ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Potential Protective Effects of Ginkgo Biloba and Rosemary on Hepatoencephalopathy and Chromosomal Aberrations Induced By Manganese Chloride in Rats

Shereen M. Mahmoud[1], Hoda I. Bahr[2]

1. Department of Forensic Medicine and Toxicology**,** Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

2. Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Manuscript Info

Manuscript History:

Received: 11 January 2015
Final Accepted: 25 February 2015
Published Online: March 2015

Key words:

Manganese, Ginkgo Biloba, Rosemary, Hepatoencephalopathy, oxidative stress

Corresponding Author

Shereen Mohamed Mahmoud

Abstract

Manganese is an essential nutrient for biological systems. However, its excess is potentially toxic resulting in neurodegenerative disorder, clinically known as ‘‘manganism’’ which distinctive for hepatoencephalopathy. The purpose of the present study was to investigate the toxic impacts of manganese chloride exposure on rat's liver and brain tissues, and the relative efficacy of Ginkgo biloba and Rosemary in averting such encephalohepatic damage. Rats were divided into 4 groups; control group had free access to water and food materials; manganese chloride group has received 50 mg/kg body weight/day in the drinking water (50 mg in 100 ml of water); Ginkgo biloba + manganese chloride group received a dose of 200 mg of Ginkgo biloba /kg body weight/day, orally dissolved in distilled water; and Rosemary + manganese chloride group received a dose of 10 mg of Rosemary /kg body weight/day, orally dissolved in distilled water by gavage all for 45 successive days. Oxidative stress biomarkers were evaluated in serum and tissues (liver and brain). Manganese chloride induced lipid peroxidation, DNA fragmentation, chromosomal aberrations and inhibited butrylcholinesterase activity. Concerning the brain and liver antioxidant system, glutathione S -transferase , catalase activities, superoxide dismutase and glutathione contents were reduced in Manganese chloride group, while all of these parameters were sustained in Ginkgo biloba + Manganese chloride and Rosemary + Manganese chloride groups. Our results indicate that both Ginkgo biloba and Rosemary are efficacious in reducing Manganese chloride deleterious effects on liver and brain tissues and also exerted a genoprotective effect.

INTRODUCTION

Manganese (Mn) is a transition metal that is essential for normal cell growth and development **(Au et al., 2008)**. It is commonly found in the environment as a constituent of dyes, fertilizers, fungicides, paints, lacquers, glazes, dry batteries, fireworks and rubber and wood preservatives as well as it is critical for optimal immune functioning, metabolism, reproduction, digestion, bone growth, and blood clotting **(Dos Santos et al., 2010)**. Mn is playing an important role in a number of physiological processes by serving as a constituent of some enzymes and an activator of others involved in the regulation of amino acid, protein, lipid, and carbohydrate metabolism (**Zheng et al., 2011)**. Additionally, Mn functions as a cofactor for a variety of metalloenzymes such as arginase, which

is responsible for urea production in the liver, glutamine synthetase, an astrocyte-specific enzyme **(Burton and Guilarte, 2009)**, mitochondrial protein Mn superoxide dismutase (MnSOD), a critical enzyme in attenuating oxidative stress, glutathione synthetase **(Taylor et al., 2006)**, phosphoenolpyruvate decarboxylase, an essential enzyme in gluconeogenesis **(Crossgrove and Zheng, 2004)**, as well as those involved in neurotransmitter synthesis and metabolism **(Golub et al., 2005)**. Central nervous system is an important target for Mn toxicity; the brain has the capability of retaining it for longer periods of time than in other organs, probably due to its difficulty in eliminating the excess of this metal. Mn may cross the blood - brain barrier, and it is delivered to different brain regions, via an axonal transport system. Exposure to excessively high Mn levels causes neurodegenerative disorder referred to as ''manganism'' **(Aschner et al., 2007; Dukhande et al., 2006)**, it is a motor syndrome in which distinctive for hepatic encephalopathy.

