

HOMOCYSTEINE, METHYLENETETRAHYDROFOLATE REDUCTASE AND RISK OF SCHIZOPHRENIA: A META-ANALYSIS.

Elevated plasma homocysteine concentration has been suggested as a risk factor for schizophrenia, but the results of epidemiological studies have been inconsistent. The most extensively studied genetic variant in the homocysteine metabolism is the 677C>T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene, resulting in reduced enzyme activity and, subsequently, in elevated homocysteine. A meta-analysis of eight retrospective studies (812 cases and 2,113 control subjects) was carried out to examine the association between homocysteine and schizophrenia. In addition, a meta-analysis of 10 studies (2,265 cases and 2721 control subjects) on the homozygous (TT) genotype of the MTHFR 677C>T polymorphism was carried out to assess if this association is causal. A 5 micromol/l higher homocysteine level was associated with a 70% (95% confidence interval, CI: 27-129) higher risk of schizophrenia. The TT genotype was associated with a 36% (95% CI: 7-72) higher risk of schizophrenia compared to the CC genotype. The performed meta-analyses showed no evidence of publication bias or excessive influence attributable to any given study. In conclusion, our study provides evidence for an association of homocysteine with schizophrenia. The elevated risk of schizophrenia associated with the homozygous genotype of the MTHFR 677C>T polymorphism provides support for causality between a disturbed homocysteine metabolism and risk of schizophrenia.

Mol Psychiatry. 2006 Feb;11(2):143-9

HOMOCYSTEINE-REDUCING STRATEGIES IMPROVE SYMPTOMS IN CHRONIC SCHIZOPHRENIC PATIENTS WITH HYPERHOMOCYSTEINEMIA.

BACKGROUND: An elevated homocysteine level is reported to be a risk factor for several diseases, including Alzheimer's and cerebrovascular disease. Recently, several studies have reported that homocysteine levels are elevated in many schizophrenic patients. Homocysteine levels can be lowered by oral folic acid, B-12, and pyridoxine. **METHODS:** Forty-two schizophrenic patients with plasma homocysteine levels >15 micromol/L were treated with these vitamins for 3 months and placebo for 3 months in a study with a randomized, double-blind, placebo-controlled, crossover design. **RESULTS:** Homocysteine levels declined with vitamin therapy compared with placebo in all patients except for one noncompliant subject. Clinical symptoms of schizophrenia as measured by the Positive and Negative Syndrome Scale declined significantly with active treatment compared with placebo. Neuropsychological test results overall, and Wisconsin Card Sort (Categories Completed) test results in particular, were significantly better after vitamin treatment than after placebo. **CONCLUSIONS:** A subgroup of schizophrenic patients with hyperhomocysteinemia might benefit from the simple addition of B vitamins.

Biol Psychiatry. 2006 Jan 17

PLASMA TOTAL HOMOCYSTEINE LEVEL AND BONE MINERAL DENSITY: THE HORDALAND HOMOCYSTEINE STUDY.

BACKGROUND: Plasma total homocysteine (tHcy) has been associated with hip fracture but not directly with bone mineral density (BMD). We examined the association of hip BMD with levels of plasma tHcy, folate, and vitamin B12 and the methylenetetrahydrofolate reductase (MTHFR) 677C-->T and 1298A-->C polymorphisms. **METHODS:** Bone mineral density was measured between 1997 and 2000 in 2,268 men and 3,070 women, aged 47 to 50 and 71 to 75 years, from the Hordaland Homocysteine Study cohort. Low BMD was defined as BMD in the lowest quintile for each sex and age group. Linear, logistic, and generalized additive regression models were used. **RESULTS:** Plasma levels of tHcy were inversely related to BMD among middle-aged and elderly women ($P<.001$) but not among men. The multiple adjusted odds ratio for low BMD among subjects with high (≥ 15 micromol/L [≥ 2.02 mg/L]) compared with low (< 9 micromol/L [< 1.22 mg/L]) tHcy level was 1.96 (95% confidence interval, 1.40-2.75) for women and was not significant for men. Additional adjustments for plasma folate level or intake of calcium and vitamin D did not substantially alter the results. Plasma folate level was associated with BMD in women only. We observed no association between BMD and vitamin B12 level or the MTHFR polymorphisms. **CONCLUSIONS:** Elevated tHcy and low folate levels were associated with reduced BMD in women but not in men. These findings suggest that tHcy may be a potential

modifiable risk factor for osteoporosis in women.

Arch Intern Med. 2006 Jan 9;166(1):88-94

EVALUATION OF PLASMA HOMOCYSTEINE AND RISK OF AGE-RELATED MACULAR DEGENERATION.

PURPOSE: To assess the relationship between plasma levels of homocysteine and age-related macular degeneration (AMD). **DESIGN:** Cross-sectional, case-control study. **METHODS:** Fasting plasma homocysteine levels were measured at two centers in 934 individuals who were participating in an ancillary study of the Age-Related Eye Disease Study. There were 547 cases and 387 control subjects, who were determined by fundus photography. Conditional logistic regression analyses were conducted to assess the association of homocysteine with AMD. **RESULTS:** Median values of homocysteine were higher among advanced AMD cases (9.51 mmol/l) compared with persons with no AMD (8.81 mmol/l; $P = .01$). Values of >12 mmol/l vs $< \text{or} = 12$ mmol/l were also associated with an increased risk of AMD ($P = .023$), when controlled for other covariates. **CONCLUSION:** Results are consistent with a possible small, independent association between higher homocysteine levels and AMD. Homocysteine may be a modifiable risk factor for AMD.

Am J Ophthalmol. 2006 Jan;141(1):201-3

HOMOCYSTEINE AND ITS DETERMINANTS IN NONDIALYZED CHRONIC KIDNEY DISEASE PATIENTS.

This cross-sectional study aimed to investigate the prevalence of hyperhomocysteinemia, the determinants of plasma total homocysteine concentrations, and the relationship of total homocysteine with nutritional parameters in a sample of patients with chronic kidney disease (CKD) and not yet on dialysis. The study was done with outpatients from the Nephrology Division of the Federal University of Sao Paulo and Oswaldo Ramos Foundation. Sixty-six patients with CKD (70% male; age 58.6 ± 15.6 years [mean \pm standard deviation]) with moderate to severe renal impairment (creatinine clearance = 29.8 ± 14.3 mL/min [0.5 ± 0.24 mL/sec]), clinically stable, and older than 18 years were included. A group of 20 healthy subjects from the clinic staff was also studied for reference values for plasma homocysteine, folate, and vitamin B-12 concentration. Fasting blood samples were collected to determine plasma total homocysteine, folate, vitamin B-12, and creatinine. To calculate creatinine clearance, a 24-hour urine collection sample was obtained. The assessment of nutritional status included anthropometric parameters. Pearson correlation, Mann-Whitney test, and multiple linear regression analysis were used for statistical analyses. The main results showed that the concentration of total homocysteine in the patients was significantly increased compared with the healthy subjects (3.4 ± 1.7 vs 1.41 ± 0.42 mg/L [25.4 ± 12.2 vs 10.4 ± 3.1 micromol/L]; $P < 0.001$). Plasma folate and plasma vitamin B-12 were in the normal range and did not differ between patients and healthy individuals. A high prevalence of hyperhomocysteinemia (total homocysteine >1.89 mg/L [14 micromol/L]) was found in the patients (89%). Plasma total homocysteine did not correlate with any of the nutritional parameters studied and did not differ between patients in terms of whether they were using or not using folic acid supplementation (3.07 ± 1.09 vs 3.55 ± 1.78 mg/L [22.7 ± 8.1 vs 26.3 ± 13.2 micromol/L]; $P = 0.47$), although plasma folate was significantly higher in the supplemented group (12.6 ± 3.0 vs 8.0 ± 3.6 ng/mL [28.5 ± 6.8 nmol/L vs 18.1 ± 8.2 nmol/L]; $P < 0.001$). According to the multiple regression analysis, the determinants of total homocysteine were only plasma folate, plasma vitamin B-12, and creatinine clearance ($r^2 = 0.20$). In conclusion, a high prevalence of hyperhomocysteinemia was found in our sample of nondialyzed patients with CKD. The determinants of total homocysteine levels were plasma folate, plasma vitamin B-12, and creatinine clearance. No association between nutritional parameters and total homocysteine was observed.

