Evaluation of Antioxidant Effect of *Nigella sativa* oil on Monosodium Glutamate-Induced Oxidative Stress in Rat Brain

Neveen, A. Noor* and Iman, M. Mourad
Zoology department, Faculty of science, Cairo University, Egypt
*neveen.nour5@gmail.com

Abstract: Oxidative stress is a characteristic feature in a number of neurodegenerative disorders. The present study evaluates the antioxidant effect of *Nigella sativa* oil (NSO) in comparison to that of vitamin C (vit.C) in the cortex and hippocampus of rats pretreated with monosodium glutamate (MSG) as an animal model of oxidative stress. The intraperitoneally injected MSG (4 mg/g body wt.) for 6 consecutive days induced significant decreases in cortical and hippocampal catalase activity and cortical glutathione-S-transferase (GST) activitity and glutathione reduced (GSH) level after 4 weeks. Oral administration of vit.C (200 mg/kg) to stressed rats restored catalase activity, increased GST activity and decreased malondialdehyde (MDA) level after 4 weeks in the cortex. Oral administration of NSO (1 ml/kg) for 4 weeks to MSG-treated rats increased cortical and hippocampal catalase activity and cortical GSH content but significantly inhibited GST activity and increased MDA level in the cortex. Combined administration of vit.C and NSO revealed nonsignificant changes in cortical and hippocampal parameters, as compared to control levels, except for a significant decrease in hippocampal GSH content. In conclusion, although there are some antioxidant effects of NSO, the pro-oxidant effect of NSO cannot be ruled out in the present MSG model of oxidative stress. [Journal of American Science. 2010;6(12):13-19]. (ISSN: 1545-1003).

Key words: Oxidative stress, monosodium glutamate, vitamin C, *Nigella sativa* oil, cortex, hippocampus.

1. Introduction

Oxidative stress is a characteristic feature in a number of neurodegenerative disorders such as stroke, Parkinson's disease and Alzheimer's disease.[1,2] The brain is particularly vulnerable to oxidative stress injury because of its high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids, relatively low antioxidant capacity, the abundance of redox-active transition metal ions,[3] and non-replicating nature of its neuronal cells.[4]

Certain brain regions are highly enriched in non-heme iron, which is catalytically involved in the production of oxygen free radicals,[5] thus increasing the risk of neurodegenerative disease. It has been shown that the regions like cortex and hippocampus are more susceptible to oxidative damage when compared to cerebellum. [6,7]

Reactive oxygen species (ROS) are generated continuously in nervous system during normal metabolism and neural activity. When the balance between ROS (for example: superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen) and antioxidant system is lost, oxidative stress results.[8,9]

In order to scavenge ROS, different defense systems exist in the brain, such as enzymatic (superoxide dismutase, glutathione peroxidase and catalase), non enzymatic (glutathione and uric acid) and dietary (vitamins A, E and C, β – carotene, quinones and flavons) antioxidants. If ROS are not effectively eliminated, they can cause peroxidation of cell membrane phospholipids, proteins (receptors and enzymes) and DNA.[9]

Monosodium glutamate (MSG), the sodium salt of glutamate, is commonly used as a flavor enhancer,[10-12] to increase palatability and food selection in a meal.[13] Many people throughout the world ingest large doses of MSG and in many countries there are no limitation on the amount of MSG that can be added to food.[14]

Glutamate is the main excitatory neurotransmitter in rat brain.[15] It has been shown that MSG administration during neonatal period increase lipid peroxidation in the midbrain of adult rats.[16] Bawari *et al*[16] suggested that oxidative stress caused by excitotoxin- generated free radicals sustained and progressed during development until adulthood. In addition, Park *et al*.[17] reported that MSG could impair memory retention and induce damage in the hypothalamic neurons in mice. Moreover, alteration due to MSG in mitochondria lipid peroxidation and antioxidant status in different brain regions is well documented.[18]

Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body. It is a well known antioxidant in animal tissues.[19] It decreases the adverse effects of ROS implicated in chronic diseases including neurodegenerative diseases.[20] Neurons maintain relatively high intracellular concentrations of ascorbic acid.[21] The reduced form of the vitamin C (ascorbate) is an especially effective antioxidant owing to its high

electron – donating power and ready conversion back to the active reduced form,[7] in addition to the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species.[22]

Nigella sativa, commonly known as black seeds, belongs to the botanical family of *Ranunculaceae*. In recent years, the plant has been investigated to justify its broad traditional therapeutic value.[9] Many favorable biological properties of *Nigella sativa* have been reported such as anti-inflammatory,[23] antioxidative,[24,25] antitumour,[26] antiulcerogenic,[27] and hepatoprotective,[28] properties. The biological activity of *Nigella sativa* seeds is attributed to its essential oil components.[23]

The aim of the present study is to evaluate the antioxidant properties of *Nigella sativa* oil (NSO) in comparison to that of the established antioxidant, vit. C and whether the combination of both treatments could increase the effect of each other or not. The activities of catalase and glutathione-S-transferase (GST), as well as, the levels of glutathione reduced (GSH) and malondialdehyde (MDA) were measured in the cortex and hippocampus of rats pre-treated with monosodium glutamate (MSG) as an animal model of oxidative stress and after treatment with vit. C and/or NSO.

2. Materials and Methods:

Animals

Male albino rats (*Rattus norvegicus*) weighing 120-180 g were used as experimental animals. The animals were obtained from animal house of the National Research Center. They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

Chemicals

L- glutamic acid sodium salt monohydrate (MSG), was purchased from WINLAB, U.K. and vitamin C **(**L-ascorbic acid) was from SAS chemicals co. India. Phosphate buffer and reagent kits were purchased from Bio-diagnostic Company, Egypt. *Nigella sativa* oil was obtained by pressing of seeds on cold to keep the active constituents unaltered.

Experimental design

The animals were divided into 5 groups. Animals of group (1) served as control and were daily administered distilled water throughout the experimental protocol. Animals of groups 2, 3, 4 and 5 were injected intraperitoneally (i.p.) with 4 mg/g body wt. MSG for six consecutive days.[29] At the 7th day, animals of the 2nd, 3rd, 4th and 5th groups were daily administered orally with distilled water, vit.C (200 mg/kg)[30], NSO (1 ml/kg)[31] and both vit C (200 mg/kg) and NSO (1 ml/kg) respectively, for 4 weeks.

Handling of tissue samples

Both treated and control animals were sacrificed after being fasted overnight. The brain of each animal was quickly removed and rapidly transferred to an ice-cold Petri dish and dissected to obtain the cortex and hippocampus.[32,33] Each brain area was weighed and frozen until analyzed.

The hippocampus and cortex were homogenized in 4 and 6 ml of ice cold phosphate buffer (50 mM pH 7.4, 0.1% tritonX and 0.5 mM EDTA), respectively. The homogenate was centrifuged at 1753 g for 15 minutes at 4ºC using a high speed cooling centrifuge (Type 3K-30, Sigma, Germany). The clear supernatant was separated and used for analysis. Catalase activity,[34,35] GST activity,[36] GSH level,[37] and Thiobarbituric acid reactive substances (expressed as malondialdehyde, MDA content),[38] were determined by using reagent kits.

Statistics

The data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparisons. Analysis was made using the SPSS statistical software. The *p*-values less than 0.05 were considered statistically significant. Percentage difference representing the percent of variation with respect to the control was calculated.

% difference = (treated mean–control mean/control mean)×100.

3. Results

The data represented in Table (1) showed a significant decrease in cortical and hippocampal catalase activity in MSG-injected group as compared to control group. These decreases in the enzyme activity persisted after treatment of MSG-injected rats with vit.C, being nonsignificant and significant in the cortex and hippocampus, respectively as compared to control animals. However, the oral administration of NSO to MSG-treated rats induced a significant increase in the enzyme activity in both cortex and hippocampus in comparison to the control, MSG and vit.C-treated groups. The combined administration of vitamin C and NSO to MSG-treated rats returned the enzyme activity to nearly control values in the cortex and hippocampus.

