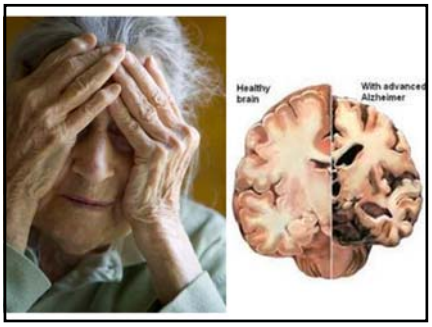
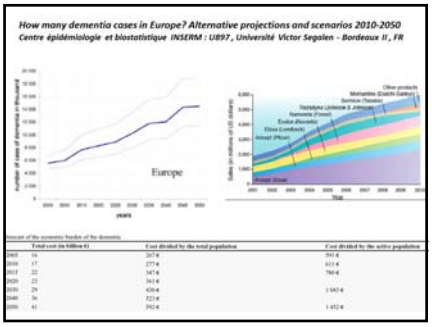
Università degli Studi del Molise

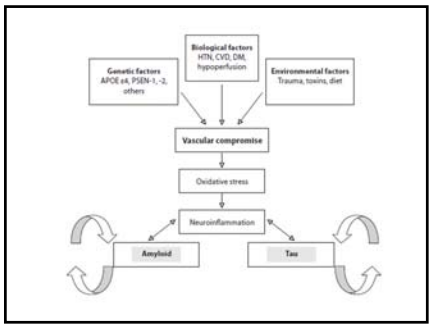
Consiglio Nazionale delle Ricerche
Istituto di Scienze Neurologiche

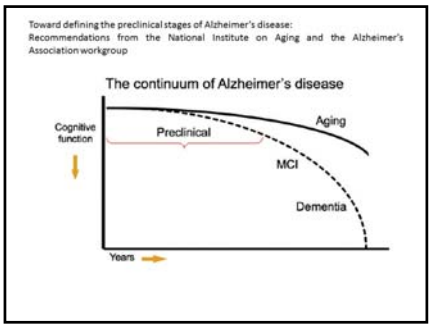
Food for the brain,
nutritional strategies against
brain ageing and cognitive decline

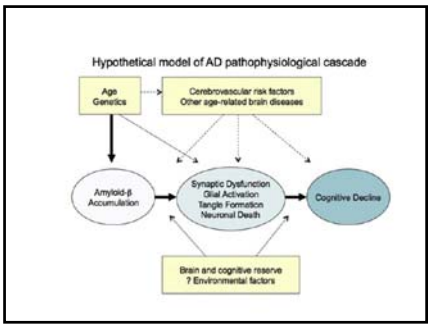
Giovanni Scapagnini, MD, PhD

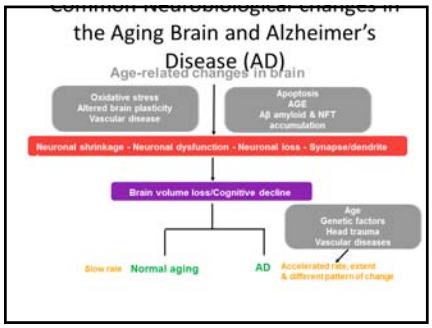


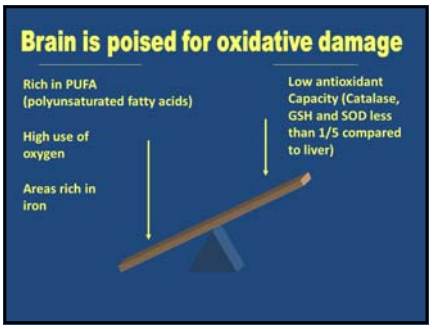


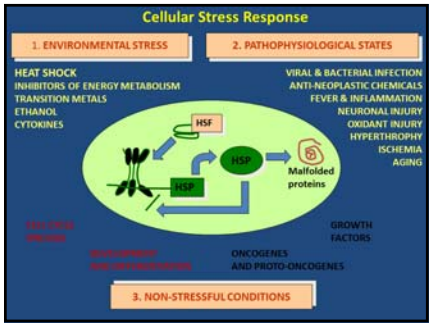


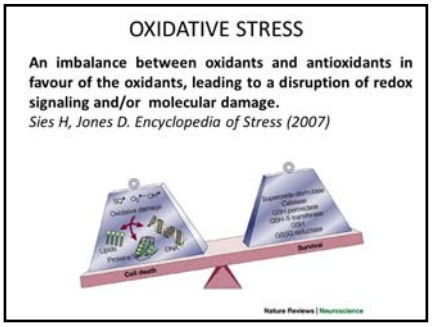


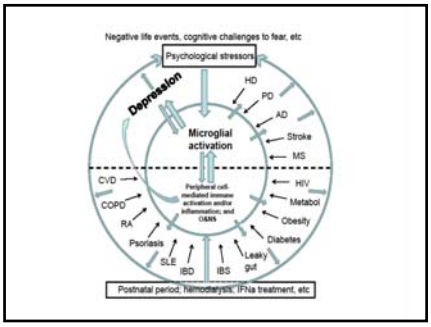










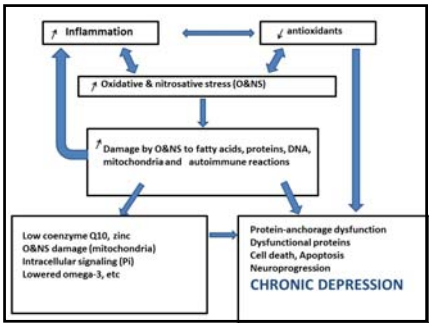


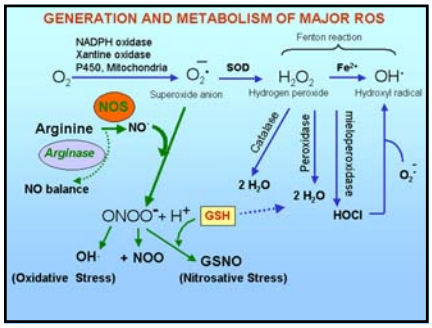
Oxidative and nitrosative pathways in depression