J Am Diet Assoc. 2006 Feb;106(2):267-70

ASSOCIATION OF PLASMA HOMOCYSTEINE WITH CORONARY ARTERY CALCIFICATION IN DIFFERENT CATEGORIES OF CORONARY HEART DISEASE RISK.

OBJECTIVE: To investigate the association of plasma homocysteine with coronary artery calcification (CAC) in strata based on 10-year risk of coronary heart disease (CHD) in a cohort enriched in persons with hypertension. **PARTICIPANTS AND METHODS:** Fasting plasma homocysteine was measured by liquid chromatography electrospray tandem mass spectrometry. Coronary artery calcification was measured noninvasively by electron beam computed tomography and CAC score calculated using the method of Agatston et al. The 10-year CHD risk was calculated based on the Framingham risk score. The association of homocysteine with log-transformed CAC score was assessed in the pooled sample and within each risk stratum by linear regression after adjustment for conventional risk factors. **RESULTS:** In the 1,071 participants studied, homocysteine was associated with CAC quantity ($P = .01$) after adjustment for CHD risk factors (age, male sex, total and high-density lipoprotein cholesterol, diabetes, history of smoking, body mass index, and systolic blood pressure), serum creatinine, and statin and hypertension medication use. When the association was assessed in strata based on 10-year CHD risk, homocysteine was significantly ($P = .003$) associated with CAC quantity in participants at intermediate 10-year risk of CHD (6%-20%) independent of other risk factors but not in those at lower risk or higher risk. **CONCLUSION:** Plasma homocysteine is associated with quantity of CAC independent of CHD risk factors. When studied in categories of 10-year CHD risk, the association was significant in participants at intermediate risk but not in those at low or high risk. Plasma homocysteine levels may have clinical

utility as a marker of CHD risk in such individuals.

Mayo Clin Proc. 2006 Feb;81(2):177-82

SERUM HOMOCYSTEINE, FOLATE AND RISK OF STROKE: KUOPIO ISCHAEMIC HEART DISEASE RISK FACTOR (KIHD) STUDY.

BACKGROUND: Homocysteine and folate have been suggested to have opposite effects on the risk of stroke, although the results are controversial. **DESIGN AND METHODS:** The purpose of this study was to assess the effects of serum total homocysteine (tHcy) and serum folate levels on the risk of stroke in a prospective cohort study. The subjects were 1,015 men aged 46-64 years and free of prior stroke, examined in 1991-1993 in the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. **RESULTS:** At baseline the mean serum tHcy concentration was 10.9 micromol/l (SD 3.4). During an average follow-up time of 9.6 years, 49 men experienced a stroke, of which 34 were ischaemic. In Cox proportional hazards models, men in the highest tHcy third had a risk factor-adjusted hazard rate ratio (RR) of 2.77 [95% confidence interval (CI): 1.23-6.24] for any stroke and 2.61 (95% CI: 1.02-6.71) for ischaemic stroke, compared with men in the lowest third. The mean baseline serum folate concentration was 10.4 nmol/l (SD 4.1). Men in the highest third of serum folate (>11.2 nmol/l) had an adjusted RR for any stroke of 0.35 (95% CI: 0.14-0.87) and for ischaemic stroke of 0.40 (95% CI: 0.15-1.09), compared with men in the lowest third. **CONCLUSION:** Elevated serum tHcy is associated with increased risk of all strokes and ischaemic strokes in middle-aged eastern Finnish men free of prior stroke. On the other hand, high serum folate concentration may protect against stroke.

Eur J Cardiovasc Prev Rehabil. 2005 Aug;12(4):369-75

DO MATERNAL FOLATE AND HOMOCYSTEINE LEVELS PLAY A ROLE IN NEURODEVELOPMENTAL PROCESSES THAT INCREASE RISK FOR SCHIZOPHRENIA?

OBJECTIVE: Evidence from many different lines of research supports the hypothesis that schizophrenia is a disorder of development with etiological factors implicated as early as the second trimester in utero. We suggest that low maternal folate, acting to increase homocysteine levels, may provide a functional link between many of the identified prenatal risk factors and the hypothesized mechanisms whereby neurodevelopmental patterning deviates toward a schizophrenic potential. **METHODS:** PubMed was searched from the present back to 1963, when elevated homocysteine was identified as a pathogen in homocystinuria as first described by Carson and colleagues (*Arch Dis Child* 1963;38:425-36). All articles for homocystinuria, homocysteine, folate, and development with schizophrenia were evaluated. **RESULTS:** The findings from this review support the hypothesis that maternal low folate and high homocysteine levels may provide a potential teratogenic mechanism that increases the risk for developing schizophrenia. **CONCLUSION:** The potential role of maternal folate deficiency and hyperhomocystinemia in the genesis of schizophrenia would extend the range of their known teratogenic effects. Given the potential for preventive treatment offered by this hypothesis, we believe further investigation into this mechanism is warranted.

Harv Rev Psychiatry. 2005 Jul-Aug;13(4):197-205

HOMOCYSTEINE AS A PREDICTIVE FACTOR FOR HIP FRACTURE IN ELDERLY WOMEN WITH PARKINSON'S DISEASE.

PURPOSE: Incidence of hip fractures among elderly patients with Parkinson's disease is high. Recent studies have found that levodopa induces hyperhomocysteinemia in Parkinson's disease. Hyperhomocysteinemia is considered to be a risk factor for osteoporotic fractures in elderly men and women. Very high plasma homocysteine levels are a feature of homocystinuria, characterized by the early onset of osteoporosis. To determine the association between plasma homocysteine concentration and the risk of hip fracture in Parkinson's disease patients receiving levodopa, we prospectively studied a cohort of elderly women with Parkinson's disease. **METHODS:** We studied 199 elderly women with Parkinson's disease receiving levodopa therapy, from whom blood samples had been obtained to measure plasma homocysteine. Age-adjusted incidence rates of hip fractures were calculated for quartiles of plasma homocysteine concentrations. Cox proportional-hazard regression was used to calculate hazard ratios for quartiles of homocysteine values. **RESULTS:** The mean duration of follow-up was 4.9 years. Hip fractures occurred in 66 patients. The age-adjusted incidence rates per 1,000 person-years for hip fractures, from the lowest to the highest quartile of plasma homocysteine levels, were 1.59 (95% confidence interval [CI], 1.01-2.24), 1.57 (95% CI, 0.98-2.19), 1.21 (95% CI, 0.61-1.72), and 26.98 (95% CI, 16.48-37.24). The risk of hip fractures was greater in the highest quartile than that in the lowest, and the risk was almost 2.4 times higher. **CONCLUSION:** These findings suggest that the homocysteine concentration is an important risk factor for hip fractures in Parkinson's disease patients receiving levodopa.

Am J Med. 2005 Nov;118(11):1250-5

HOMOCYSTEINE AS A PREDICTIVE FACTOR FOR HIP FRACTURE IN STROKE PATIENTS.