Mn is generally believed to exert cellular toxicity via a number of mechanisms, including disruption of mitochondrial function **(Malecki, 2001)**, initiation of oxidative stress and induction of free radical production by direct or indirect formation of reactive oxygen species (ROS) **(Chtourou et al., 2010; Milatovic et al., 2011)**; changes in the functions of all neurotransmission pathways, disruption of cellular calcium/iron homeostasis **(Reaney and Smith, 2005)**, and trace minerals **(Chen et al., 2006)**, initiation of cytochrome c release and induction of apoptosis **(Roth et al., 2002)**.

ROS generated in tissues and sub-cellular compartments are efficiently scavenged by the antioxidant defense system, which protect cells from DNA damage, protein oxidation, and lipid peroxidation (LPO). Mn exposure enhanced the rate of ROS generation in mitochondria, increased single strand breaks of mitochondrial DNA and decreased GSH levels in mitochondria and brain homogenates in a dose-dependent manner **(Jiao et al., 2008)**.

Current approaches to minimize the severity of Mn toxicity include enhancement of its sequestration and elimination by using different treatment methods. Considering the relationship of Mn exposure with oxidative stress and elemental homeostasis, we can speculate that the administration of antioxidants and natural biomolecules may be protective in Mn toxicity. To our knowledge, an alternative approach for the management of Mn toxicity is yet to be discovered. Ginkgo biloba (G. biloba) is one of the most popular herbal medicines in which extracts have been utilized therapeutically for decades with a uniquely broad spectrum of activity **(Kelly et al., 2005; Mahadevan and Park, 2008)**. Numerous studies have shown that *G. biloba* has antioxidant **(Arushanian and Beˇıer, 2008)**, free radical scavenging and hepatic and neuroprotective effects **(Saleem et al., 2008)**. The studies of the underlying principle behind the therapeutic action of *G. biloba* on chronic ailments, such as neurodegenerative diseases, has focused on its antioxidant properties.

As well the use of plants is as old as mankind and plant food-derived antioxidants are increasingly proposed as important dietary antioxidant factors. Natural products are cheap and claimed to be safe. *Rosemary* (R. officinalis) is a common house - hold plant grown in many parts of the world and gaining interest for its pharmacological properties **(Mulinaccia et al., 2009; Nabekuraa et al., 2010)**. *R. officinalis* has been intensively studied during the last 10 years; there is evidence that its aqueous extract has a large number of pharmacological properties, including hepatoprotective and antioxidant activities **(Bakirel et al., 2008; Pereira et al., 2005; Sotelo-Félix et al., 2002).**

Therefore, this study aimed to (1) characterize the encephalohepatic changes induced by subchronic exposure to $MnCl_2$ (2) evaluate the possible protective effect of *G. biloba* and RM against $MnCl_2$ toxicity in rats and (3) evaluate the antigenotoxic potential of *R. officinalis* extracts and *G. biloba* against oxidative DNA damage induced by $MnCl_2$ toxicity.

1.MATERIALS AND METHODS

2.1. Experimental materials:

Manganese Chloride Tetrahydrate ($MnCl_2 \cdot 4H_2O$; 99.99%) was purchased From LOBA CHEMIE PVT.LTD. P.Box No. 2042, MUMBAI, INDIA.

Ginkgo biloba (200 mg) was manufactured by PHARAONIA PHARMACEUTICALS FOR EMA PHARM PHARMACEUTICALS INC., EGYPT.

Fresh Rosemary leaves were purchased from a local market in CAIRO, EGYPT.

Reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), lipid peroxidase (MDA), uric acid, butyryl cholinesterase (BchE), ammonia (NH3), protein and albumin were purchased from BIODIAGNOSTIC INC., GIZA, EGYPT.

2.2. Experimental design:

2.2.1. Preparation of $MnCl_2$ - $4H_2O$ solution

$MnCl_2$ - $4H_2O$ solution was freshly prepared by dissolving $MnCl_2$ in drinking water (50 mg of $MnCl_2$ in 100 ml of water) at a concentration of 50 mg/kg body weight **(Martins et al., 2012).**

2.2.2. Preparation of Ginkgo biloba solution

G. biloba was freshly prepared as a suspension with distilled water and stored at 4º C. The suspension was given orally via a gastro-esophageal gavage, once daily for 45 successive days at doses of 200 mg/100 ml/kg body weight **(Lian Wang et al., 2013)**.