- 1. Lowered antioxidants in MDD
- 2. Increased damage to proteins, fatty acids and DNA in MDD
- 3. Autoimmune responses against self-epitopes that govern intracellular signaling in MDD

Connsequences of lowered antioxidant levels

1. more free radicals, O&NS and damage by O&NS
2. less anti-inflammatory capacity (NFKB, COX-2, cytokines)
3. less neurotrophic & neuroprotective capacity ---→ increased neuroprogression
4. depression and fatigue: zinc and CoQ10





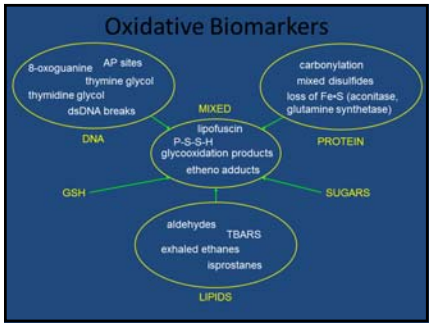
An antioxidant is any substance that is present in low concentrations compared to those of an oxidizable substrate and significantly delays or prevents oxidation of that substance.
Halliwell B, Gutteridge JMC (2007)

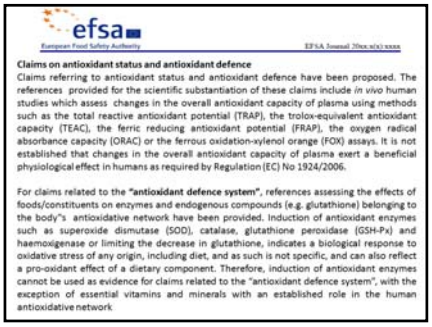
There are no universal antioxidants able to efficiently quench any type of reactive oxygen species

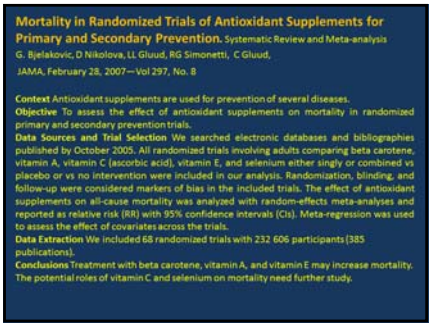
Determination of antioxidant activity/capacity

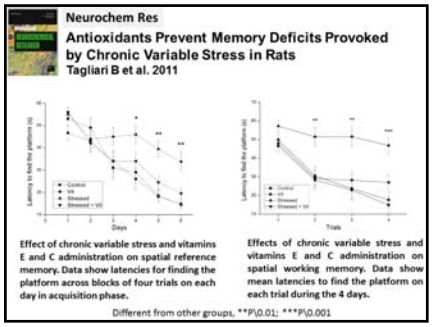
Models based on HAT mechanisms
- Oxygen Radical Absorbance Capacity Assay (ORAC)
- Total Radical-Trapping Antioxidant Parameter Assay (TRAP)
- Crocin Bleacching Assay

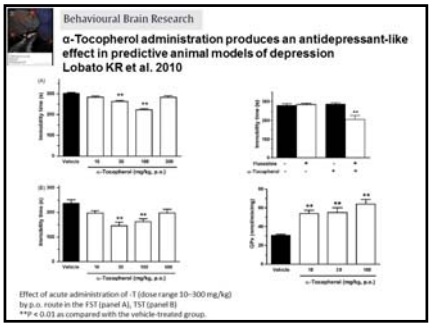
Models based on SET mechanisms
- Trolox Equivalent Antioxidant Capacity Assay (TEAC)
- 2,2-Diphenyl-1-Picryhydrazyl Radical Assay (DPPH)
- Ferric Reducing Antioxidant Power Assay (FRAP)

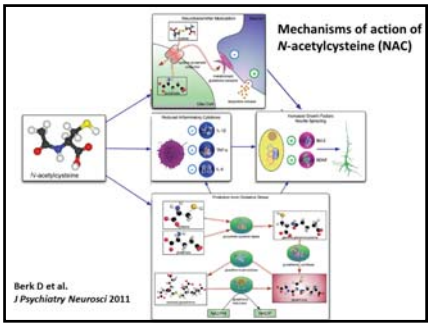

Oxidative Biomarkers
8-oxoguanine
AP sites
thymine glycol
thymidine glycol
dsDNA breaks
DNA
carbonylation
mixed disulfides
loss of Fe-S (aconitase, glutamine synthetase)
PROTEIN
MIXED
lipofuscin
P-S-S-H
glycooxidation products
etheno adducts
GSH
SUGARS
aldehydes
TBARS
exhaled ethanes
isprostanes
LIPIDS