Risk of hip fractures in stroke patients is higher than that in a reference population. Hyperhomocysteinemia is regarded as a risk

factor for ischemic stroke. The high prevalence of osteoporosis among patients with homocystinuria suggests that hyperhomocysteine may also increase the risk of fractures. To determine the association between homocysteine concentration and the risk of hip fractures, we studied a cohort of stroke patients with hemiplegia. Age-adjusted incidence rates of a hip fracture were calculated for quartiles of homocysteine concentrations. Cox proportional-hazard regression was used to calculate hazard ratios for quartiles of homocysteine levels. The initial enrolment of 433 hemiplegic patients with ischemic stroke, older than 65 years old, were followed for up to 10 years. The mean plasma homocysteine concentration at the enrolment was 14.1 +/- 5.2 micromol/L. There were 33 hip fractures among men and 46 among women during the mean follow-up period of 9.0 years. The age-adjusted incidence rates per 1,000 person-years for hip fractures increased almost linearly from 2.89 in the lowest to 27.87 in the highest quartiles of homocysteine levels. We conclude that hyperhomocysteinemia is one of the risk factors for hip fractures in stroke patients.

Bone. 2005 Apr;36(4):721-6

Sun protection

**DETECTION OF REACTIVE OXYGEN SPECIES IN THE SKIN OF LIVE MICE AND RATS EXPOSED TO UVA LIGHT:
 A RESEARCH REVIEW ON CHEMILUMINESCENCE AND TRIALS FOR UVA PROTECTION.**

The harmful effects of ultraviolet (UV) exposure on the skin are associated with the generation of reactive oxygen species (ROS) such as superoxide anion radical ($O(2)^{\cdot-}$), hydrogen peroxide ($H(2)O(2)$), hydroxyl radical ($\cdot OH$), and singlet oxygen ($(1)O(2)$) as well as with lipid peroxides and their radicals (LOOH and LOO \cdot). To give direct proof that such ROS are generated in UV-exposed skin, we proposed the in vivo detection and imaging method in which both a sensitive and specific chemiluminescence (CL) probe, such as CLA, and an ultralow-light imaging apparatus with a CCD camera were used. With this method we found that $O(2)^{\cdot-}$ is formed intrinsically and that $(1)O(2)$ and $O(2)^{\cdot-}$ are generated in the UVA-exposed skin of mice. In addition, we indicated that antioxidative ability against ROS in the skin of hairless rats decreased as age increased. Using these findings, we demonstrated the protective abilities of sodium ascorbate, caffeic acid, essential aroma oils, and zinc(ii) ion and its complexes, which we administered to mice both topically and orally. We present a review for the current state of our research proposing the sensitive CL method as a useful in vivo tool in photobiological research for the detection of oxidative stress as well as for the evaluation of antioxidative agents to the skin.

Photochem Photobiol Sci. 2005 Sep;4(9):715-20

ULTRAVIOLET RADIATION EXPOSURE AND RISK OF MALIGNANT LYMPHOMAS.

BACKGROUND: The incidence of malignant lymphomas has been increasing rapidly, but the causes of these malignancies remain poorly understood. One hypothesis holds that exposure to ultraviolet (UV) radiation increases lymphoma risk. We tested this hypothesis in a population-based case-control study in Denmark and Sweden. **METHODS:** A total of 3,740 patients diagnosed between October 1, 1999, and August 30, 2002, with incident malignant lymphomas, including non-Hodgkin lymphoma, chronic lymphocytic leukemia, and Hodgkin lymphoma, and 3,187 population controls provided detailed information on history of UV exposure and skin cancer and information on other possible risk factors for lymphomas. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated by logistic regression. Statistical tests were two-sided. **RESULTS:** Multivariable-adjusted analyses revealed consistent, statistically significant negative associations between various measures of UV light exposure and risk of non-Hodgkin lymphoma. A high frequency of sun bathing and sunburns at age 20 years and 5-10 years before the interview and sun vacations abroad were associated with 30%-40% reduced risks of non-Hodgkin lymphoma (e.g., for sunbathing four times a week or more at age 20 versus never sunbathing, OR = 0.7, 95% CI = 0.6 to 0.9; for two or more sunburns a year at age 20 versus no sunburns, OR = 0.6, 95% CI = 0.5 to 0.8). These inverse associations increased in strength with increasing levels of exposure (all $P(\text{trend}) < 0.01$). Similar, albeit weaker, associations were observed for Hodgkin lymphoma. There were no clear differences among non-Hodgkin lymphoma subtypes, although associations were stronger for B-cell than for T-cell lymphomas. A history of skin cancer was associated with a doubling in risks of both non-Hodgkin and Hodgkin lymphoma. **CONCLUSIONS:** A history of high UV exposure was associated with reduced risk of non-Hodgkin lymphoma. The positive association between skin cancer and malignant lymphomas is, therefore, unlikely to be mediated by UV exposure.

J Natl Cancer Inst. 2005 Feb 2;97(3):199-209

SUN EXPOSURE AND MORTALITY FROM MELANOMA.

BACKGROUND: Melanoma incidence and survival are positively associated, both geographically and temporally. Solar elastosis, a histologic indicator of cutaneous sun damage, has also been positively associated with melanoma survival. Although these observations raise the possibility that sun exposure increases melanoma survival, they could be explained by an association between incidence and early detection of melanoma. We therefore evaluated the association between measures of skin screening and death from cutaneous melanoma. **METHODS:** Case subjects ($n = 528$) from a population-based study of cutaneous melanoma were followed for an average of more than 5 years. Data, including measures of intermittent sun exposure, perceived awareness of the skin, skin self-screening, and physician screening, were collected during in-person interviews and review of histopathology and histologic parameters (i.e., solar elastosis, Breslow thickness, and mitoses) for all of the lesions. Competing risk models were used to compute risk of death (hazard ratios [HRs], with 95% confidence intervals [CIs]) from melanoma. All statistical tests were two-sided. **RESULTS:** Sunburn, high intermittent sun exposure, skin awareness histories, and solar elastosis were statistically significantly inversely associated with death from melanoma. Melanoma thickness, mitoses, ulceration, and anatomic location on the head and neck were statistically significantly positively associated with

melanoma death. In a multivariable competing risk analysis, skin awareness (with versus without, HR = 0.5, 95% CI = 0.3 to 0.9, P = .022) and solar elastosis (present versus absent, HR = 0.4, 95% CI = 0.2 to 0.8, P = .009) were strongly and independently associated with melanoma death after adjusting for Breslow thickness, mitotic index, and head and neck location, which were also independently associated with death. CONCLUSIONS: Sun exposure is associated with increased survival from melanoma.

J Natl Cancer Inst. 2005 Feb 2;97(3):195-9

THE GREEN TEA POLYPHENOL (-)-EPIGALLOCATECHIN GALLATE AND GREEN TEA CAN PROTECT HUMAN CELLULAR DNA FROM ULTRAVIOLET AND VISIBLE RADIATION-INDUCED DAMAGE.

BACKGROUND: Antioxidant compounds in green tea may be able to protect against skin carcinogenesis and it is of interest to investigate the mechanisms involved. A study was therefore conducted to determine whether the isolated green tea polyphenol (-)-epigallocatechin gallate (EGCG) could prevent ultraviolet radiation (UVR)-induced DNA damage in cultured human cells. This work was then extended to investigate whether drinking green tea could afford any UVR protection to human peripheral blood cells collected after tea ingestion. **METHODS:** The alkaline comet assay was used to compare the DNA damage induced by UVR in cultured human cells with and without the presence of EGCG. The same assay technique was then employed to assess UVR-induced DNA damage in peripheral leucocytes isolated from 10 adult human volunteers before and after drinking 540 ml of green tea. **RESULTS:** Initial trials found that EGCG afforded concentration-dependent photoprotection to cultured human cells with a maximal activity at a culture concentration of 250 microM. The cells types tested (lung fibroblasts, skin fibroblasts and epidermal keratinocytes) demonstrated varying susceptibility to the UVR insult provided. The in vivo trials of green tea also demonstrated a photoprotective effect, with samples of peripheral blood cells taken after green tea consumption showing lower levels of DNA damage than those taken prior to ingestion when exposed to 12 min ultraviolet A (UVA) radiation. **CONCLUSION:** The studies showed that green tea and/or some constituents can offer some protection against UV-induced DNA damage in human cell cultures and also in human peripheral blood samples taken post-tea ingestion.