2.2.3. Rosemary extraction method

The air dried leaves were coarsely powdered. 8 gm of *R. officinalis* powder were soaked in 100 ml hot water (88º C) in water bath for 6 h. Then filtered by capron silic cloth 150 µ and the filtrate were stored in dark bottles in refrigerator at (4º C). These procedures were repeated weekly. During 24 hours each rat was orally administrated 10 ml/kg body weight once daily for 45 successive days by gavage **(Haloui et al., 2000)**.

2.2.4. Animals

Twenty eight healthy adult male Sprague-Dawley rats, weighing about 180 ± 20 g and aged 7–8 weeks, were purchased from the Medical RESEARCH INSTITUTE, ZAGAZIG UNIVERSITY, EGYPT. All animals were housed in gang cages maintained in a room with controlled environment conditions and a 12-h light – dark cycle. The animal experiments were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals: **(2010)**. All efforts were made to minimize the number of animals used and their suffering.

"Guide for the Care and Use of Laboratory Animals"

Eighth Edition. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Institute for laboratory animal research. Division on earth and life studies. NATIONAL RESEARCH COUNCIL OF THE NATIONAL ACADEMIES. THE NATIONAL ACADEMIES PRESS. WASHINGTON, D.C.www.nap.edu ISBN: 0-309-15401-4

2.2.5. Animals grouping and treatment

After 2 weeks of acclimatization, all animals were randomly divided into 4 experimental groups of 7 rats each. Rats in the control or vehicles-received group had free access to water and food materials; those in the $MnCl_2$ treated group were received 50 mg/kg body weight in drinking water (50 mg of $MnCl_2$ in 100 ml of water) for 45 successive days; those in the *G. biloba* + $MnCl_2$ treated group received a dose of 200 mg of *G. biloba* /100 ml/kg body weight /day, orally dissolved in distilled water ; and those in the *R. officinalis* + $MnCl_2$ treated group received a dose of 10 ml of *R. officinalis* /kg body weight /day, orally dissolved in distilled water by gavage for 45 successive days.

2.3. Blood and organs sampling:

Twenty-four hours after the last dose of saline or $MnCl_2$, the rats were anesthetized with diethylether **(Reyes et al., 2009)**, Then, blood samples were collected from the inner canthus of the eye by heparinized capillary tube into 2 ml clean test tube. Following standing in room temperature for at least 30 min, the blood was centrifuged at 5000 rpm for 10 min at that point the serum was separated, transferred into eppendorf tubes, and stored at -20º C prior to determination of biochemical parameters. Immediately after the collection of blood samples, the animals were euthanized, and their livers and brains were quickly excised, rinsed in ice-cold saline and used immediately or stored frozen at -80º C until analysis.

2.4. Biochemical serum analysis:

Using commercially available diagnostic kits (BIODIAGNOSTIC CO., EGYPT), the activities of the serum lipid peroxidase (MDA), Total protein, albumin, uric acid and butrylcholinesterase (BchE) were spectrophotometrically determined following the manufacturer's instructions.

2.5. Preparation of tissue homogenate as crude enzyme source:

2.5.1. Assessment of oxidative stress markers.

Liver and brain were surgically removed then immediately rinsed with ice cold 1.15% KCL solution, pre-cut into small pieces and taken for homogenization employing several strokes in a POTTER-ELVEJHAM homogenizer using a Teflon pestle, in the appropriate buffer, to obtain 10% (w/v) tissue homogenate. Throughout the homogenization process, the tissue homogenate was maintained on crushed ice in an ice bucket. The tissue

homogenate was then centrifuged in a refrigerated high-speed centrifuged at 4oC and at 10,000 x g for 20 minutes **(Muthuviveganandavel et al, 2011).** Aliquots of the supernatant were utilized for the spectrophotometrical assessment of the levels of the following: catalase (CAT) activity as the rate of disintegration of hydrogen peroxide **(Aebi, 1984)**, glutathione reduced (GSH) by using Ellman's ragent **(Beutler et al., 1963)**, lipid peroxidation (LPO) assessed as the production of the thiobarbituric acid reactive substances (TBARS) in the presence of BHT **(ohkawa et al., 1979)**, glutathione-s-transferase (GST) activity as the rate of GSH conjugation of CDNB **(Habig et al., 1974)**, superoxide dismutase (SOD) activity as the enzymes can inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye **(Nishikimi et al., 1972)**.

