in vitro, but DAS failed to do so. Despite the non complete nature of the present studies the picture is emerging that the antioxidative stress properties of garlic might result from the positive contributions of its different sulfur components each in a different step and not necessarily from the contribution of only one of them. Studies are in progress to further this hypothesis. Supported by CONICET, Argentina.

P1C61 THE ANTIOXIDATIVE MECHANISMS OF TEA IN THE PREVENTION OF CANCER

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The preventive effects and antioxidative mechanisms of green tea and tea mixture (a mixture of whole tea extract, polyphenols and tea pigments) on N-nitrosodiethylamine (DEN) induced carcinogenesis were studied in a rats model treated with multiple small doses of DEN (Solt Farber). The results showed that oral administration of 2% green tea and 0.5% tea-mixture as drinking water both resulted in a significant reduction of DEN induced glutamyl transpeptidaose-positive foci in the liver and peroxidation products (malondiadehyde, MDA; acetaldehyde, ACT; acetone, ACON; propionphenylhydrazine, PP) formed in the rat liver microsome ($p < 0.01$). On the other hand, it was found that drinking green tea significantly increased the activities of superoxide dismutase (SOD), NAD(P)H-quinone reductase (QR) and glutatione S-transferase (GST) in the liver ($p < 0.01$). Whereas drinking tea mixture also increased the activities of catalase (CAT), SOD, QR and GST ($p < 0.01$).

Based on the above results, various tea ingredients were screened for their anticarcinogenic activity by measuring QR activity in Hepa-G2 cell culture. The results showed that tea polyphenols, tea pigments and tea mixture were all able to induce the activity of QR significantly. The QR activity increased 35.8%, 58.7%, 74.6% (2 mg/l) and 73.0%, 60.4%, 60.6% (10 mg/l) respectively, Among the single-component ingredients of tea tested, theanubigens, EGCG and ECG also enhanced the activity of QR dramaticly. The increasing rates were 30.3%, 53.1%, 50.7% (10 mg/l) respectively. But EGC, theaflavins, tea polysaccharide and tea caffeine, showed no apparent effects on QR activity. It's concluded that, among those tea ingredients studied, the multi-component ingredients were more effective than the single-component ones.

P1C62 EFFECTS OF WINE COMPLEX POLYPHENOLS AGAINST LIVER OXIDATIVE DNA DAMAGE IN RATS TREATED WITH 2-NITROPROPANE

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2-Nitropropane (2NP) is an hepatocarcinogen for which there is strong evidence of a mechanism of action mediated by oxidative DNA damage The protection afforded by antioxidants against carcinogenesis mediated by oxygen radicals might therefore be due to the prevention of oxidative DNA damage. To predict whether wine complex polyphenols (WCP) might exert preventive effects on oxidative DNA damage and hepatotoxicity induced with 2NP, we administered a mixture of WCP to rats 2 wk before a single challenge with the carcinogen. The animals were treated for 14 consecutive days per os with WCP (57.2 mg/kg/d). Controls received water alone. Hepatic nuclear levels of 8-Hydroxy-2-deoxyguanosine (8OHdG), a reliable marker for oxidative DNA damage, were subsequently examined. The 8OHdG was significantly increased by 81%, 73%, and 131%, in rats sacrificed 6, 9 or 15 hr following the 2NP injection respectively. The levels of 8OHdG were significantly decreased by WCP (57.2 mg/kg/d) from $47.88 \times 10^{-6} \pm 3.17$ (S.E.) (n = 5) to $33.32 \times 10^{-6} \pm 2.52$ (S.E.) (n = 5) in liver of rats sacrificed 15 hr following the 2NP injection. Our results demonstrated that WCP have a protective effect on oxidative DNA damage induced by 2NP and this effect might be due to a scavenging effect on active oxygen species of these polyphenolic compounds.

P1C63 ANTIOXIDANT PROPERTIES AND RADICAL SCAVENGING ACTIVITIES OF PHENOLIC COMPOUNDS EXTRACTED FROM *VITIS VINIFERA* CELL CULTURES

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Numerous epidemiological studies in France have shown a negative correlation between moderate red wine consumption and the incidence of cardiovascular diseases. Furthermore, there is increasing evidence that oxidized low-density lipoproteins may be responsible for promoting atherogenesis.