Photodermatol Photoimmunol Photomed. 2005 Feb;21(1):15-22

GREEN TEA POLYPHENOLS PREVENT ULTRAVIOLET LIGHT-INDUCED OXIDATIVE DAMAGE AND MATRIX METALLOPROTEINASES EXPRESSION IN MOUSE SKIN.

Chronic exposure of solar ultraviolet (UV) light to human skin results in photoaging. UV-induced oxidative damage and induction of matrix metalloproteinases (MMP) have been implicated in this process. Because polyphenols from green tea (GTP) prevent other cutaneous adverse effects of UV radiation we hypothesized that UV irradiation-induced oxidative damage and induction of MMP might be prevented in vivo in mouse skin by oral administration of GTP. GTP was administered in drinking water (0.2%, wt/vol) to SKH-1 hairless mice, which were then exposed to multiple doses of UVB (90 mJ per cm², for 2 mo on alternate days) following in vivo photoaging animal protocol. Treatment of GTP resulted in inhibition of UVB-induced protein oxidation in vivo in mouse skin, a hallmark of photoaging, when analyzed biochemically, by immunoblotting, and immunohistochemistry. GTP treatment also inhibited UVB-induced protein oxidation in vitro in human skin fibroblast HS68 cells, which supports in vivo observations. Moreover, oral administration of GTP also resulted in inhibition of UVB-induced expression of matrix degrading MMP, such as MMP-2 (67%), MMP-3 (63%), MMP-7 (62%), and MMP-9 (60%) in hairless mouse skin. These data suggest that GTP as a dietary supplement could be useful to attenuate solar UVB light-induced premature skin aging.

J Invest Dermatol. 2004 Jun;122(6):1480-7

URSOLIC ACID INDUCES APOPTOSIS THROUGH MITOCHONDRIAL INTRINSIC PATHWAY AND CASPASE-3 ACTIVATION IN M4BEU MELANOMA CELLS.

Over the coming years, skin cancer could become a significant public health problem. Previous results indicate that ursolic acid (UA), a pentacyclic triterpene acid, has pleiotropic biologic activities such as antiinflammatory and antiproliferative activities on cancer cells. As UA represents a promising chemical entity for the protection of human skin, in agreement with tests done by the cosmetic industry, we investigated its effects on the M4Beu human melanoma cell line. In this report, we demonstrated for the first time that UA had a significant antiproliferative effect on M4Beu, associated with the induction of an apoptotic process, characterized by caspase-3 activation, the downstream central effector of apoptosis. We demonstrated that UA-induced apoptosis was dependent on the mitochondrial intrinsic pathway, as shown by transmembrane potential collapse (DeltaPsi_m) and by alteration of the Bax-Bcl-2 balance, with a concomitant increase in Bax expression and decrease in Bcl-2 expression. We also showed that UA-induced DeltaPsi_m was associated with apoptosis-inducing factor leakage from mitochondria. Taken together, our results suggest that UA-induced apoptosis on M4Beu cells is accomplished via triggering of mitochondrial pathway. In conclusion, UA could be an encouraging compound in the treatment or prevention of skin cancer and may represent a new promising anticancer agent in the treatment of melanoma.

Int J Cancer. 2005 Mar 10;114(1):1-11

Eukaryotic cellular machineries including the genome face continuous challenge from environmental deleterious agents, as well as from the by products of their own metabolism. Our skin is the most important barrier. It protects us from xenobiotic and genotoxic agents including ultraviolet (UV) solar radiation and potential carcinogens, which are notorious for causing skin cancer. There is a rise in non-melanoma skin cancer (NMSC), which is diagnosed in more than a million people every year in the United States alone, and is also prevalent in the other Western countries. In addition to sunscreens, chemoprevention of skin cancer by natural non-toxic compounds is suggested as an effective strategy to prevent the incidence of skin cancer. Our extensive animal studies on silibinin, a non-toxic bioactive component in milk thistle, suggest that it has a strong potential to prevent skin cancer incidence, promotion and progression in response to chemical carcinogens and tumour promoters as well as UV radiation. Our data suggest that silibinin has multiple targets in the cell, and can be protective against the harmful effects of cytotoxic agents such as reactive oxygen species and inflammation. Further, silibinin modulates mitogenic and survival signalling, p53, Cip1/p21 and other cell cycle regulatory molecules to prevent UVB-induced skin carcinogenesis. Our ongoing studies also suggest the positive effect of silibinin on the repair of UVB-induced DNA damage in mouse skin. Overall, the protective efficacy of silibinin against skin cancer is supported by sound mechanistic rationale in animal and cell culture studies, and suggests its potential use for humans.

Eur J Cancer. 2005 Sep;41(13):1969-79

A HISTORY OF THE THERAPEUTIC USE OF LIQUORICE IN EUROPE.

Liquorice root has been used in Europe since prehistoric times, and is well documented in written form starting with the ancient Greeks. In this review we compare the independent development of medical uses of this botanical drug in several ancient cultures, attempting to show the rationality of specific indications across different ethnic groups with different cultural backgrounds. Identical specific indications in different cultures highlight universally reproducible therapeutic effects that are beyond those of a mere placebo. In the first part of the review, historical sources dealing with liquorice (Scythian, Greek, Roman, and from the Middle Ages in Germany, Italy, Spain, England) have been considered. In the second part, the historical records of diseases treated with liquorice have been presented. Finally, a comparison between traditional use in and outside Europe, with the most important recent scientific studies concerning its use, is presented.

J Ethnopharmacol. 2005 Jul 14;99(3):317-24

EFFECTS OF GLYCYRRHIZIN ON UVB-IRRADIATED MELANOMA CELLS.

It is known that liquorice root is rich in compounds which exert several pharmacological actions. In the present study, we evaluated the effect of glycyrrhizin (the main constituent of liquorice root) and of its metabolite aglycone, 18beta-glycyrrhetic acid, on UVB-irradiated human melanoma cells: SKMEL-2 from metastatic tissue and SKMEL-28 from primary malignant melanoma. Tests performed (Trypan blue exclusion test, MTT and Western blot) showed that glycyrrhizin is not toxic for both types of cells. In SKMEL-28 cells, Bcl-2 expression was low after UVB irradiation, but it was increased when treated with glycyrrhizin. On the contrary, in the SKMEL-2 cell culture, Bcl-2 expression was not modified by the substances under study. The results show that glycyrrhizin treatment might offer protection from the damage induced in humans by UVB radiation, while it seems to be ineffective on metastatic cells. Further studies must be performed to understand the mechanism of the protective effect.

In Vivo. 2005 Jan-Feb;19(1):319-22

COMPARISON OF ANTIOXIDANT ACTIVITY OF EXTRACT FROM ROOTS OF LICORICE (GLYCYRRHIZA GLABRA L.) TO COMMERCIAL ANTIOXIDANTS IN 2% HYDROQUINONE CREAM.