Regarding GST activity (Table 2), the present data showed that the cortical GST activity decreased significantly after MSG injection. The oral administration of vit. C alone and vit. C+ NSO to MSG-treated animals induced significant and

nonsignificant increases in GST activity, respectively in comparison to control group. Meanwhile, the oral administration of NSO to MSG-treated rats did not alter the significant decrease in the enzyme activity due to MSG injection alone. On the other hand, GST activity in the hippocampus did not show any significant changes during the experimental period.

As shown in Table (3), the cortical GSH level decreased significantly after MSG treatment. However, this decrease in GSH level persisted significantly after vitamin C administration in comparison to the control. The oral administration of NSO alone and vit. C+NSO induced significant and non significant increases in GSH levels, respectively relative to the control group. In contrast to the cortex, the data did not record any significant changes in the hippocampal GSH content after MSG injection. The combined administration of both vit. C and NSO showed a significant decrease in the GSH content as compared to the control value.

Data in Table (4) revealed a nonsignificant change in the cortical lipid peroxidation after MSG injection as determined by MDA formation. However, vit. C administration to MSG-injected rats induced a significant decrease in MDA level. Meanwhile, NSO administration provoked a significant increase in MDA level as compared to control, MSG and vit.C-treated groups. The combined administration of vit.C and NSO to MSG-treated rats did not induce any significant change in MDA level in relation to control and MSG-treated groups. MDA content in the hippocampus presented a significant decrease due to MSG injection. Administration of vit.C did not change the decrease in MDA level obtained as a result of MSG injection alone. Nonsignificant changes were observed in MDA level as a result of NSO and vit.C+NSO treatments to MSG-treated rats as compared to control group.

Table (1): Effect of daily oral administration of vit. C and/or NSO on catalase activity (U/g tissue) in the cortex and hippocampus of rats treated with MSG.

Area	Control	MSG		Vit.C		NSO		(Vit. C+ NSO)		F-test
		Value	% D	value	% D	value	% D	value	% D	
Cortex	A 2.74±0.07 (7)	B 1.93±0.06 (7)	-29.56	AB 2.42±0.10 (8)	-11.68	C 3.53±0.28 (6)	+28.83	A 2.87±0.13 (8)	+4.74	*
Hippocampus	A 9.70±0.58 (7)	B 7.16±0.63 (5)	-26.19	B 7.67±0.29 (7)	-20.93	C 11.58±0.15 (6)	+19.38	AC 10.65±0.18 (6)	+9.79	*

Values represent mean±S.E.M. with the number of animals between parentheses.
*: $p < 0.05$ significant.
A, B and C: different letters mean significant changes.
%D: percentage difference between treated and control.

Table (2): Effect of daily oral administration of vit. C and/or NSO on GST activity (U/g tissue) in the cortex and hippocampus of rats treated with MSG.

Area	Control	MSG		Vit.C		NSO		(Vit. C+ NSO)		F-test
		value	%D	value	% D	value	% D	value	% D	
Cortex	A 0.30±0.03 (6)	B 0.09±0.01 (7)	-70.00	C 0.50±0.04 (6)	+66.67	B 0.10±0.02 (6)	-66.67	AC 0.46±0.06 (6)	+53.33	*
Hippocampus	0.10±0.01 (6)	0.06±0.01 (6)	-40.00	0.08±0.02 (7)	-20.00	0.10±0.02 (6)	0.00	0.06±0.01 (6)	-40.00	n.s.

Values represent mean±S.E.M. with the number of animals between parentheses.
n.s.: $p > 0.05$ nonsignificant. *: $p < 0.05$ significant.
A, B and C: different letters mean significant changes.
%D: percentage difference between treated and control.

Table (3): Effect of daily oral administration of vit. C and/or NSO on GSH content (mg/g tissue) in the cortex and hippocampus of rats treated with MSG.