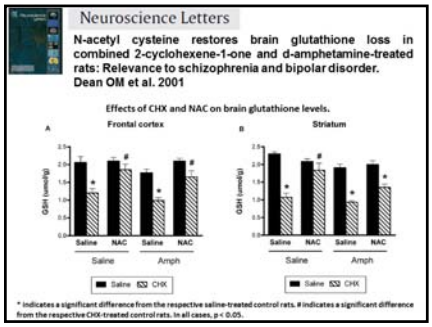

efsa
European Food Safety Authority
EFSA Journal 20xx;x(x):xxxx
Claims on antioxidant status and antioxidant defence
Claims referring to antioxidant status and antioxidant defence have been proposed. The references provided for the scientific substantiation of these claims include *in vivo* human studies which assess changes in the overall antioxidant capacity of plasma using methods such as the total reactive antioxidant potential (TRAP), the trolox-equivalent antioxidant capacity (TEAC), the ferric reducing antioxidant potential (FRAP), the oxygen radical absorbance capacity (ORAC) or the ferrous oxidation-xylenol orange (FOX) assays. It is not established that changes in the overall antioxidant capacity of plasma exert a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.
For claims related to the **"antioxidant defence system"**, references assessing the effects of foods/constituents on enzymes and endogenous compounds (e.g. glutathione) belonging to the body"s antioxidative network have been provided. Induction of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and haemoxigenase or limiting the decrease in glutathione, indicates a biological response to oxidative stress of any origin, including diet, and as such is not specific, and can also reflect a pro-oxidant effect of a dietary component. Therefore, induction of antioxidant enzymes cannot be used as evidence for claims related to the "antioxidant defence system", with the exception of essential vitamins and minerals with an established role in the human antioxidative network

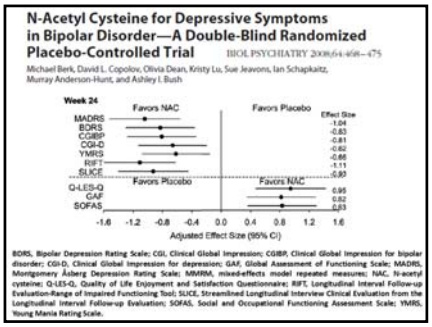

Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention. Systematic Review and Meta-analysis
G. Bjelakovic, D Nikolova, LL Gluud, RG Simonetti, C Gluud,
JAMA, February 28, 2007—Vol 297, No. 8
Context Antioxidant supplements are used for prevention of several diseases.
Objective To assess the effect of antioxidant supplements on mortality in randomized primary and secondary prevention trials.
Data Sources and Trial Selection We searched electronic databases and bibliographies published by October 2005. All randomized trials involving adults comparing beta carotene, vitamin A, vitamin C (ascorbic acid), vitamin E, and selenium either singly or combined vs placebo or vs no intervention were included in our analysis. Randomization, blinding, and follow-up were considered markers of bias in the included trials. The effect of antioxidant supplements on all-cause mortality was analyzed with random-effects meta-analyses and reported as relative risk (RR) with 95% confidence intervals (CIs). Meta-regression was used to assess the effect of covariates across the trials.
Data Extraction We included 68 randomized trials with 232 606 participants (385 publications).
Conclusions Treatment with beta carotene, vitamin A, and vitamin E may increase mortality. The potential roles of vitamin C and selenium on mortality need further study.

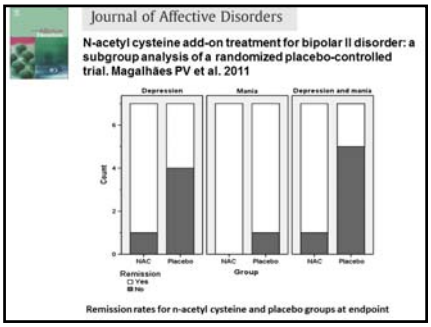

Neurochem Res
Antioxidants Prevent Memory Deficits Provoked by Chronic Variable Stress in Rats
Tagliari B et al. 2011
Effect of chronic variable stress and vitamins E and C administration on spatial reference memory. Data show latencies for finding the platform across blocks of four trials on each day in acquisition phase.
Effects of chronic variable stress and vitamins E and C administration on spatial working memory. Data show mean latencies to find the platform on each trial during the 4 days.
Different from other groups, **P\0.01; ***P\0.001


Behavioural Brain Research
α-Tocopherol administration produces an antidepressant-like effect in predictive animal models of depression
Lobato KR et al. 2010
Effect of acute administration of -T (dose range 10–300 mg/kg) by p.o. route in the FST (panel A), TST (panel B)
**P < 0.01 as compared with the vehicle-treated group.


Mechanisms of action of N-acetylcysteine (NAC)
N-acetylcysteine
Berk D et al.
J Psychiatry Neurosci 2011

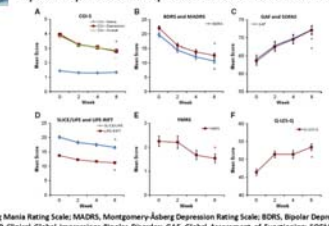

Neuroscience Letters
N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and d-amphetamine-treated rats: Relevance to schizophrenia and bipolar disorder.
Dean OM et al. 2001
Effects of CHX and NAC on brain glutathione levels.
Frontal cortex
Striatum
GSH (umol/g)
Saline
NAC
Amph
CHX
* Indicates a significant difference from the respective saline-treated control rats. # indicates a significant difference from the respective CHX-treated control rats. In all cases, p < 0.05.


N-Acetyl Cysteine for Depressive Symptoms in Bipolar Disorder—A Double-Blind Randomized Placebo-Controlled Trial
BIOL PSYCHIATRY 2008;64:468–475
Michael Berk, David L. Copolov, Olivia Dean, Kristy Lu, Sue Jeavons, Ian Schapkaitz, Murray Anderson-Hunt, and Ashley I. Bush
Week 24
Favors NAC
Favors Placebo
Effect Size
MADRS -1.04
BDRS -0.83
CGIBP -0.81
CGI-D -0.62
YMRS -0.66
RIFT -1.11
SLICE -0.80
Q-LES-Q 0.95
GAF 0.82
SOFAS 0.83
Adjusted Effect Size (95% CI)
BDRS, Bipolar Depression Rating Scale; CGI, Clinical Global Impression; CGIBP, Clinical Global Impression for bipolar disorder; CGI-D, Clinical Global Impression for depression; GAF, Global Assessment of Functioning Scale; MADRS, Montgomery Åsberg Depression Rating Scale; MMRM, mixed-effects model repeated measures; NAC, N-acetyl cysteine; Q-LES-Q, Quality of Life Enjoyment and Satisfaction Questionnaire; RIFT, Longitudinal Interval Follow-up Evaluation-Range of Impaired Functioning Tool; SLICE, Streamlined Longitudinal Interview Clinical Evaluation from the Longitudinal Interval Follow-up Evaluation; SOFAS, Social and Occupational Functioning Assessment Scale; YMRS, Young Mania Rating Scale.