Compared with others alcoholic beverages, red wine contains a high quantity of phenolic compounds. The aim of our work was to use grape cells of *Vitis vinifera* in culture in order to produce phenolic compounds similar to those recovered in wine. At the present time, we have isolated and identified flavonoid (anthocyanins, catechins) and non-flavonoid (stilbenes) compounds. Antioxidant activities were assessed by their capacity to prevent Fe^{2+}-induced lipid peroxidation in microsomes and their action on Cu^{2+}-induced lipid peroxidation in low-density lipoproteins. The ability of these compounds to act as radical scavengers was investigated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical.

The results demonstrate that all classes of phenolic compounds tested and found in red wine exhibit interesting antioxidant properties and radical scavenging activities. Furthermore, they are more efficient than Trolox, the water-soluble vitamin E analog. These data may account in part for the so-called "French paradox," i.e. that moderate drinking of red wine over a long period of time can protect against coronary heart diseases.

P1C64 EVALUATION OF THE PRODUCTION OF OXIDATIVE STRESS BY TOXIN T514 OF *KARWINSKIA HUMBOLDTIANA*

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In the present study we have analyzed the production of "oxidative stress" by toxin T514 of Buckhorn (Karwinskia humboldtiana) and its possible role in the cytotoxicity of this compound to primary liver cell cultures. The role of catalase and superoxide dismutase (SOD) as defense mechanisms against "oxidative stress" was also studied. Freshly isolated hepatocytes (>90% viability, trypan blue) were cultivated 72 h (10^6 cells/ml) in 24 wells plates and exposed to T514 (6, 12, 25 and 50 uM) in the presence or abscense of catalase and SOD. Cytotoxicity was determined by methylthiazoltetrazolium (MTT) reduction. "Oxidative stress" was evaluated by the dichlorofluorescein diacetate (DCF-DA) fluorescent probe, lipidperoxidation production and nitrobluetetrazolium test.

T514 stimulated DCF-fluorescence, which was correlated to time and concentration.

Catalase and SOD inhibited T514 cytotoxicity by 50% and 30% respectively (MTT test); implicating the superoxide anion and hydrogen peroxide in producing the changes in intracellular dichlorofluorescein fluorescence.

We suggest that "oxidative stress" is a mechanism by which T514 induces its cytotoxic effect.

P1C65 ADRIAMYCIN-INDUCED FREE RADICAL-DEPENDENT INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION

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Adriamycin is a potent, broad-spectrum antineoplastic agent whose clinical utility is limited by a dose-dependent, cumulative cardiomyopathy. Recent evidence implicates interference with mitochondrial bioenergetics in the manifestation of adriamycin cytotoxicity. Specifically, induction of the mitochondrial permeability transition (MPT) appears to be the initiating event in adriamycin-induced metabolic failure. The purpose of the present investigation was to assess the role of redox cycling and liberation of oxygen free radicals in mediating the induction of the MPT by adriamycin *in vitro*. The rate of generation of superoxide anion free radicals from sub-mitochondrial particles (SMP) incubated with glutamate plus malate was stimulated by adding either cyanide, antimycin A, or rotenone. Conversely, free radical generation by SMP with succinate as substrate was inhibited by rotenone but not by cyanide or antimycin A. From this we conclude that adriamycin redox cycles at complex I of the electron transport chain to liberate oxygen free radicals. Furthermore, induction of the MPT by adriamycin was dependent on the energizing substrate, the rate of induction of the MPT correlating with rate of free radical generation. Even under the most severe conditions, cyclosporine A afforded complete protection against induction of the MPT without altering the rate of oxygen radical generation. We conclude that induction of the MPT by adriamycin is mediated by oxygen free radicals and that the protection afforded by cyclosporine A its not due to an antioxidant activity of this drug. (Supported by HL-58016.)