Area	Control	MSG		Vit.C		NSO		(Vit. C+ NSO)		F-test
		Value	% D	Value	% D	Value	% D	Value	% D	
Cortex	A 26.36±0.95 (5)	B 13.64±0.97 (6)	-48.25	B 16.59±1.45 (7)	-37.06	C 36.78±1.85 (6)	+39.53	AC 32.39±2.18 (6)	+22.88	*
Hippocampus	A 53.64±1.16 (6)	A 52.75±1.31 (6)	-1.66	AB 47.68±1.34 (6)	-11.11	A 51.98±1.59 (6)	-3.09	B 44.03±2.31 (6)	-17.92	*

Values represent mean±S.E.M. with the number of animals between parentheses.

*: $p < 0.05$ significant.
A, B and C: different letters mean significant changes.
%D: percentage difference between treated and control.

Table (4): Effect of daily oral administration of vit. C and/or NSO on MDA content (nmol/g tissue) in the cortex and hippocampus of rats treated with MSG.

Area	Control	MSG		Vit.C		NSO		(Vit. C+ NSO)		F-test
		value	%D	Value	%D	Value	%D	Value	%D	
Cortex	A 197.75±2.84 (6)	A 194.91±4.93 (5)	-1.44	B 163.43±1.94 (6)	-17.36	C 230.23±7.86 (6)	+16.42	AB 180.89±4.14 (6)	-8.53	*
Hippocampus	AC 175.52±2.51 (6)	B 155.03±4.07 (6)	-11.67	B 156.73±4.02 (6)	-10.71	AB 170.58±4.20 (5)	-2.81	C 191.93±4.17 (6)	+9.35	*

Values represent mean±S.E.M. with the number of animals between parentheses.
*: $p < 0.05$ significant.
A, B and C: different letters mean significant changes.
%D: percentage difference between treated and control.

4. Discussion:

The present data showed that the i.p. injection of MSG for 6 consecutive days to adult rats induced significant decreases in cortical and hippocampal catalase activity as well as cortical GST activity and GSH content after 4 weeks, as compared to control rats. Singh *et al*.[29] reported that repeated monosodium glutamate doses showed prolonged and delayed effects on the mitochondrial free radical scavenger system and the consequential membrane damage as inferred from altered levels of Mn-superoxide dismutase (Mn-SOD), catalase, glutathione peroxidase (GPx), glutathione reduced (GSH), lipid peroxidation and uric acid content in mitochondria in different brain regions of male rats.

Thus, it could be suggested that the i.p. injection of MSG administration may provide a successful model of oxidative stress and hence may be used to investigate the antioxidant properties of several natural products.

Decreased catalase activity has been shown to be associated with oxidative stress in brain regions.[39,40] It functions to catalyze the decomposition of H_2O_2 to water and oxygen.[41]

Singh *et al*.[29] investigated the effect of i.p. MSG administration for 6 consecutive days on mitochondrial lipid peroxidation and antioxidant status in adult rat cerebral hemisphere after 30 and 45 days. In the present study, the effect of MSG is investigated in the cerebral cortex and hippocampus of adult male rats and the decrease in catalase activity and glutathione reduced content in the cerebral cortex is in accordance with the results of Singh *et al*.[29] in the cerebral hemisphere. Thus, the cerebral cortex could be the target area for MSG effects in the cerebral hemisphere. Furthermore, the present results agree with the more recent study of Farombi and Onyema,[12] who indicated a decrease in brain GSH content and catalase activity after i.p. injection of adult rats with MSG for 10 days.

Kono and Fridovich,[42] suggested that increased production of free radicals may lead to depletion or inactivation of catalase enzyme. Further reports have shown that mitochondria generate superoxide and related ROS during glutamate receptor over activation.[43,44] During oxidative stress, the superoxides destroy the iron-sulfur centers and thereby irreversibly deactivate the iron containing enzymes.[45,46]

Thus, the decrease in cortical and hippocampal catalase activity in MSG-injected group may be due to depletion or irreversible deactivation of the catalase enzyme by superoxide generation.

GSH (a tripeptide comprised of glutamate, cysteine and glycine) plays a critical role in protecting cells from oxidative stress and xenobiotics. GSH can function as an antixodant in many ways. It can react non-enzymatically with superoxide,[47] nitric oxide,[48] hydroxyl radical,[49] and peroxynitrite.[50] Thus, it functions directly as a free radical scavenger. Therefore, the decrease in cortical GSH content observed in the present study may be due to its consumption in scavenging the generated ROS.