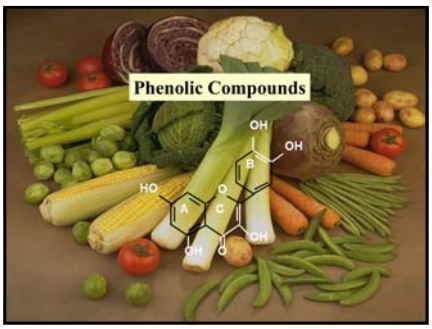

Journal of Affective Disorders
N-acetyl cysteine add-on treatment for bipolar II disorder: a subgroup analysis of a randomized placebo-controlled trial. Magalhães PV et al. 2011
Depression
Mania
Depression and mania
Count
NAC
Placebo
Remission
Yes
No
Group
Remission rates for n-acetyl cysteine and placebo groups at endpoint

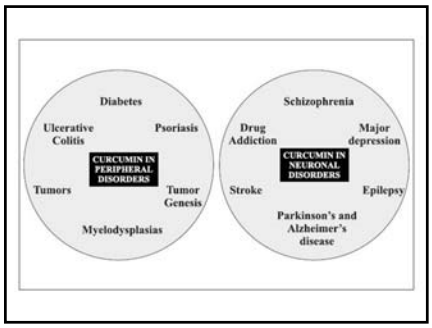
Journal of Affective Disorders

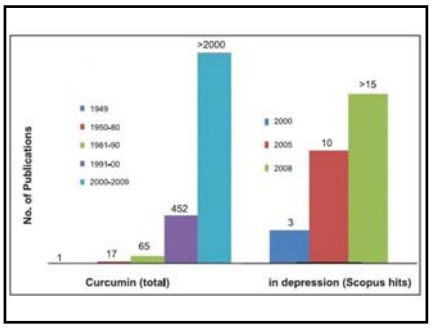
The efficacy of N-acetylcysteine as an adjunctive treatment in bipolar depression: An open label trial. Berk M et al. 2011



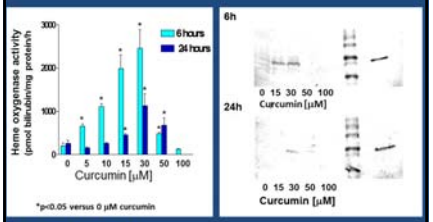
YMRS, Young Mania Rating Scale; MADRS, Montgomery-Åsberg Depression Rating Scale; BDRS, Bipolar Depression Rating Scale; CGI-BP Clinical Global Impressions-Bipolar Disorder; GAF, Global Assessment of Functioning; SOFAS, Social and Occupational Assessment Scale; LIFE-RIFT, Range of Impaired Functioning Tool; SLICE-LIFE, Streamed Longitudinal Interval Clinical Evaluation for the Longitudinal Interview Follow-Up Evaluation; Q-LES-Q, Quality of Life Enjoyment and Satisfaction Questionnaire



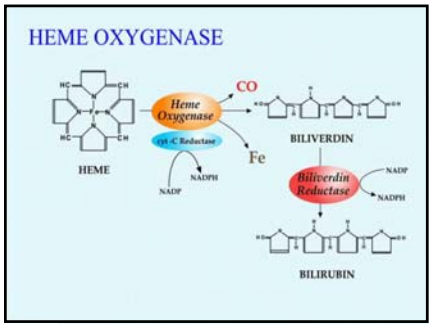




Effect of curcumin on heme oxygenase activity and HO-1 protein expression in astrocytes



Scapagnini G et al. Mol Pharmacol.



Bilirubin and biliverdin possess strong antioxidant activities

Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, and Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043-1046, 1987.

Clark JE, Foresti R, Green CJ, and Motterlini R. Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. *Biochem J* 348 Pt 3: 615-619, 2000.

Chen K, Gunter K, and Maines MD. Neurons overexpressing heme oxygenase-1 resist oxidative stress- mediated cell death. *J Neurochem* 75: 304-313, 2000.

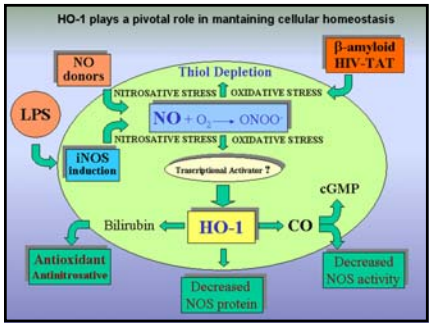
Carbon monoxide has vascular and anti-inflammatory effects. In the CNS it is a potential neurotransmitter.

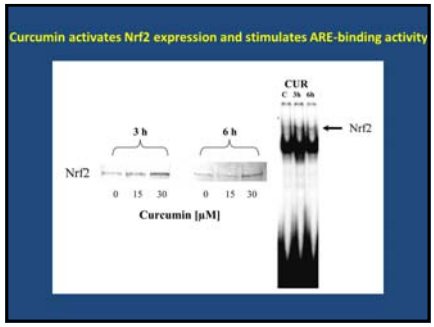
Kharitonov VG, Sharma VS, Pilz RB, Magde D, Koesling D. Basis of guanylate cyclase activation by carbon monoxide. *Proc. Natl. Acad. Sci. U.S.A.* 92: 2568-2571, 1995.