Powdered dry roots of licorice (*Glycyrrhiza glabra* L.) were extracted with methanol. Licorice extract was tested for antioxidative activity in comparison with antioxidants (sodium metabisulfite and BHT) at 0.1%, 0.5%, 1.0%, and 2.0% w/w in 2% w/w hydroquinone cream. The systems were incubated in a dark room at 25 degrees +/- 0.5 degrees C and 45 degrees +/- 0.5 degrees C for three months. The physical stability and the percentages of hydroquinone remaining after two weeks and one, two, and three months were determined by UV spectrophotometer at 294 nm according to official standard procedures. The experiment revealed that oxidation degradation of hydroquinone was accelerated by heat even with the existence of antioxidants. The higher percentages of remaining hydroquinone were observed for higher antioxidant concentration but showed lower physical stability in the formulation in the presence of commercial antioxidants, especially in the cases of 1.0% and 2.0% BHT. In the third month, at 25 degrees +/- 0.5 degrees C and 45 degrees +/- 0.5 degrees C, the extract demonstrated more antioxidant activity from two other commercial antioxidants at all concentrations, with about 43-53% and 34-46%, respectively, more hydroquinone remaining than in the control system (p<0.001). In the third month, the preparation containing 0.1%, 0.5%, 1.0%, and 2.0% extract gave good physical formulation stability with about 72%, 76%, 78%, and 81 % hydroquinone remaining at 25 degrees +/- 0.5 degrees C and 51%, 55%, 60%, and 63% hydroquinone remaining at 45 degrees +/- 0.5 degrees C, respectively.

This suggested the possibility of using a licorice extract at 0.5% and 1.0% as an effective natural antioxidant for substances that are oxidation-susceptible.

J Cosmet Sci. 2003 Nov-Dec;54(6):551-8

Superoxide dismutase (SOD)

EXTRACELLULAR SUPEROXIDE DISMUTASE (EC-SOD) BINDS TO TYPE I COLLAGEN AND PROTECTS AGAINST OXIDATIVE FRAGMENTATION.

The antioxidant enzyme extracellular superoxide dismutase (EC-SOD) is mainly found in the extracellular matrix of tissues. EC-SOD participates in the detoxification of reactive oxygen species by catalyzing the dismutation of superoxide radicals. The tissue distribution of the enzyme is particularly important because of the reactive nature of its substrate, and it is likely essential that EC-SOD is positioned at the site of superoxide production to prevent adventitious oxidation. EC-SOD contains a C-terminal heparin-binding region thought to be important for modulating its distribution in the extracellular matrix. This paper demonstrates that, in addition to binding heparin, EC-SOD specifically binds to type I collagen with a dissociation constant ($K(d)$) of 200 nm. The heparin-binding region was found to mediate the interaction with collagen. Notably, the bound EC-SOD significantly protects type I collagen from oxidative fragmentation. This expands the known repertoire of EC-SOD binding partners and may play an important physiological role in preventing oxidative fragmentation of collagen during oxidative stress.

J Biol Chem. 2004 Apr 2;279(14):13705-10

ADAPTIVE MECHANISMS TO OXIDATIVE STRESS DURING AGING.

Whether or not oxidative stress is the cause of the aging process, as proposed by the oxidative stress theory of aging remains unknown; but accumulated evidence overwhelmingly identifies increased oxidative stress with age as a source of damage to cellular structure and function. From an evolutionary perspective, the utilization of oxygen as a life supporting means makes oxidative stress an inescapable part of an organism's biological system. The inseparability of oxidative stress from the biological system can be viewed as an adaptive response that all aerobic organisms undergo to ward-off the potentially harmful effects of oxygen and its derivatives, including free radicals. The organism's adaptive mechanisms include an intricate network of defenses that regulate and guard against any over-acting oxidative reactions to ensure its survival. This review discusses and illustrates several adaptive responses at various levels (from gene regulation to physical exercise) that organisms use as part of their survival strategy.

Mech Ageing Dev. 2006 Feb 21

CELLS DISCOVER FIRE: EMPLOYING REACTIVE OXYGEN SPECIES IN DEVELOPMENT AND CONSEQUENCES FOR AGING.

The free radical theory of aging states that aging results from the accumulated damage caused by reactive oxygen species (ROS). Herein, we provide a critique of the theory that aims to point out the theory's weaknesses and put forward ideas for how future experiments must adjust to several emerging concepts. In the same way fire is dangerous and nonetheless humans learned how to use it, it now appears that cells evolved mechanisms to control and use ROS. The way ROS are used as signaling molecules in many crucial biological functions suggests ROS are not unwanted by-products of metabolism. We hypothesize that the connection between ROS and cellular processes like growth, proliferation, and apoptosis may explain why long-lived animals appear to have lower levels of ROS production: the longer development of long-lived animals may lead to lower steady state levels of ROS. With age, antioxidant systems become deregulated, just like so many other cellular components, and so oxidative damage occurs. Therefore, the production of ROS is not merely a cause of havoc but rather a complex and critical system whose disruption in disease and aging leads to oxidative damage. Potential roles of ROS in aging are discussed under this model.

Exp Gerontol. 2006 Jan;41(1):1-10

AGEING FREE RADICALS AND CELLULAR STRESS.

A number of theories have attempted to account for ageing processes in various species. Following the << rate of living >> theory of Pearl, Harman suggested fifty years ago that the accumulation of oxidants could explain the alteration of physical and cognitive functions with ageing. Oxygen metabolism leads to reactive species, including free radicals, which tend to oxidize surrounding molecules such as DNA, proteins and lipids. As a consequence various functions of cells and tissues can be altered, leading to DNA instability, protein denaturation and accumulation of lipid byproducts. Oxidative stress is an adaptive

process which is triggered upon oxidant accumulation and which comprises the induction of protective and survival functions. Experimental evidence suggests that the ageing organism is in a state of oxidative stress, which supports the free radical theory. A number of other theories have been proposed ; some of these are actually compatible with the free radical theory. Caloric restriction is among the best models to increase life span in many species. While the relationship between caloric restriction and corrected metabolic rate is controversial, the decrease in ROS production by mitochondria appears to be experimentally supported. The ROS and mitochondrial theories of ageing appear to be compatible. Genetic models of increased life span, particularly those affecting the Foxo pathway, are usually accompanied by an increased resistance to oxidative insult. The free radical theory is not consistent with programmed senescence theories involving the cell division dependent decrease in telomere length ; however, oxidants are known to alter telomere structure. An appealing view of the role of oxidative stress in ageing is the trade-off principle which states that a phenotypic trait can be evolutionarily conserved because of its positive effects on development, growth or fertility, and despite its negative effect on somatic functions and ageing. It is likely that most cellular stresses which comprise adaptive and toxic functions follow such a rule.

Med Sci (Paris). 2006 Mar;22(3):266-72

ALZHEIMER'S DISEASE, OXIDATIVE INJURY, AND CYTOKINES.

Alzheimer's disease is infrequently a genetically driven disease. Rather it is the product of free radical injury inflicted over decades after an initial insult to the central nervous system (CNS). The brain is uniquely sensitive to oxidative injury. A variety of insults to the CNS are now associated with Alzheimer's disease. These include hypertension, diabetes, and head trauma. These then cause a cytokine cascade and microlocalized inflammation in the CNS, that in time results in clinical Alzheimer's disease. By the ninth decade of life over half of the population manifests Alzheimer's disease. Prevention or reversal of this pathophysiology will lie in administration of effective antioxidant therapy with specific treatments when etiologies are known.

J Alzheimers Dis. 2004 Dec;6(6):651-7

REGULATION OF SUPEROXIDE-PRODUCING NADPH OXIDASES IN NONPHAGOCYtic CELLS.