2.5.2. Determination of Ammonia.

Ammonia was measured in serum and cerebral cortex. Blood (150 µL) was taken from the tail vein in the morning (9:00 AM). The cerebral cortex was homogenized and deproteinized in 5 volumes of ice-cold, 6% trichloroacetic acid, and kept on ice for 15 minutes. After centrifugation at 12,000 *xg* for 10 minutes at 4°C, the supernatants were collected, neutralized with 2 mol/L KHCO3, and centrifuged at 12,000 x*g* for 10 minutes at 4°C (**Jover et al., 2006**). Ammonia was measured on the neutralized supernatants using biodignostic kit.

2.5.3. Tissue homogenization and butyrylcholinesterase extraction.

For butyrylcholinesterase extraction, small pieces of liver and brain stored at -80º C were thawed slowly at 4º C and homogenized (10% w/v) in ice-cold Tris-saline buffer (50 mM Tris-HCl, 1 M NaCl, and 50 mM $MgCl_2$, pH 7.4) containing 0.5% (w/v) Triton X-100 and supplemented with a cocktail of proteinase inhibitors **(Sa´ez-Valero et al., 1993**). The suspension was then centrifuged at 5000 rpm for 1 hr at 4º C to recover a cholinesterase rich fraction. BchE activity was determined by using biodignostic kit.

2.6. Molecular assessment

2.6.1. Chromosomal aberrations

The genotoxic effect on liver and brain was evaluated by cytogenetic assay according to the method of **(Nagpure et al., 2007).**

2.7. DPA assay

The diphenylamine (DPA) reaction was performed by the method of **(Paradones et al., 1993)** for determination the percentage of mitochondrial DNA fragmentation.

2.8. Statistical analysis

Data were expressed as mean± SE and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Duncan analysis to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analysis was performed using SPSS statistical version 22 software package (SPSS1 Inc., USA).

3. RESULTS

3.1. Serum biochemical parameters

Our results depict that $MnCl_2$ oral administration for 45 successive days caused marked hepatic encephalopathy which evidenced by the significant ($P < 0.05$) elevation in both serum and brain ammonia compared with control rats **(Table 1)**. However, *G. biloba* and *R. officinalis* treated $MnCl_2$ challenged rats showed marked reduction of serum and brain ammonia level, which were less than the range observed in $MnCl_2$ treated rats and within the range of the control rats.

In line with ammonia, there was a marked and significant ($P < 0.05$) elevation of uric acid in serum **(Table 1)** of rats treated orally with $MnCl_2$ for 45 successive days in comparison to those in control group, as well as rats in *G. biloba* and *R. officinalis* treated groups exhibit significant ($P < 0.05$) decrease in serum uric acid compared with those in $MnCl_2$ and control groups.

3.2. Indices of antioxidant status and butyrylcholinesterase activity

$MnCl_2$ treated rats showed marked elevation ($P < 0.05$) in MDA level in serum, liver and brain tissues compared to those in control group, as well as this findings were restored into normal ranges in *G. biloba* + $MnCl_2$ and *R. officinalis* + $MnCl_2$ treated rat groups **(Table 1)**.

In relation to control rats, the $MnCl_2$ treated group had significant reduction in liver and brain GSH content, GST activity, CAT activity and SOD activity ($P < 0.05$).