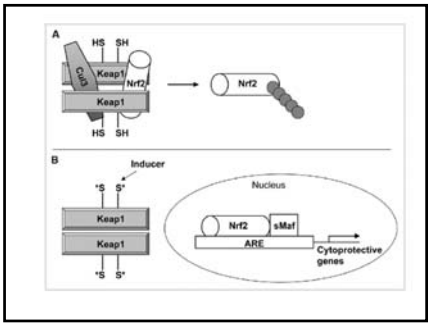
Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, Green CJ. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ. Res.* 90: 17-24, 2002.

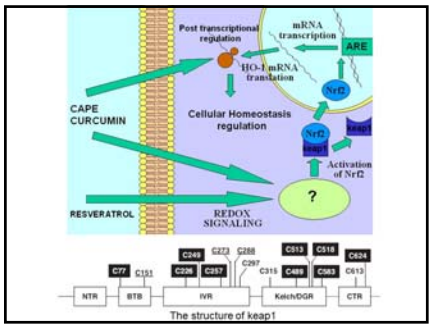
Otterbein LE. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat. Med.* 6: 422–428, 2000

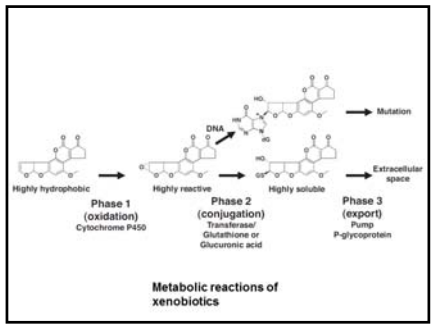
Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381-384, 1993

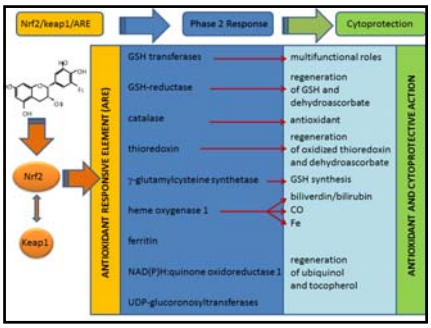

HO-1 plays a pivotal role in mantaining cellular homeostasis
NO donors
β-amyloid HIV-TAT
Thiol Depletion
NITROSATIVE STRESS
OXIDATIVE STRESS
LPS
NO + O2 → ONOO
iNOS induction
Trascriptional Activator ?
cGMP
Bilirubin
HO-1
CO
Antioxidant Antinitrosative
Decreased NOS activity
Decreased NOS protein

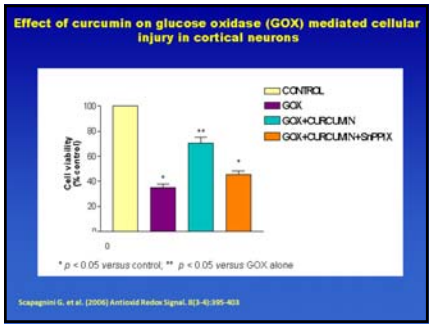

Curcumin activates Nrf2 expression and stimulates ARE-binding activity
CUR
C 3h 6h
Nrf2
3 h
6 h
Nrf2
0 15 30 0 15 30
Curcumin [μM]

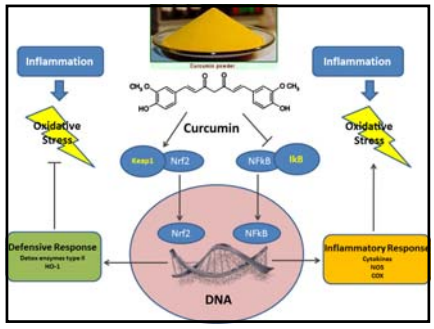

A
HS SH
Cul3
Keap1
Nrf2
Keap1
HS SH
Nrf2
B
Inducer
S S
Nucleus
Keap1
Keap1
Nrf2
sMaf
ARE
Cytoprotective genes
S S


Post transcriptional regulation
mRNA transcription
ARE
HO-1 mRNA translation
Nrf2
CAPE
CURCUMIN
Cellular Homeostasis regulation
Nrf2
keap1
Keap1
Activation of Nrf2
?
RESVERATROL
REDOX SIGNALING
C77 C151 C226 C257 C249 C273 C288 C297 C315 C489 C513 C518 C583 C624 C613
NTR BTB IVR Kelch/DGR CTR
The structure of keap1


Mutation
DNA
Highly hydrophobic
Highly reactive
Highly soluble
Extracellular space
Phase 1 (oxidation) Cytochrome P450
Phase 2 (conjugation) Transferase/ Glutathione or Glucuronic acid
Phase 3 (export) Pump P-glycoprotein
Metabolic reactions of xenobiotics


Nrf2/keap1/ARE
Phase 2 Response
Cytoprotection
ANTIOXIDANT RESPONSIVE ELEMENT (ARE)
GSH transferases
multifunctional roles
GSH-reductase
regeneration of GSH and dehydroascorbate
catalase
antioxidant
thioredoxin
regeneration of oxidized thioredoxin and dehydroascorbate
γ-glutamylcysteine synthetase
GSH synthesis
heme oxygenase 1
biliverdin/bilirubin
CO
Fe
ferritin
NAD(P)H:quinone oxidoreductase 1
regeneration of ubiquinol and tocopherol
UDP-glucoronosyltransferases
Nrf2
Keap1
ANTIOXIDANT AND CYTOPROTECTIVE ACTION