The membrane-integrated protein gp91phox functions as the catalytic center of the superoxide-producing phagocyte NADPH oxidase. Recent studies have identified homologs of gp91phox in nonphagocytic cells, which constitute the NADPH oxidase (Nox) family. Activation of the Nox oxidases leads to production of reactive oxygen species (ROS), thereby participating in a variety of biological events, such as host defense, hormone biosynthesis, and signal transduction. The activity of the Nox enzymes is regulated by various proteins, including the small GTPase Rac; regulatory mechanisms differ dependent on the type of the Nox proteins. For example, an oxidase activator (p47phox or Noxo1) and an oxidase activator (p67phox or Noxa1) are absolutely required for superoxide production by gp91phox and Nox1, but not by Nox3. Rac, albeit probably dispensable to the Nox3 activity, plays an essential role in activation of gp91phox. Thus, functional reconstitution of Nox systems is crucial for the study of Nox regulation. Here we describe a basic method for the reconstitution of Nox systems by expression of oxidase proteins in transfectable cells.

Methods Enzymol. 2006;406:456-68

A PHYSIOLOGICALLY BASED MODEL FOR ETHANOL AND ACETALDEHYDE METABOLISM IN HUMAN BEINGS.

Pharmacokinetic models for ethanol metabolism have contributed to the understanding of ethanol clearance in human beings. However, these models fail to account for ethanol's toxic metabolite, acetaldehyde. Acetaldehyde accumulation leads to signs and symptoms, such as cardiac arrhythmias, nausea, anxiety, and facial flushing. Nevertheless, it is difficult to determine the levels of acetaldehyde in the blood or other tissues because of artifactual formation and other technical issues. Therefore, we have constructed a promising physiologically based pharmacokinetic (PBPK) model, which is an excellent match for existing ethanol and acetaldehyde concentration-time data. The model consists of five compartments that exchange material: stomach, gastrointestinal tract, liver, central fluid, and muscle. All compartments except the liver are modeled as stirred reactors. The liver is modeled as a tubular flow reactor. We derived average enzymatic rate laws for alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), determined kinetic parameters from the literature, and found best-fit parameters by minimizing the squared error between our profiles and the experimental data. The model's transient output correlates strongly with the experimentally observed results for healthy individuals and for those with reduced ALDH activity caused by a genetic deficiency of the primary acetaldehyde-metabolizing enzyme ALDH2. Furthermore, the model shows that the reverse reaction of acetaldehyde back into ethanol is essential and keeps acetaldehyde levels approximately 10-fold lower than if the reaction were irreversible.

Alcohol. 2005 Jan;35(1):3-12

REDUCTION OF DIABETES-INDUCED RENAL OXIDATIVE STRESS BY A CANTALOUPE MELON EXTRACT/GLIADIN BIOPOLYMERS, OXYKINE, IN MICE.

Oxidative stress is implicated as an important mechanism by which diabetes causes nephropathy. Oxykine is the cantaloupe melon extract rich in vegetal superoxide dismutase covered by polymeric films of wheat matrix gliadin. In this study, we examined whether chronic oral administration of oxykine could prevent the progression of diabetic nephropathy induced by oxidative stress using preclinical rodent model of type 2 diabetes. We used female db/db mice and their non-diabetic db/m littermates. The mice were divided into the following three groups: non-diabetic db/m; diabetic db/db, and diabetic db/db treated with oxykine. Blood glucose level, body weight, urinary albumin, and urinary 8-hydroxydeoxyguanosine (8-OHdG) were measured during the experiments. Histological and 8-OHdG immunohistochemical studies were performed on 12 weeks from the beginning of treatment. After 12 weeks of treatment, the levels of blood glucose and the body weight were not significantly different between the oxykine-treated group and the non-treated db/db group, however both groups kept significantly high levels rather than db/m mice. The relative mesangial area calculated by mesangial area/total glomerular area ratio was significantly ameliorated in the oxykine treated group compared with non-treated db/db group. The increases in urinary albumin and 8-OHdG at 12 weeks of treatment were significantly inhibited by chronic treatment with oxykine. The 8-OHdG immunoreactive cells in the glomeruli of non-treated db/db mice were more numerous than that of oxykine-treated db/db mice. In this study, treatment of oxykine ameliorated the progression and acceleration of diabetic nephropathy for rodent model of type 2 diabetes. These results indicated that the oxykine reduced the diabetes-induced oxidative stress and renal mesangial cell injury. In conclusion, oxykine might be a novel approach for the prevention of diabetes nephropathy.

Biofactors. 2005;23(2):85-95

INFLUENCE OF AN ORALLY EFFECTIVE SOD ON HYPERBARIC OXYGEN-RELATED CELL DAMAGE.

In a prospective, double-blind, randomised placebo-controlled study, we tested the hypothesis that a new formulation consisting of wheat gliadin chemically combined with a vegetal (thus orally effective) preparation of superoxide dismutase (SOD) allows to prevent hyperbaric oxygen (HBO)-induced oxidative cell stress. Twenty healthy volunteers were exposed to 100% oxygen breathing at 2.5 ATA for a total of 60 min. DNA strand breaks (tail moments) were determined using the alkaline version of the comet assay. Whole blood concentrations of reduced (GSH) and oxidised (GSSG) glutathione and F2-isoprostanes, SOD, glutathione peroxidase (GPx) and catalase (Cat) activities and red cell malondialdehyde (MDA) content were determined. After HBO exposure the tail moment ($p = 0.03$) and isoprostane levels ($p = 0.049$) were significantly lower in the group that received the vegetal formulation. Neither SOD and Cat nor GSH and GSSG were significantly affected by this preparation or HBO exposure. By contrast, blood GPx activity, which tended to be lower in the SOD-group already before the HBO exposure ($p = 0.076$), was significantly lower afterwards ($p = 0.045$). We conclude that an orally effective SOD-wheat gliadin mixture is able to protect against DNA damage, which coincided with reduced blood isoprostane levels, and may therefore be used as an antioxidant.

Free Radic Res. 2004 Sep;38(9):927-32

ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF A CUCUMIS MELO LC. EXTRACT RICH IN SUPEROXIDE DISMUTASE ACTIVITY.

The present study was conducted to evaluate in vitro and in vivo the antioxidant and anti-inflammatory properties of a cantaloupe melon (*Cucumis melo* LC., Cucurbitaceae) extract (CME) selected for its high superoxide dismutase activity. Peritoneal macrophages were pre-activated in vitro with 300 IU of interferon-gamma (IFN-gamma) and were then challenged in culture with IgG1/anti-IgG1 immune complexes (IgG1IC) in presence of various CME extracts. The subsequent production of free radicals (superoxide anion, nitric oxide, and peroxynitrite) and of pro-(TNF-alpha) and anti-(IL-10) inflammatory cytokines was evaluated. The CME inhibited in a dose-dependent manner the production of superoxide anion with a maximal effect at 100 microg/ml. This inhibitory effect of CME appeared to be closely linked to the SOD activity because it was dramatically decreased after heat inactivation of the SOD activity (HI-CME). In addition, the CME inhibited the production of peroxynitrite strengthening the antioxidant properties of this CME rich in SOD activity. The production of the pro- and anti-inflammatory cytokines, namely TNF-alpha and IL-10, being conditioned by the redox status of macrophages we also evaluated the effect of CME and HI-CME on the IgG1IC-induced cytokine production. When the SOD activity was present in the CME it promoted the IgG1IC-induced production of IL-10 instead of TNF-alpha. These data demonstrated that, in addition to its antioxidant properties, the anti-inflammatory properties of the CME extract were principally related to its capacity to induce the production of IL-10 by peritoneal macrophages. The particular properties of wheat gliadin (*Triticum vulgare*, Poaceae) for the oral delivery of functional proteins led us to test it in a new nutraceutical formula based on its combination with the CME thus monitoring the SOD activity release during the gastro-intestinal digestive process. In these experiments C57BL/6 mice were supplemented orally everyday during 28 days with: (1) the placebo, (2) the CME extract alone, (3) the gliadin, (4) the CME/gliadin combination, or (5) the HI-CME/gliadin combination (SOD inactivated). At the end of the supplementation period all the animals were injected intra-peritoneal (i.p.) with the pro-inflammatory cytokine IFN-gamma (300 IU) and peritoneal macrophages were harvested 24 h after to test their capacities to produce free radicals, TNF-alpha and IL-10 after triggering with IgG1IC. We demonstrated that animals supplemented during 28 days with the CME/gliadin combination were protected against the pro-inflammatory properties of IFN-gamma while the other products were inefficient. These data did not only indicate that the SOD activity is important for the antioxidant and anti-inflammatory properties of the CME extract, but also demonstrated that when the SOD activity is preserved during the digestive

