagnic and anticarcinogenic action
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Cancer Letters (CANCER LETT.) (Ireland) 1986, 30/3 (329-336)

Ellagic acid (EA), a plant phenol, is reported to possess antimutagenic and anticarcinogenic activity. In the present study, explants of esophagus, trachea, colon, forestomach and bladder from young male Sprague-Dawley **rats** were incubated in medium containing (sup 3H)EA (4.5 muCi/ml) for 24 h at 37degreeC. DNA from these explants was extracted, purified and quantitated to determine (sup 3H)EA binding to the DNA. Significant covalent binding of (sup 3H)EA to DNA occurred in all the explants. Calf thymus DNA incubated in 0.05 M sodium phosphate buffer containing (sup 3H)EA covalently bound (sup 3H)EA in a concentration dependent manner. Furthermore covalent binding of (sup 3H)EA to calf thymus DNA was inhibited by the addition of unlabeled EA that was concentration dependent over a range of 50-150 muM and by the addition of unlabeled adenosine, cytidine, guanosine or thymidine at a concentration of 1.0 mM. **These results suggest that one of the mechanisms by which EA inhibits mutagenesis and carcinogenesis is by forming adducts with DNA, thus masking binding sites to be occupied by the mutagen or carcinogen.**

39. Inhibition of N-methyl-N-nitrosourea-induced mutagenicity and DNA methylation by ellagic acid
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Proceedings of the National Academy of Sciences of the United States of America (PROC. NATL. ACAD. SCI. U. S. A.) (United States) 1986, 83/21 (8039-8043)

Ellagic acid, a naturally occurring plant phenol, inhibits the activity of the direct-acting mutagen N-methyl-N-nitrosourea (MeNU) in Salmonella typhimurium TA100. Ellagic acid at 0.10, 0.25, 0.50, and 1.00 mM inhibited the mutagenicity of MeNU (0.40 mM) by 3%, 13%, 45%, and 60%, respectively. Ellagic acid (3 mM) also inhibited the mutagenic activity of N,N-dimethylnitrosamine (25-200 mM) in the presence of pyrazole-induced **rat** liver fraction S-9. The effect of ellagic acid on DNA methylation was studied by incubating 0, 0.72, 1.32, 2.64, and 6.60 mM ellagic acid with DNA (0.9 mM nucleotide) and (sup 3H)MeNU (0.66 mM). HPLC analysis of DNA hydrolysates showed that ellagic acid caused a dose-dependent 36-84% decrease in Osup 6-methylguanine but only a 20% decrease in the 7-methylguanine adduct. Under conditions where methylation at the Osup 6 position of guanine in double-stranded DNA was inhibited 65% by ellagic acid, no significant inhibition of either Osup 6- or 7-methylguanine formation was detected in single-stranded DNA. Affinity-binding studies revealed that (sup 3H)ellagic acid binds equally to double-stranded or single-stranded DNA but that poly(dA-dT) binds 1.5 times as much ellagic acid as does poly(dG-dC). The binding of ellagic acid to DNA is dependent on the concentration of both ellagic acid and DNA. The specific inhibition of Osup 6-methylguanine formation only in double-stranded DNA and the relatively low inhibition of 7-methylguanine formation rule out the possibility that ellagic acid prevents DNA alkylation by scavenging the electrophilic intermediate generated in the hydrolysis of MeNU. **The results suggest that ellagic acid inhibition of MeNU-induced mutagenicity is due to specific inhibition of methylation at the Osup 6 position of guanine through an ellagic acid-duplex DNA affinity-binding mechanism.**

40. LIPID PEROXIDATION **

Protective effect of curcumin, ellagic acid and bixin on radiation induced toxicity

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Indian Journal of Experimental Biology (INDIAN J. EXP. BIOL.) (India)
1996, 34/9 (845-847)

While body irradiation of **rats** (10 Gy as five fractions) found to produce lung fibrosis within 2 months as seen from increased lung collagen hydroxyproline and histopathology. Oral administration of antioxidants curcumin, ellagic acid, bixin and alpha-tocopherol at a concentration 200 mumole/kg body weight significantly reduced the lung collagen hydroxypyroline in these animals. In serum and liver lipid peroxidation which were found to be increased by irradiation was reduced significantly by antioxidant treatment. The liver superoxide dismutase and glutathione

peroxidase activity were also found to be increased and catalase activity decreased in irradiated control. Superoxide dismutase activity reduced significantly by antioxidant treatment while catalase activity was found to be increased with alpha-tocopherol treatment. The increased frequency of micronucleated polychromatic erythrocytes after whole body irradiation of **mice** was found to be significantly reduced with antioxidants.