Glutathione-S-transferases (GSTs) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional groups.[51] Subsequently, the observed decline in the cortical GST activity, in the present study, may be ascribed to the reduction in the level of its substrate, GSH.

Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes.[52] MDA is the major

aldehyde metabolite of lipid peroxidation.[53] The present nonsignificant change in cortical MDA level in MSG-treated group may confirm the delayed effect of MSG exposure and this result is in line with the results of Singh *et al*.[29] as they found significant increase in the level of mitochondrial lipid peroxidation in the cerebral hemisphere at the 45th day of MSG administration and not at the 30th day.

In the present study, it was found that the oral administration of vit. C to MSG-injected rats restored cortical catalase activity to nearly control level, whereas it reversed the significant decrease in cortical GST activity due to MSG injection to a significant increase (+66.67%). Meanwhile, GSH content in the cortex was not affected by vit. C treatment to stressed rats. However, cortical MDA level was decreased significantly after vit. C administration as compared to control group.

Vitamin C is a well known antioxidant and has been shown to protect various tissues against the damage caused by ROS.[19] In addition, many studies have indicated that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer and cataract, probably through antioxidant mechanisms.[54] Moreover, Huang *et al*.[55] reported that vitamin C (500 mg ascorbate/day) supplementation in non-smokers reduced lipid peroxidation.

Therefore, the present increase in GST activity and restoration of catalase activity to nearly control level in addition to the decrease in MDA content in the cortex could indicate that the antioxidant properties of vit. C may be mediated on one hand by an increase in antioxidant enzymatic activity and by a decrease in lipid peroxidation on the other hand.

Within the brain, ascorbate levels are not homogenous; in humans, the highest levels are found in the hippocampus, amygdala, and hypothalamus.[56] Therefore, the mild oxidative state observed, in the present study, in the hippocampus due to MSG administration may be due to the inherent high content of vit. C in this brain area.

Treatment of oxidatively stressed rats, in the present study, with NSO increased catalase activity in the cortex and hippocampus as well as the GSH content in the cortex. Meanwhile, a significant inhibition of cortical GST activity accompanied by an increase in MDA was obtained.

Thymoquinone (TQ) is the main constituent of NSO. It was intensively studied and ascribed to possess antioxidant properties.[57] On the other hand, Khader *et al*.[58] reported that TQ like other quinone compounds, can be considered to be a redox-cycler which is metabolized *in vitro* to hydroquinones or semiquinone radicals by cellular oxidoreductases leading to the production of ROS. This may explain the present increase in cortical MDA content after treatment of MSG-injected rats with NSO.

It has been reported that the exposure to ROS and nitrogen species may raise the GSH content by increasing the GSH synthesis rate.[59,60] Thus, the increase in cortical GSH content may be an attempt to counteract the increase in MDA level as a defense mechanism by brain cells against free radicals generation.

Cortical and hippocampal catalase activity showed significant increases due to NSO treatment of stressed rats. In agreement with the present result, Kanter *et al*.[61] found that NSO prevented inhibition of catalase activity following experimental spinal cord injury in rats. Moreover, Al-Majed *et al*.[62] found that thymoquinone is beneficial in restoring declined hippocampal SOD and catalase due to ischemia insult.

Although, there are some antioxidant effects of NSO which are represented in the increase of GSH content and catalase activity, the pro-oxidant effect of NSO cannot be ruled out in the present MSG model of oxidative stress.

Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids,[63] and must have neutralized the ROS generated in the aqueous phase before lipid peroxidation was initiated.[12]

Therefore, it could be concluded that the combined administration of vitamin C and NSO to stressed rats, in the present study, may represent a synergestic antioxidant effect between the water soluble vit. C and lipid soluble components of NSO. Moreover, vit. C may counteract the pro-oxidant effects of NSO. However, further studies are recommended for the evaluation of the antioxidant properties of NSO in different models of oxidative stress.