process by its combination with wheat gliadin it is possible to elicit in vivo the pharmacological effects of this antioxidant enzyme.

*J Ethnopharmacol.*_2004 Sep;94(1):67-75

NEURITIC REGENERATION AND SYNAPTIC RECONSTRUCTION INDUCED BY WITHANOLIDE A.

We investigated whether withanolide A (WL-A), isolated from the Indian herbal drug Ashwagandha (root of *Withania somnifera*), could regenerate neurites and reconstruct synapses in severely damaged neurons. We also investigated the effect of WL-A on memory-deficient mice showing neuronal atrophy and synaptic loss in the brain. Axons, dendrites, presynapses, and postsynapses were visualized by immunostaining for phosphorylated neurofilament-H (NF-H), microtubule-associated protein 2 (MAP2), synaptophysin, and postsynaptic density-95 (PSD-95), respectively. Treatment with A beta(25-35) (10 microM) induced axonal and dendritic atrophy, and pre- and postsynaptic loss in cultured rat cortical neurons. Subsequent treatment with WL-A (1 microM) induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and postsynapses in the neurons. WL-A (10 micromol kg(-1) day(-1), for 13 days, p.o.) recovered A beta(25-35)-induced memory deficit in mice. At that time, the decline of axons, dendrites, and synapses in the cerebral cortex and hippocampus was almost recovered. WL-A is therefore an important candidate for the therapeutic treatment of neurodegenerative diseases, as it is able to reconstruct neuronal networks.

Br J Pharmacol. 2005 Apr;144(7):961-71

NEUROPROTECTIVE EFFECTS OF WITHANIA SOMNIFERA ON 6-HYDROXYDOPAMINE INDUCED PARKINSONISM IN RATS.

6-Hydroxydopamine (6-OHDA) is one of the most widely used rat models for Parkinson's disease. There is ample evidence in the literature that 6-OHDA elicits its toxic manifestations through oxidant stress. In the present study, we evaluated the anti-parkinsonian effects of *Withania somnifera* extract, which has been reported to have potent anti-oxidant, anti-peroxidative and free radical quenching properties in various diseased conditions. Rats were pretreated with 100, 200 and 300 mg/kg b.w. of the *W. somnifera* extract orally for 3 weeks. On day 21, 2 microL of 6-OHDA (10 microg in 0.1% in ascorbic acid-saline) was infused into the right striatum while sham operated group received 2 microL of the vehicle. Three weeks after 6-OHDA injections, rats were tested for neurobehavioral activity and were killed 5 weeks after lesioning for the estimation of lipidperoxidation, reduced glutathione content, activities of glutathione-S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase, catecholamine content, dopaminergic D2 receptor binding and tyrosine hydroxylase expression. *W. somnifera* extract was found to reverse all the parameters significantly in a dose-dependent manner. Thus, the study demonstrates that the extract of *W. somnifera* may be helpful in protecting the neuronal injury in Parkinson's disease.

Hum Exp Toxicol. 2005 Mar;24(3):137-47

CHOLINESTERASE INHIBITING WITHANOLIDES FROM WITHANIA SOMNIFERA.

A total of two new (1, 2) and four known (3-6) withanolides were isolated from the whole plant of *Withania somnifera*. Their structures were elucidated on the basis of spectroscopic techniques and were characterized as 6alpha,7alpha-epoxy-3beta,5alpha,20beta-trihydroxy-1-oxowitha-24-enolide (1), 5beta,6beta-epoxy-4beta,17alpha,27-trihydroxy-1-oxowitha-2,24-dienolide (2), withaferin-A (3), 2,3-dihydroxywithaferin-A (4), 6alpha,7alpha-epoxy-5alpha,20beta-dihydroxy-1-oxowitha-2,24-dienolide (5), and 5beta,6beta-epoxy-4beta-hydroxy-1-oxowitha-2,14,24-trienolide (6), respectively. Compounds 2, 3, 5, and 6 displayed inhibitory potential against butyrylcholinesterase, but only compounds 3, 4, and 6 were found to be active against acetylcholinesterase.

Chem Pharm Bull (Tokyo). 2004 Nov;52(11):1358-61

ANTICARCINOGENIC ACTIVITY OF WITHANIA SOMNIFERA DUNAL AGAINST DALTON'S ASCITIC LYMPHOMA.

The effect of ethanolic extract of the root of *Withania somnifera* Dunal (REWS) against Dalton's Ascitic Lymphoma has been evaluated in Swiss albino mice. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with REWS. The hematological parameters were also corrected by REWS in tumour-induced mice. These observations are suggestive of the protective effect of REWS in Dalton's Ascitic Lymphoma (DAL).

ANTIOXIDANT APPROACH TO DISEASE MANAGEMENT AND THE ROLE OF 'RASAYANA' HERBS OF AYURVEDA.

The disease preventive and health promotive approach of 'Ayurveda', which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic and finds increasing acceptability in many regions of the world. Ancient Ayurvedic physicians had developed certain dietary and therapeutic measures to arrest/delay ageing and rejuvenating whole functional dynamics of the body system. This revitalization and rejuvenation is known as the 'Rasayan chikitsa' (rejuvenation therapy). Traditionally, Rasayana drugs are used against a plethora of seemingly diverse disorders with no pathophysiological connections according to modern medicine. Though, this group of plants generally possesses strong antioxidant activity, only a few have been investigated in detail. Over about 100 disorders like rheumatoid arthritis, hemorrhagic shock, CVS disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis and AIDS have been reported as reactive oxygen species mediated. In this review, the role of free radicals in these diseases has been briefly reviewed. 'Rasayana' plants with potent antioxidant activity have been reviewed for their traditional uses, and mechanism of antioxidant action. Fifteen such plants have been dealt with in detail and some more plants with less work have also been reviewed briefly.

J Ethnopharmacol. 2005 Jun 3;99(2):165-78

IMPORTANCE OF HEMOGLOBIN CONCENTRATION TO EXERCISE: ACUTE MANIPULATIONS.

An acute reduction of blood hemoglobin concentration ([Hb]), even when the circulating blood volume is maintained, results in lower [Formula: see text] and endurance performance, due to the reduction of the oxygen carrying capacity of blood. Conversely, an increase of [Hb] is associated with enhanced [Formula: see text] and endurance capacity, that is also proportional to the increase in the oxygen carrying capacity of blood. The effects on endurance capacity appear more pronounced and prolonged than on [Formula: see text]. During submaximal exercise, there is a tight coupling between O₂ demand and O₂ delivery, such that if [Hb] is acutely decreased muscle blood flow is increased proportionally and vice versa. During maximal exercise with either a small or a large muscle mass, neither peak cardiac output nor peak leg blood flow are affected by reduced [Hb]. An acute increase of [Hb] has no effect on maximal exercise capacity or [Formula: see text] during exercise in acute hypoxia. Likewise, reducing [Hb] in altitude-acclimatized humans to pre-acclimatization values has no effect on [Formula: see text] during exercise in hypoxia.