41. Protective effect of curcumin, ellagic acid and bixin on radiation induced lipid peroxidation
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Journal of Experimental and Clinical Cancer Research (J. EXP. CLIN. CANCER RES.) (Italy) 1995, 14/4 (427-430)

Serum and tissue lipid peroxides in **rats** were found to increase after gamma irradiation; such an increase was found to be time and dose dependent. Serum lipid peroxide level in the control animals was 2.86 nmol/ml, which augmented to 4.2 nmole/ml 144 h after irradiation. Liver lipid peroxide was increased from 0.25 nmole/mg protein to 5.6 nmole/mg protein after 96 hrs, while the kidney lipid peroxide underwent a twofold increase. Increase in serum lipid peroxide was also found to be dose dependent. After administration of radiation dose of 14 Gy the value raised to 12.2 nmole/ml. Lipid peroxides of liver and kidney showed moderate change. Oral administration of curcumin, **ellagic acid**, bixin and alpha-tocopherol to **rats** significantly reduced the increased serum and liver lipid peroxides induced by radiation ($P < 0.001$). Cancer patients undergoing therapeutic irradiation were found to have significant increase of serum lipid peroxide.

42, Carcinogenesis 1994 Sep;15(9):2065-8
Ellagic acid induces NAD(P)H:quinone reductase through activation of the antioxidant responsive element of the **rat** NAD(P)H:quinone reductase gene.
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Induction of cellular detoxification enzymes can increase detoxification of carcinogens and reduce carcinogen-induced mutagenesis and tumorigenesis. To determine if the dietary anticarcinogen ellagic acid induced enzymes which detoxify xenobiotics and carcinogens, we examined the effect of ellagic acid on the expression of the phase II detoxification enzyme NAD(P)H:quinone reductase (QR). QR is induced by xenobiotics and antioxidants interacting with the xenobiotic responsive and antioxidant responsive elements of the 5' regulatory region of the QR gene. Ellagic acid is structurally related to the antioxidants

which induce QR and we proposed that ellagic acid would induce QR expression through activation of the antioxidant responsive element of the QR gene. **Rats** fed ellagic acid demonstrated a 9-fold increase in hepatic and a 2-fold increase in pulmonary QR activity, associated with an 8-fold increase in hepatic QR mRNA. To determine if this increase in QR mRNA was due to activation of the antioxidant responsive element, transient transfection studies were performed with plasmid constructs containing various portions of the 5' regulatory region of the **rat** QR gene. These transfection studies confirmed that ellagic acid induces transcription of the QR gene and demonstrated that this induction is mediated through the antioxidant responsive element of the QR gene.

43. The effects of **ellagic acid** and 13-cis-retinoic acid on N-nitrosobenzylmethylamine-induced esophageal tumorigenesis in **rats**
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Cancer Letters (CANCER LETT.) (Ireland) 1991, 56/2 (117-124)

Ellagic acid (EA) and 13-cis-retinoic acid (CRA), both alone and in combination, were tested for their ability to inhibit N-nitrosobenzylmethylamine-induced tumors in the **rat** esophagus. Groups of male **rats** were fed AIN-76A diet containing EA (4 g/kg), CRA (240 mg/kg), or a combination of EA and CRA (4 g/kg and 240 mg/kg), respectively, for 25 weeks. Two weeks after initiation of the diets, NBMA (0.5 mg/kg per injection) was administered s.c. once weekly for 15 weeks. After 25 weeks on the diets, the animals were necropsied. The incidence of esophageal tumors was 97-100% in all NBMA-treated groups. The multiplicity of tumors in NBMA-treated groups was reduced significantly by EA (60%), but not by CRA, or by EA + CRA. These results demonstrate that EA and CRA do not act synergistically to inhibit NBMA-induced esophageal tumorigenesis.

44. Dietary **ellagic acid** reduces the esophageal microsomal metabolism of methylbenzylnitrosamine
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Cancer Letters (CANCER LETT.) (Ireland) 1989, 44/1 (39-44)

Dietary **ellagic acid** has been shown to reduce the incidence of methylbenzylnitrosamine-induced esophageal carcinoma in the **rat**. Methylbenzylnitrosamine (MBN) is a naturally occurring carcinogen which requires cytochrome P-450 dependent activation to be mutagenic. We examined whether the reduction in tumor incidence observed with dietary **ellagic acid** was associated with alterations in the cytochrome P-450 dependent microsomal metabolism of MBN. Dietary **ellagic acid** was shown to

significantly reduce total esophageal and hepatic microsomal cytochome P-450 ($P < 0.05$) and significantly reduce the esophageal microsomal metabolism of MBN ($P < 0.05$). The addition of **ellagic acid** in vitro also resulted in a significant inhibition ($P < 0.05$) of the esophageal microsomal metabolism of MBN. In contrast, dietary **ellagic acid** and the addition of **ellagic acid** in vitro did not alter the hepatic microsomal metabolism of MBN. **The reduced rate of MBN metabolism by the esophageal microsomes from the ellagic acid fed rats may contribute to the decreased incidence of esophageal carcinoma observed in these animals.**