Corresponding author
Neveen, A. Noor
Zoology department, Faculty of science, Cairo University, Egypt
neveen.nour5@gmail.com

5. References:

1. Andersen JK (2004) Nat. Med. 10 Suppl, S18-S25
2. Ji B-S, Ji H & Liu G-Q (2004) *Acta Pharmacol Sin* 25, 297-300
3. Butterfield DA & Stadtman ER (1997) *Adv Cell Aging Gerontol* 2, 161-191
4. Evans PH (1993) *Br Med Bull* 49, 577-587
5. Hill JM & Switzer RC (1984) *Neuroscience* 11, 595-603

6. Mandavilli BS & Rao KS (1996) *Biochem Mol Biol Int* 40, 507-514.
7. Venkataraman P, Muthuvel R, Krishnamoorthy G, Arunkumar A, Sridhar M, Srinivasan N, Balasubramanian K, Aruldhas M M & Arunakaran, J (2007) *Neuro Toxicol* 28, 490-498
8. Allen RG (1991) *Proc Soc Exp Biol Med* 196, 117-129
9. Ilhan A, Gurel A, Armutcu F, Kamisli S & Iraz M (2005) *Neuropharmacology* 49, 456-464
10. Kurihara K & Kashiwayanagi M (1998) *Ann NY Acad Sci* 855, 393-397
11. Chaudari N & Roper SD (1998) *Ann NY Acad Sci* 855, 398-405
12. Farombi EO & Onyema OO (2006) *Hum Exp Toxicol* 25, 251-259
13. Bellisle F, Dalix AM, Chappuis AS, Rossi F & Fiquet P (1996) *Appetite* 26, 267-276
14. Diniz YS, Faine LA, Galhardi C.M, Rodrigues HG, Ebaid GX, Burneiko RC, Cicogna, AC & Novelli, ELB (2005) *Nutrition* 21, 749-755
15. Rotta LN, Schmidt AP, Mello e Souza T, Nogueira, CW, Souza KB, Izquierdo IA, Perry ML & Souza DO (2003) *Neurochem Res* 28, 1181-1186
16. Bawari M, Babu GN, Ali MM & Misra UK (1995) *Neuroreport* 6, 650-652
17. Park CH, Choi SH, Piao Y, Kim S, Lee YJ, Kim HS, Jeong, SJ, Rah JC, Seo JH, Lee JH, Chang K, Jung YJ & Suh YH (2000) *Toxicol Lett* 115, 117-125
18. Singh K & Ahluwalia P (2003) *J Nutr Sci Vitaminol[Tokyo]* 49, 145-148
19. Kajo S (2004) *Curr Med Chem* 11, 1041-1064
20. Muthuvel R, Venkataraman P, Krishnamoorthy G, Gunadharini DN, Kanagaraj P, Jone Stanley A, Srinivasan N, Balasubramanian K, Aruldhas MM & Arunakaran J (2006) *Clin Chim Acta* 365, 297-303
21. Qiu S, Li L, Weeber EJ & May JM (2007) *J Neurosci Res* 85, 1046-1056
22. Tsao CS (1997) In *Vitamin C in health and disease* (Packer L & Fuchs J, eds), pp. 25-58, Marcel Dekker Inc. New York
23. Hajhashemi V, Ghannadi A & Jafarabadi H (2004) *Phytother. Res* 18, 195-199
24. Burits M & Bucar F (2000) *Phytother Res* 14, 323-328
25. Meral I, Yener Z, Kahraman T & Mert N (2001) *J Vet Med A Physiol Pathol Clin Med* 48, 593-599
26. Salomi MJ, Nair SC & Panikkar KR (1991) *Nutr Cancer* 16, 67-72
27. Kanter M, Demir H, Karakaya C & Ozbek H (2005) *World J Gastroenterol* 11, 6662-6666
28. Kanter M, Coskun O & Budancamanak M (2005) *World J Gastroenterol* 11, 6684-6688
29. Singh P, Mann KA, Mangat HK & Kaur G (2003) *Mol Cell Biochem* 243, 139-145
30. Ramanathan K, Balakumar BS & Panneerselvam C (2002) *Hum Exp Toxicol* 21, 675-680
31. Ali BH (2004) *Am J Chin Med* 23, 49-55
32. Zeman W & Innes JRM (1963) Graigie's Neuroanatomy of The Rat. Academic Press, New York, London
33. Glowinski J & Iversen LL (1966) *J Neurochem* 13, 655-669
34. Fossati P, Prencipe L & Berti G (1980) *Clin Chem* 26, 227-231
35. Aebi, H (1984) *Methods Enzymol* 105, 121-126
36. Habig WH, Pabst MJ & Jakoby WB (1974) *J Biol Chem* 249, 7130-7139
37. Beutler E, Duron O & Kelly BM (1963) *J Lab Clin Med* 61, 882-888
38. Ohkawa H, Ohishi N & Yagi K (1979) *Anal Biochem* 95, 351-358
39. Arivazhagan P, Shila S, Kumaran S & Panneerselvam C (2002) *Exp Gerontol* 37, 803-811
40. Shila S, Kokilavani V, Subathra M & Panneerselvam C (2005) *Toxicology* 210, 25-36
41. Chelikani P, Fita I & Loewen PC (2004) *Cell Mol Life Sci* 61, 192-208
42. Kono Y & Fridovich I (1982) *J Biol Chem* 257, 5751-5754
43. Dugan LL, Sensi SL, Canzoneiro LMT, Handran SD, Rothman SM, Lin TS, Goldberg MP & Choi, DW (1995) *J Neurosci* 15, 6377-6388
44. Patel M, Day BJ, Crapo JD, Fridovich I & McNamara JO (1996) *Neuron* 16, 345-355
45. Gardner PR & Fridovich I (1991) *J Biol Chem* 266, 1478-1483
46. Qing-You L, Pederson C, Day BJ & Patel M (2001) *J Neurochem* 78, 746-755
47. Winterbourn CC & Metodiewa D (1994) *Arch Biochem Biophys* 314, 284-290
48. Clancy RM, Levartovsky D, Leszczynska-Piziak J, Yegudin J & Abramson SB (1994) *Proc Nat Acad Sci USA* 91, 3680-3684
49. Bains JS & Shaw CA (1997) *Brain Res Rev* 25, 335-358.
50. Koppal T, Drake J, Yatin S, Jordan B, Varadarajan S, Bettenhausen L & Butterfield DA (1999) *J Neurochem* 72, 310-317
51. Yousef MI (2004) *Toxicology* 199, 47-57
52. Cheeseman, KH (1993) *Mol Aspects Med* 14, 191-197.