Effect of curcumin on glucose oxidase (GOX) mediated cellular injury in cortical neurons
CONTROL
GOX
GOX+CURCUMIN
GOX+CURCUMIN+SnPP IX
Cell viability (% control)
100
80
60
40
20
0
* p < 0.05 versus control, ** p < 0.05 versus GOX alone
Scapagnini G. et al. (2006) Antioxid Redox Signal. 8(3-4):395-403


Inflammation
Inflammation
Oxidative Stress
Curcumin
Oxidative Stress
Keap1
Nrf2
NFkB
IkB
Nrf2
NFkB
Defensive Response
Inflammatory Response
DNA


(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(19) World Intellectual Property Organization International Bureau
(43) International Publication Date 10 September 2004 (10.09.2004)
PCT
(10) International Publication Number WO 2004/075883 A1
CURCUMIN

Epidemiological studies showed that in India, where curcumin is widely used in daily diet, there is a reduced age-adjusted prevalence of AD (in patients between 70 and 79 years of age is 4.4 fold less than that of the United States), as well as a lower prevalence of Parkinson's disease.

Chandra V, Pandav R, Dodge HH, Johnston JM, Belle SH, DeKosky ST, and Ganguli M. **Incidence of Alzheimer's disease in a rural community in India: the Indo-US study.** *Neurology* 57: 985-989, 2001.

Muthane U, Yasha TC, and Shankar SK. **Low numbers and no loss of melanized nigral neurons with increasing age in normal human brains from India.** *Ann Neurol* 43: 283-287, 1998.

Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. **Curry consumption and cognitive function in the elderly.** *Am J Epidemiol* 164(9):898-9, 2006

PNAS

Proc Natl Acad Sci U S A. 2007 July 31; 104(31): 12849–12854.

Innate immunity and transcription of MGAT-III and Toll-like receptors in Alzheimer's disease patients are improved by bisdemethoxycurcumin

Fiala M. et al.

Department of Medicine, Greater Los Angeles Veteran's Affairs Medical Center

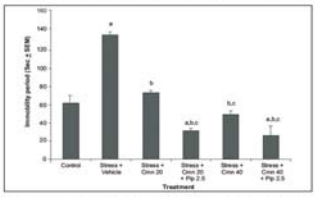
J Clin Psychopharmacol. 2008 Feb;28(1):110-3.

Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease.

Baum L. et al

Pharmacology, Biochemistry and Behavior

Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Bhutani MK et al. 2009



Effect of curcumin and its combination with piperine on forced swim-induced immobility period in rats. ap≤0.05 as compared with control group; bp≤0.05 as compared with stress (S)+vehicle group, cp≤0.05 as compared with stress (S)+ curcumin (20).

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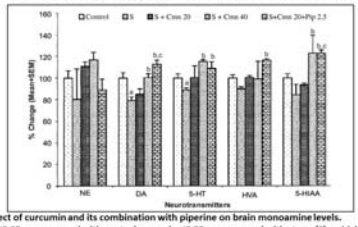
Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Bhutani MK et al. 2009



Effect of curcumin and its combination with piperine on brain monoamine levels. ap≤0.05 as compared with control group, bp≤0.05 as compared with stress (S)+vehicle group, cp≤0.05 as compared with stress (S)+curcumin (20).

BRAIN RESEARCH

Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Xu Y et al. 2007

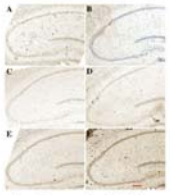


Table 1 – The effects of curcumin on 5-HT$_{1A}$ receptor mRNA expression in stressed rats

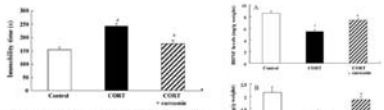
Group	Dose (mg/kg)	Brain region 5-HT$_{1A}$ receptor mRNA expression		
		CA1	CA3	DG
Control		100.0 ± 2.2	100.0 ± 1.3	100.0 ± 1.5
Stress+ vehicle		80.7 ± 2.1**	77.5 ± 0.7	57.2 ± 1.3**
Curcumin	5	72.9 ± 1.9	75.8 ± 1.4	66.8 ± 1.2
	10	80.1 ± 1.7*	79.1 ± 1.2	77.1 ± 1.7*
	20	85.7 ± 0.9*	85.2 ± 0.8	84.5 ± 1.1*
Imipramine	10	91.4 ± 1.3*	85.6 ± 1.3	89.1 ± 2.1**

Values are mean ± S.E.M. and expressed as a percentage of the control group (n=5–6). *p<0.01, compared with control group; *p < 0.05, *p < 0.01, compared with stress+ vehicle group.

The effects of curcumin on BDNF immunoreactivity in hippocampal neurons of stressed rats. (A) Control group. (B) Stress+vehicle group. (C) 5 mg/kg curcumin. (D) 10 mg/kg curcumin. (E) 20 mg/kg curcumin. (F) 10 mg/kg imipramine.

Neuroscience Letters

Curcumin reverses corticosterone-induced depressive-like behavior and decrease in brain BDNF levels in rats. Huang Z et al. 2011

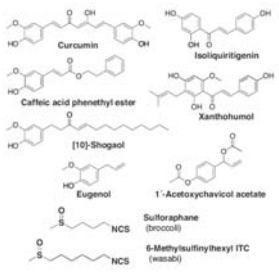


Effect of curcumin on the immobility time of CORT-treated rats in the forced swim test. The rats were administered with CORT (40 mg/kg, s.c.) once daily for 21 days, and curcumin (20 mg/kg, p.o.) was given to the animals 30 min prior to the CORT injection. Values are given as mean±SEM (n = 8). #p < 0.01 compared with the control group; *p < 0.01 compared with the CORT group.