45. OTHER **

Screening of selected flavonoids and phenolic acids in 19 berries.
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Food Research International 1999 , 32 (5) 345-353

An HPLC method for simultaneous analysis of flavonoids and phenols was applied to 19 different edible berries (wild and cultivated var.) originating from Finland. Relative contents of flavonoids and phenols varied widely among berries studied. Quercetin was the major flavonoid determined in many berries; quercetin content was highest (GREATER THAN 100 mg/100 g) in blueberries (cv. Northblue) followed by cranberries, lingonberries, chokeberries and crowberries. **Ellagic acid** was the major phenol determined in red raspberries, Arctic bramble, and cloudberries; levels were GREATER THAN 160 mg/100 g in all 3 types of berry. Strawberries also contained high **ellagic acid** levels (GREATER THAN 40 mg/100 g). Principal component analysis was used to classify the berries studied on the basis of their phenols and flavonoids profiles. Results suggested that most berries studied form good sources of quercetin and **ellagic acid** which have potential antioxidative and anticarcinigenic properties.

46. Involvement of lipid peroxidation in necrosis of skin flaps and its suppression by **ellagic acid**
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Plastic and Reconstructive Surgery (PLAST. RECONSTR. SURG.) (United States) 1994, 94/7 (1027-1037)

To evaluate the pathogenesis of lipid peroxidation in skin-flap necrosis

and to select a novel herbal antioxidant to suppress lipid peroxidation and salvage the flaps, in vitro and in vivo experiments were instituted. In vitro studies revealed (1) the potentiality of the cutaneous microsomal system (vesicular fragment of endoplasmic reticulum) to generate oxyradicals by FeClinf 3 (oxidative agent), since NADPH-dependent lipid peroxidation was elevated time-dependently, (2) suppression of microsomal lipid peroxidation by herbal antioxidants (dose- and time-dependently), further supporting the theory of oxyradical-induced lipid peroxidation in the skin, **and (3) that ellagic acid showed the strongest response**, with curcumin, chlorogenic acid, and alpha-tocopherol (tocopherol) being moderate, and ferulic acid and gallic acid remaining weakest. Thus ellagic acid, curcumin, chlorogenic acid, and tocopherol at doses of 10, 60, 80 and 100 muM (twice LD50, the dose which could inhibit lipid peroxidation by 50 percent) were chosen for in vivo assessments, respectively. In vivo studies were performed using **rat** back skin random flaps (70 x 15 mm and based anteriorly) and circular island flaps (20 mm in diameter and raised on superficial epigastric vessels). Control flaps were painted with a Tris-ethanol solution, and test flaps were painted with either ellagic acid, curcumin, chlorogenic acid, or tocopherol (above- mentioned doses per 250 mul of Tris-ethanol per 300 mmsup 2 of flap surface 1 hour before the operation and once a day for 3 postoperative days). Doses, frequency, and period of drug application were based on in vitro and in vivo pilot experiments. The results were as follows: (1) a direct and time- dependent relation was noticed between lipid peroxide levels and the rate of necrosis in both types of flap; (2) time-dependent elevation of lipid peroxide levels of skin, subcutaneous fat, and exudate of island flaps during ischemia and those of skin and subdermal fat after reperfusion indicated pre- and post-reflow states of lipid peroxidation rather than the original conception of merely reperfusion state; and (3) in good agreement with the results of in vitro experiments, **ellagic acid** exerted the strongest effect to suppress lipid peroxide levels of skin and to augment the viability of random flaps more than that of island flaps. These results suggest (1) that in the field of flap research and testing of flap-salvaging drugs, combined in vitro and in vivo experiments would help not only in better understanding the pathophysiology of the flap necrosis but also in finding the proper method and dose of drug administration; (2) that since even **ellagic acid** failed to salvage the flaps completely, it seems other factors might be involved, which further studies may aim at; and (3) that more experiments such as topical application of radioisotope-labeled ellagic acid should be done to determine the rate and depth of cutaneous absorption of this compound for clinical use.