P1C66 DNA DAMAGE AND CELL DEATH INDUCED BY SALSOLINOL THROUGH OXIDATIVE STRESS

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Salsolinol (SAL), a novel dopaminergic catechol tetrahydroisoquinoline neurotoxin, has been speculated to contribute to Parkinson's disease and neuropathology of chronic alcoholism. In the present study, we found that SAL mediated the strand scission in øX174 RFI or pBR322 supercoiled DNA in the presence of Cu (II), which was protected by catalase or the copper chelator, bathocuproinedisulfonic acid (BCS). Furthermore, SAL in combination with Cu (II) produced hydroxyl radical as determined by hydroxylation of salicylic acid. Treatment of PC12 cells with SAL led to cell death which was exacerbated by Cu (II). BCS again ameliorated the Cu (II)-dependent cytotoxicity of SAL. Cells exposed to SAL underwent apoptosis as determined by microscopic morphology and internucleosomal DNA fragmentation. Apoptotic cell death was further confirmed by terminal transferse-mediated dUTP nick end labeling (TUNEL). Transfection of PC12 cells with the anti-apoptotic gene, *bcl-xL* abrogated the cell death by SAL. While SAL-induced cytotoxicity was significantly enhanced by Cu (II), the extent of apoptotic death in PC12 cells exposed to SAL in combination with cupric ion was lower than that attained with the catechol alone. From these findings, it seems likely that SAL in the presence of molecular oxygen undergoes redox cycling catalyzed by Cu (II) with concomitant generation of reactive oxygen species which may contribute to the overall neurotoxicity exerted by this catechol isoquinoline. Although SAL alone can cause apoptotic cell death, the transition metal ion such as Cu (II) may divert the SAL-induced cell damage to necrosis by generating massive production of reactive oxygen species. Supported by the Genetic Engineering grant from the Ministry of Education, Republic of Korea.

P1C67 CORRELATION BETWEEN GLUTATHIONE S-TRANSFERASE ACTIVITY AND LIPID PEROXIDE FORMATION IN LIVER OF WEANLING RATS UNDER PARACETAMOL TREATMENT

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Newborn animals are known to be relatively less susceptible than the adults to paracetamol-induced liver damages. The mechanism(s) by which growing animal protect its cellular macromolecules against paracetamol toxic metabolites is not well understood. When the toxic metabolite of paracetamol i.e. N-acetyl-p-benzoquinonimine (NAPQI) is produced in large amounts it can cause depletion of hepatic cellular glutathione (GSH) stores and thereby available to bind to cellular macromolecules leading to cytotoxic effects. Evidence show that age-related differences in the rate of GSH depletion has little influence on paracetamol detoxification. Whereas cytosolic GSH S-transferase (GST) activity plays an important role in detoxification of paracetamol. Hepatic cytosolic GST activity in growing rats is normally between 60–70% of that in adults. A differential induction in GST occurs in adult and growing rat liver in response to a single i.p. dose of paracetamol (500 mg/kg). The extent of enzyme induction in adult and growing tissues is 1.8 and 40% respectively. GST induction in growing tissue was associated with a surge in paracetamol-GSH conjugate formation *in vivo*. HPLC analysis of liver cytosols obtained from control and paracetamol-treated rates showed that paracetamol-GSH conjugation is stimulated in growing rats in response to drug (24 fold as compared to adults). Age-related difference in GSH conjugate formation could be attributed to the specific induction in weanling liver GST (40%). Time-course studies showed that concomitant with GST induction the rate of lipid peroxide formation which was increased significantly 3 h after drug administration returned to normal values after 24 h. These results suggest that paracetamol-dependent induction in specific GST isoforms in immature animals is an important mechanism involved in inactivation of NAPQI as well as other lipid peroxide products, hereby confer relative resistance to animals at this age.

P1C68 COPPER SULFATE PRETREATMENT INHIBITS MPP+-INDUCED STRIATAL LIPID PEROXIDATION (LPO) IN MICE

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1-methyl-4-phenylpyridinium (MPP+) is the major metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) a drug which induces a Parkinson-like damage in several animal species. It is now accepted that MPP+ is responsible of the neurotoxic effects of MPTP in dopaminergic nigrostriatal pathway. On the other hand, it has been demonstrated that copper deficiency produces impairment of the many processes dependent on the metal. Our group reported that MPTP decreases copper content (50%) in *corpus striatum*, suggesting an important role of copper on dopaminergic neurotoxicity of MPTP. In this study we evaluated the possible antagonist effect of copper sulfate pretreatment in MPP+-induced LPO. Adult male C57-Black mice (25–30 g) were injected with copper sulfate (2.5 mg/kg). After 24 h, mice were injected i.p. into their right lateral ventricle with MPP+ and 2 hours later they were killed by