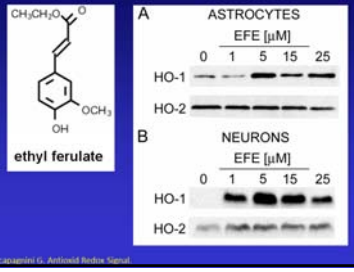
Effect of curcumin on brain-derived neurotrophic factor (BDNF) protein levels in the hippocampus (A) and frontal cortex (B) of CORT-treated rats.

MECHANISMS PROPOSED FOR ANTIDEPRESSANT ACTIVITY OF CURCUMIN

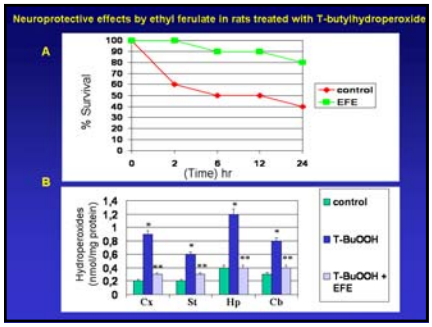
- Monoamine oxidase (MAO) inhibitory property of curcumin
- Modulating the serotonin and dopamine neurotransmission in brain
- Increasing the levels of neurotrophic factors, particularly brain derived neurotrophic factor (BDNF)
- Antiinflammatory and antioxidant property

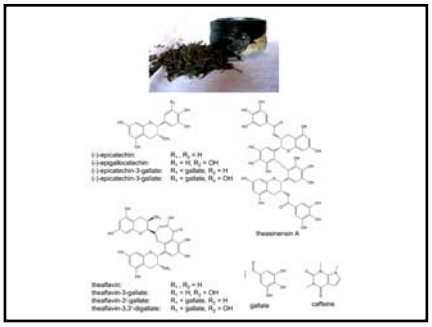


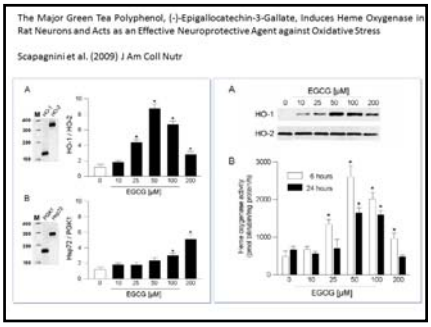
Effect of ethyl ferulate on HO-1 protein expression in astrocytes and neurons

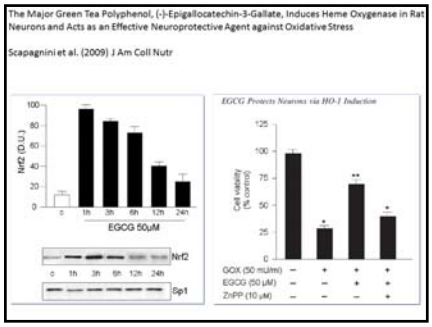


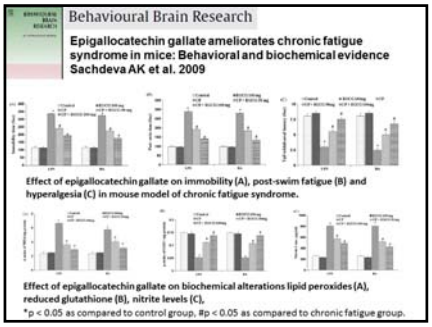
Scapagnini G. Antioxid Redox Signal.

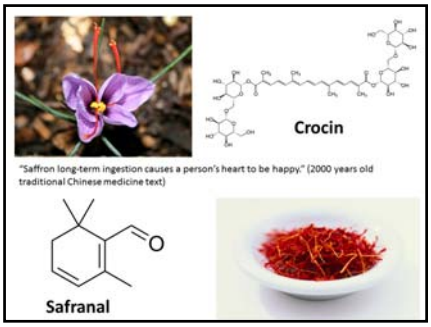

Neuroprotective effects by ethyl ferulate in rats treated with T-butylhydroperoxide
A
% Survival
control
EFE
(Time) hr
B
Hydroperoxides (nmol/mg protein)
control
T-BuOOH
T-BuOOH + EFE
Cx
St
Hp
Cb

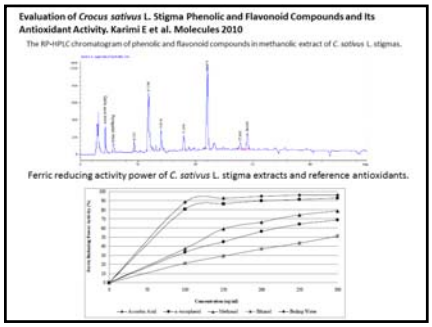


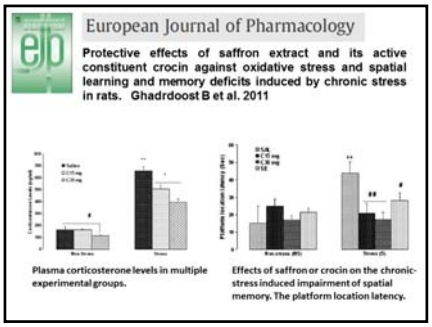

The Major Green Tea Polyphenol, (-)-Epigallocatechin-3-Gallate, Induces Heme Oxygenase in Rat Neurons and Acts as an Effective Neuroprotective Agent against Oxidative Stress
Scapagnini et al. (2009) J Am Coll Nutr
EGCG [μM]
HO-1
HO-2
6 hours
24 hours
EGCG [μM]