Respir Physiol Neurobiol. 2006 Mar 2

ANTIBACTERIAL EFFICACY OF WITHANIA SOMNIFERA (ASHWAGANDHA) AN INDIGENOUS MEDICINAL PLANT AGAINST EXPERIMENTAL MURINE SALMONELLOSIS.

In the present study, we evaluated the antibacterial activity of ashwagandha [*Withania somnifera* L. Dunal (Solanaceae; root and leaves)], an Indian traditional medicinal plant against pathogenic bacteria. Both aqueous as well as alcoholic extracts of the plant (root as well as leaves) were found to possess strong antibacterial activity against a range of bacteria, as revealed by in vitro Agar Well Diffusion Method. The methanolic extract was further subfractionated using various solvents and the butanolic sub-fraction was found to possess maximum inhibitory activity against a spectrum of bacteria including *Salmonella typhimurium*. Moreover, in contrast to the synthetic antibiotic (viz. chloramphenicol), these extracts did not induce lysis on incubation with human erythrocytes, advocating their safety to the living cells. Finally, the antibacterial efficacy of the extracts isolated from plant (both root and leaves) was determined against experimental salmonellosis in Balb/C mice. Oral administration of the aqueous extracts successfully obliterated salmonella infection in Balb/C mice as revealed by increased survival rate as well as less bacterial load in various vital organs of the treated animals.

Phytomedicine. 2005 Mar;12(3):229-35

MECHANISMS OF CARDIOPROTECTIVE EFFECT OF WITHANIA SOMNIFERA IN EXPERIMENTALLY INDUCED MYOCARDIAL INFARCTION.

The present study was designed to evaluate the cardioprotective potential of hydro-alcoholic extract of *Withania somnifera* on the basis of haemodynamic, histopathological and biochemical parameters in the isoprenaline-(isoproterenol) induced myocardial necrosis in rats and to compare with Vitamin E, a known cardioprotective antioxidant. Wistar albino male rats (150-200 g) were divided into six main groups: sham, isoprenaline control, *Withania somnifera*/Vitamin E control and *Withania somnifera*/Vitamin E treatment groups. *Withania somnifera* was administered at doses 25, 50 and 100 mg/kg and Vitamin E at a dose of 100 mg/kg, orally for 4 weeks. On days 29 and 30, the rats in the isoprenaline control and *Withania somnifera*/Vitamin E treatment groups were given isoprenaline (85 mg/kg), subcutaneously at an interval of 24 hr. On day 31, haemodynamic parameters were recorded and the hearts were subsequently removed and processed for histopathological and biochemical studies. A significant decrease in glutathione ($P < 0.05$), activities of superoxide dismutase, catalase, creatinine phosphokinase and lactate dehydrogenase

($P < 0.01$) as well as increase in lipid peroxidation marker malonyldialdehyde level ($P < 0.01$) was observed in the hearts of isoproterenol control group rats as compared to sham control. However, we have not observed any significant changes in activity of glutathione peroxidase and protein levels. Left ventricular dysfunction was seen as a decrease in heart rate, left ventricular rate of peak positive and negative pressure change and elevated left ventricular end-diastolic pressure in the control group was recorded. On histopathological examination, myocardial damage was further confirmed. Our data show that *Withania somnifera* (25, 50, and 100 mg/kg) exerts a strong cardioprotective effect in the experimental model of isoprenaline-induced myonecrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardioprotective effect. Among the different doses studied, *Withania somnifera* at 50 mg/kg dose produced maximum cardioprotective effect.

Basic Clin Pharmacol Toxicol. 2004 Apr;94(4):184-90

GROWTH INHIBITION OF HUMAN TUMOR CELL LINES BY WITHANOLIDES FROM WITHANIA SOMNIFERA LEAVES.

Ayurvedic medicines prepared in India consist of *Withania somnifera* roots as one of the main ingredients. It is consumed as a dietary supplement around the world. The leaves of *W. somnifera* were used in the treatment of tumors and inflammation in several Asian countries. We have isolated twelve withanolides such as withaferin A (1), sitoindoside IX (2), 4-(1-hydroxy-2, 2-dimethylcyclopropanone)-2, 3-dihydrowithaferin A (3), 2, 3-dihydrowithaferin A (4), 24, 25-dihydro-27-desoxywithaferin A (5), physagulin D (1 \rightarrow 6)-beta-D-glucopyranosyl- (1 \rightarrow 4)-beta-D-glucopyranoside (6), 27-O-beta-D-glucopyranosylphysagulin D (7), physagulin D (8), withanoside IV (9), and 27-O-beta-D-glucopyranosylviscosalactone B (10), 4, 16-dihydroxy-5beta, 6beta-epoxyphysagulin D (11), viscosalactone B (12) from the leaves of this species. Compounds 1-12 and diacetylwithaferin A (13) were tested for their antiproliferative activity on NCI-H460 (Lung), HCT-116 (Colon), SF-268 (Central Nervous System; CNS) and MCF-7 (Breast) human tumor cell lines. The inhibitory concentration to afford 50% cell viability (IC₅₀) for these compounds was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Withaferin A and its derivatives exhibited inhibitory concentrations (50%) ranging from 0.24 +/- 0.01 to 11.6 +/- 1.9 microg/mL. Viscosalactone B (12) showed the 50% inhibition at concentrations ranging from 0.32 +/- 0.05 to 0.47 +/- 0.15 microg/mL whereas its 27-O-glucoside derivative (10) exhibited IC₅₀ between 7.9 +/- 2.9 and 17.3 +/- 3.9 microg/ml. However, Physagulin D type withanolides showed either weak or no activity at 30 microg/mL. Therefore, incorporation of withanolides in the diet may prevent or decrease the growth of tumors in human.

Life Sci. 2003 Nov 21;74(1):125-32

EVALUATION OF THE EFFECT OF WITHANIA SOMNIFERA ROOT EXTRACTS ON CELL CYCLE AND ANGIOGENESIS.

In the Indian System of Medicine, the medicinal plant, *Withania somnifera* Dunal (Solanaceae) finds application for numerous ailments including cancer. This study explores the mechanism(s) underlying this property. The hydroalcoholic extract of the roots (WS) was partitioned between chloroform (WS-chloroform) and water (WS-water). Further, WS-chloroform was fractionated (A1-A12) by reverse-phase column chromatography and their withanolide content was quantified by high-performance liquid chromatography (HPLC). Preliminarily, the anti-proliferative activity of all the extracts and fractions was screened against human laryngeal carcinoma (Hep2) cells by microculture tetrazolium assay (MTT). Two extracts (WS and WS-chloroform) and three fractions (A4, A5 and A6) negatively affected Hep2 viability at the concentration of 25 mug/ml and these were further investigated pharmacologically. Flow cytometry revealed cell cycle block and accumulation of hypoploid (sub G1) cells as the mode of anti-proliferative activity of all but A4. Their anti-angiogenic potential was investigated by a chickchorio-allantoic membrane (CAM) wherein a significant inhibition ($p < 0.0001$) of vascular endothelium growth factor (VEGF), induced neovascularization was recorded. The effect was confirmed in vivo by mouse sponge implantation method. These findings suggest that the roots of *Withania somnifera* possess cell cycle disruption and anti-angiogenic activity, which may be a critical mediator for its anti-cancer action.

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