47. Hydrolyzable tannins: Potent inhibitors of hydroperoxide production and tumor promotion in mous skin treated with 12-O-tetradecanoylphorbol-13-acetate in vivo
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International Journal of Cancer (INT. J. CANCER) (United States) 1992 51/3 (425-432)

The anti-oxidant and the anti-tumor promotion activities of several hydrolyzable tannins (HTs), including a commercial tannic-acid (TA) mixture, were examined in mouse skin treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) in vivo. A single application of TPA gradually increases the hydroperoxide (HPx)-producing activity of the epidermis, which is maximally stimulated at 3 days and returns to control levels at 9 days. **Pretreatments with TA and ellagic acid (EA) strongly inhibit, in a dose-dependent manner, this HPx response to TPA**. Total inhibition by TA lasts for about 16 hr, beyond which it is substantially reduced but not completely lost. TA can also reduce the level of epidermal HPx when it is applied 36 hr after the tumor promoter. EA is an antioxidant 10 times more potent than TA and n-propyl gallate (PG), which are equally effective against TPA-induced HPx production. Gallic acid is the least effective of the HTs in inhibiting HPx formation. TA also inhibits the production of HPx induced by several structurally different tumor promoters and the greater HPx responses produced by repeated TPA treatments. When applied 20 min before each promotion treatment, twice a week for 45 weeks, several HTs inhibit the incidence and yield of papillomas and carcinomas promoted by TPA in initiated skin. Overall, TA is more effective than EA and PG in inhibiting skin tumor promotion by TPA, suggesting that the anti-oxidant effects of HTs are essential but not sufficient for their anti-tumor-promotion activity.

48. Evaluation of strawberry cultivars for **ellagic acid** content.
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HortScience vol. 26 (1): p.66-68
Publication Year: 1991

Ellagic acid in tissue extracts of green and red-ripe strawberries was detected and quantified by HPLC. **Ellagic acid** content of green fruit pulp ranged from 1.32 to 8.43 mg/g DW (mean 3.36 mg/g) and in achenes of green fruit from 1.32 to 20.73 mg/g (mean 7.24). **Ellagic acid** content of red fruit pulp for 35 cultivars and selections at one location ranged from 0.43 to 4.64 mg/g (mean 1.55) and for 15 clones at another location from 0.43 to 3.47 mg/g (mean 1.45). Ellagic acid content in achenes from red-ripe fruit ranged from 1.37 to 21.65 mg/g (mean 8.46) for 34 clones at one location and from 2.81 to 18.37 mg/g (mean 8.93) for 15 clones at another location. Leaf **ellagic acid** content ranged from 8.08 to 32.30 mg/g (mean 14.71) for 13 clones examined. Large differences in **ellagic acid** content were found among cultivars, but tissue values were not consistent

within cultivars. Values from one tissue type did not correlate consistently with values for the other tissues. Sufficient variation was found among cultivars to suggest that increased **ellagic acid** levels may be achieved in progeny from crosses with selected parental material. 6 ref.

49. The effects of pH and **rat** intestinal contents on the liberation of **ellagic acid** from purified and crude ellagitannins
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Journal of Natural Products (Lloydia) (J. NAT. PROD. LLOYDIA) (United States) 1991, 54/4 (946-952)

This study was undertaken to measure the liberation in vitro of ellagic acid (2), a naturally occurring inhibitor of carcinogenesis, from precursor ellagitannins under conditions found in the gut tract. Enzymes, namely beta-glucosidase, esterases, and alpha-amylase, were incubated with raspberry extract. In addition, raspberry extract and casuarictin (1) were treated at different pH's and with the contents of small intestine and cecum from **rats** fed AIN-76A diet. The esterase activity of the enzyme samples was measured spectrophotometrically using p-nitrophenol acetate as the substrate, and the amount of **ellagic acid** (2) released from all samples was analyzed by hplc. The hydrolysis of the ellagitannins was not catalyzed by any of the purified enzymes tested, and components of the raspberry extract were found to inhibit the purified esterases noncompetitively. Casuarictin (1) was hydrolyzed to yield high quantities of **ellagic acid** (2) when placed in buffer at pH 7 and 8, or when incubated with cecal contents for two hours. The release of **ellagic acid** (2) from the raspberry extract was optimal at pH 8, and maximal release in cecal contents occurred with 1 h. Small intestinal contents had no significant effect on **ellagic acid** liberation from either casuarictin (1) or raspberry extract.

Ellagic acid is a polyphenol found abundantly in various fruits, nuts and vegetables. Ellagic acid is active in antimutagenesis assays, and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents, and TPA-induced tumor promotion in mouse skin. Ellagic acid functions through a variety of mechanisms, including inhibition of microsomal P-450 enzymes, stimulation of glutathione-S-transferase, scavenging the reactive metabolites of carcinogens, and direct binding to DNA, thus potentially masking sites that would normally interact with ultimate carcinogens.