Further, the levels of GSH content, GST activity, CAT activity and SOD activity in both the liver and brain tissues of *G. biloba* + $MnCl_2$ and RM + $MnCl_2$ treated rats were greater than those of the $MnCl_2$ treated rats **(Table 2)**. Further, as shown in **(Table 2)**, BchE activity in serum, liver and brain tissues of $MnCl_2$ treated rats was significantly lesser than that in the control group and significantly higher than that in the *G. biloba* + $MnCl_2$ and *R. officinalis* + $MnCl_2$ treated rats ($P < 0.05$).

3.3. DNA fragmentation and chromosomal aberrations

Data revealed increase in percentage of DNA fragmentation (in liver and brain tissues) in line with reduction in total protein content (in liver and brain tissues) and albumin (in serum) in $MnCl_2$ treated rats (**Table 3)** than normal control group.

In contrast *G. biloba* + $MnCl_2$ and *R. officinalis* + $MnCl_2$ treated rats showed marked reduction in DNA fragmentation (in liver and brain tissues) in line with elevation in total protein content (in liver and brain tissues) and albumin (in serum) in $MnCl_2$ treated rats (**Table 3)** than $MnCl_2$ treated rats toward the normal control group.

Table (4) and (5) showed marked elevation in chromosomal aberrations in both liver and brain tissues respectively in $MnCl_2$ treated rats which in turn subsided with the administration of *G. biloba* and *R. officinalis*.

Our data showed that, oral administration of *G. biloba* and *R. officinalis* ameliorate oxidative stress in serum, liver and brain caused by $MnCl_2$ oral administration and reduced hepatic encephalopathy and restored all alterations toward normal values.

Table (1) Showed Ammonia in Serum (µmol/l) and Brain (µmol/gm Tissue), Serum Uric Acid (mg/dl), Liver and Brain Uric Acid (mg/gm tissue) and MDA in Serum (nmol/ml), MDA in Liver and Brain (nmol/gm tissue) in The Experimental Rats.

Groups / **Parameters**		**Control**	**$MnCl_2$**	**G. biloba + $MnCl_2$**	**RM + $MnCl_2$**
Ammonia level	**Serum**	37.45 ± 1.89*	130 ± 1.36†	78.69 ± 1.5≠	48.30 ± 1.29 π
	Brain	0.22 ± 0.01*	0.59 ± 0.02†	0.29 ± 0.02≠	0.24 ± 0.01 ≠*
Uric acid	**Serum**	3.33 ± 0.18*	5.92 ± 0.27†	4.57 ± 0.17≠	3.53 ± 0.11*
	Liver	0.84 ± 0.03*	1.69 ± 0.09†	1.15 ± 0.07*	0.94 ± 0.02*

	Brain	0.31 ± 0.01*	0.86 ± 0.05†	0.54 ± 0.03≠	0.43 ± 0.02*
MDA	**Serum**	12.9 ± 0.68*	26.32 ± 0.99†	18.98 ± 0.32≠	13.22 ± 0.62*
	Liver	25.84 ± 0.66*	44.6 ± 0.88†	32.91 ± 1.22≠	25.81 ± 0.64*
	Brain	59.61 ± 2.34*	78.24 ± 1.32†	59.13 ± 1.83*	54.42 ± 2.58 *

Data are expressed as means ± S.E. Data having different superscript are significant at P < 0.05.

Table (2) Explores Reduced GSH Content (mg/gm Tissue), GST Activity (U/gm Tissue), CAT Activity (mg/gm Tissue), SOD Activity (U/gm Tissue) and MDA Level in serum (nmol/ml) and in Liver and Brain (nmol/gm Tissue) and butyrylcholine esterase activity in serum (U/L) and in liver and brain (U/gm tissue) in The Experimental Rats.