53. Paradis V, Mathurin P, Kollinger M, Imbert-Bismut F, Charlotte F, Piton A, Opolon P, Holstege A, Poynard T & Bedossa P (1997) Journal of Clinical Pathology 50, 401-406
54. Carr AC & Frei B (1999) *Am J Clin Nutr* 69, 1086-1107.
55. Huang HY, Appel LJ, Croft KD, Miller ER, Mori TA & Puddey IB (2002) *Am J Clin Nutr* 76, 549-555/
56. Hornig D (1975) *Ann NY Acad Sci* 258, 1293-1398.
57. Houghton PJ, Zarka R, de las Heras B & Hoult JR (1995) *Planta Med* 61, 33-36.
58. Khader M, Bresgen N & Eckl PM (2009) *Food Chem Toxicol* 47, 129-133.
59. Rahman I, Bel A, Mulier B, Lawson MF, Harrison DJ, Macnee W & Smith CA (1996) *Biochem Biophys Res Commun* 229, 832-837.
60. Moellering D, Mc Andrew J, Patel RP, Forman HJ, Mulcahy RT, Jo H & Darley-Usmar VM (1999) *FEBS Lett* 448, 292-296.
61. Kanter M, Coskun O, Kalayci M, Buyukbas S & Cagavi F (2006) *Hum Exp Toxicol* 25, 127-133
62. Al-Majed AA, Al-Omar FA & Nagi MN (2006) *Eur J Pharmacol* 543, 40-47.
63. Singh RP, Sharad S & Kapur S (2004) *JIACM* 5, 218-225.

6/9/2010