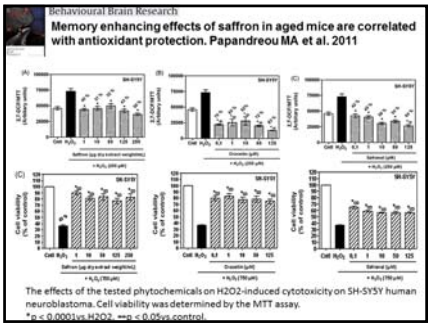

The Major Green Tea Polyphenol, (-)-Epigallocatechin-3-Gallate, Induces Heme Oxygenase in Rat Neurons and Acts as an Effective Neuroprotective Agent against Oxidative Stress
Scapagnini et al. (2009) J Am Coll Nutr
Nrf2 (D.U.)
EGCG 50μM
Nrf2
Bp1
EGCG Protects Neurons via HO-1 Induction
Cell viability (% control)
GOX (50 mU/ml)
EGCG (50 μM)
ZnPP (10 μM)


Behavioural Brain Research
Epigallocatechin gallate ameliorates chronic fatigue syndrome in mice: Behavioral and biochemical evidence
Sachdeva AK et al. 2009
Effect of epigallocatechin gallate on immobility (A), post-swim fatigue (B) and hyperalgesia (C) in mouse model of chronic fatigue syndrome.
Effect of epigallocatechin gallate on biochemical alterations lipid peroxides (A), reduced glutathione (B), nitrite levels (C),
*p < 0.05 as compared to control group. #p < 0.05 as compared to chronic fatigue group.


Crocin
"Saffron long-term ingestion causes a person's heart to be happy." (2000 years old traditional Chinese medicine text)
Safranal


Evaluation of Crocus sativus L. Stigma Phenolic and Flavonoid Compounds and Its Antioxidant Activity. Karimi E et al. Molecules 2010
The RP-HPLC chromatogram of phenolic and flavonoid compounds in methanolic extract of C. sativus L. stigmas.
Ferric reducing activity power of C. sativus L. stigma extracts and reference antioxidants.


European Journal of Pharmacology
Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Ghadrdoost B et al. 2011
Plasma corticosterone levels in multiple experimental groups.
Effects of saffron or crocin on the chronic-stress induced impairment of spatial memory. The platform location latency.


Behavioural Brain Research
Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Papandreou MA et al. 2011
The effects of the tested phytochemicals on H2O2-induced cytotoxicity on SH-SY5Y human neuroblastoma. Cell viability was determined by the MTT assay.
*p < 0.0001vs.H2O2, **p < 0.05vs.control.

Study Design and Primary Results of Saffron (*Crocus sativus*) Trials

Author	Extract Description	Daily Dose	Study Design	Comparator	Results	Tolerability	Industry Sponsored
Akhondzadeh et al (2004)	Crocus sativus (dried stigma) from Novin Zahvan Co. TVC; ethanolic (30%) extraction evaporation dried; extract not characterized	30 mg	R, DB, AC, 6 wks	Imipramine (100 mg)	Both groups showed improvement in mean HAM-D scores compared to baseline (↓ ~50% in saffron grp vs. ~58% in imipramine grp. Both p<0.0001); no significant differences between treatment groups	Dry mouth observed significantly more often in imipramine group (n=7; p=0.03), as was sedation (n=4; p=0.05)	No
Noorbala et al (2005)	Crocus sativus (dried stigma) from Novin Zahvan Co. TVC; ethanolic (30%) extraction evaporation dried; capsules; standardized to 0.3-0.35 mg safranal	30 mg	R, DB, AC, 6 wks	Fluoxetine (20 mg)	Both groups showed improvement in mean HAM-D scores compared to baseline (↓ ~54%) in saffron grp vs. ~61%↓ in fluoxetine grp); no significant differences between treatment groups	Not significant difference between groups; trend toward a decreased incidence of sexual dysfunction (p=0.10) and tremor (p=0.10) in the saffron group	No
Akhondzadeh et al (2005)	Crocus sativus (dried stigma) from Novin Zahvan Co. TVC; ethanolic (30%) extraction evaporation dried; extract not characterized	30 mg	R, DB, PC, 6 wks	Placebo	Mean HAM-D scores for the saffron group lower at week 6, compared to placebo (↓ ~50%↓ in saffron grp vs. ~22%↓ in placebo grp. p<0.001)	Not significantly different between groups; trend toward increased appetite in the saffron group (p=0.10)	No
Modabbernia et al (2006)	Crocus sativus (dried petal) from Novin Zahvan Co. TVC; ethanolic (30%) extraction evaporation dried; extract not characterized	30 mg	R, DB, PC, 6 wks	Placebo	Improvement in mean HAM-D scores in saffron group by week two (p<0.05 compared to placebo); mean HAM-D scores in saffron group lower at week 4 compared to placebo (↓ ~60%↓ in saffron grp vs. ~22%↓ in placebo grp. p<0.001)	No significant difference in frequency of side effects between groups	No

Dwyer AV et al, Alternative Medicine Review 2011

Open questions

- Information on dose-efficacy and interaction between the various antioxidants is scarce. The majority of the studies available at present have been performed at high doses and often with only one component.
- Differences in genetic make-up are likely to affect individual requirements for antioxidants. This aspect of individualized nutrition has not been paid much attention and few data are available
- Evidence is increasing that oxidant functions as signal molecules and interfere with redox sensitive signaling pathways. It is not understood how antioxidants influence such complex interplays.

The validity of oxidative stress biomarkers is still under discussion.

The principal criticisms relate to 2 main aspects:

1 Technical problems. Specificity and sensitivity of the assays, stability of the compound during sample preparation and storage, reproducibility of the analyses.

2 Association of the marker with a biochemical, pathophysiological or clinical outcome related to a disease or risk for a disease.

Trevithick CC, Chartrand MM, Wahlman J, Rahman F, Hirst M, Trevithick JR. Shaken, not stirred: bioanalytical study of the antioxidant activities of martinis. BMJ. 1999 Dec 18-25;319(7225):1600-2.

- Shaken martinis were more effective in deactivating hydrogen peroxide than the stirred variety
- Both were more effective than gin or vermouth alone