Groups / **Parameters**		**Control**	**$MnCl_2$**	**G. biloba + $MnCl_2$**	**RM + $MnCl_2$**
GSH content	**Liver**	74.92 ± 1.58*	40.19 ± 2.13†	48.76 ± 2.21≠	71.06 ± 2.47*
	Brain	47.42 ± 2.68*	21.52 ± 0.97†	37.0 ± 1.77≠	37.93 ± 0.62≠
GST activity	**Liver**	48.14 ± 1.92*	20.54 ± 0.58†	33 ± 1.27≠	38.96 ± 2 π
	Brain	6.87 ± 0.36*	4.68 ± 0.32†	6.28 ± 0.42*	7.4 ± 0.34 *
CAT activity	**Liver**	8.44 ± 0.26*	4.68 ± 0.31†	7.21 ± 0.36≠	8.48 ± 0.45 *

	Brain	6.04 ± 0.32*	3.31 ± 0.22†	4.45 ± 0.45≠	5.22 ± 0.25 ≠*
SOD activity	**Liver**	50 ± 1.79*	22.23 ± 0.99†	46. 00 ± 1.39*≠	51.57 ± 1.31* π
	Brain	37.28 ± 0.80*	20.83 ± 1.01†	32.80 ± 1.07≠	38.11 ± 1.29*
Butyryl esterase activity	**serum**	485.17 ± 13†	259.8 ± 6.93*	304.8 ± 12.7≠	263.97 ± 5.44*
	Liver	138.43 ± 3.4*	32.95 ± 0.95†	103.5 ± 3.5≠	92.71 ± 2.4π
	Brain	123.26 ± 3.81*	83.28 ± 3.20†	93.94 ± 1.4≠	68.47 ± 1.09 π

Table (3) Showed Serum Albumin and Total Protein (mg/gm Tissue) and Percentage of DNA Fragmentation (%) In Liver and Brain in the Experimental Rats.

Groups		**Control**	**$MnCl_2$**	**G. biloba + $MnCl_2$**	**RM + $MnCl_2$**
Parameters					
Serum albumin		2.66 ± 0.19*	1.25 ± 0.09†	2.25 ± 0.12*	2.58 ± 0.12*
Total protein	**Liver**	169.6 ± 1.82*	92.5 ± 1.05†	120.5 ± 0.6≠	146.07 ± 1.59 π
	Brain	66.2 ± 2.06*	36.6 ± 1.13†	54.8 ± 1.2≠	56.4 ± 1.19*
(%) Of DNA fragmentation	**Liver**	15.9 ± 1.14*	40.6 ± 2.47†	21.9 ± 1.4≠	18.9 ± 1.37*≠
	Brain	13.58 ± 0.48*	39.2 ± 0.67†	22.2 ± 1.3≠	19.3 ± 0.81 π

Data are expressed as means ± S.E. Data having different superscript are significant at P < 0.05.

Table (4) Showed The Types of Brain Chromosomal Aberration in The Experimental Rats.

Groups Brain chromosomal aberration	Control	$MnCl_2$	G. biloba + $MnCl_2$	RM + $MnCl_2$
Gap	1	11	7	2
Break	2	10	5	2
Deletion	1	10	4	3
Ring	0	9	5	3
stickiness	1	12	7	2
End to end association	1	9	5	3
Total chromosomal aberration	1.00 ± 0.25*	10.16 ± 0.47†	5.50 ± 0.50$^{\neq}$	2.50 ± 0.22$^{\pi}$

Table (5) Showed The Types of Liver Chromosomal Aberration in The Experimental Rats.

Groups Liver chromosomal aberration	Control	$MnCl_2$	G. biloba + $MnCl_2$	RM + $MnCl_2$
Gap	0	9	6	2
Break	1	8	4	1
Deletion	1	7	4	2
Ring	0	6	6	1
stickiness	1	9	5	2
End to end association	0	7	5	1
Total chromosomal aberration	0.50 ± 0.22*	7.66 ± 0.49†	5.00 ± 0.36$^{\neq}$	1.50 ± 0.22*

4. DISCUSSION

Despite being an essential element, $MnCl_2$ has been proven to cause hepatic encephalopathy when present in the body for long time; it causes alterations in the oxidative stress biomarkers and in the antioxidant pathways **(Huang et al., 2011; Liapi et al., 2008)**. The discrepancy between Mn concentrations in blood and that in its targeted tissues, particularly liver and brain, is attributable to the nature of its intracellular distribution and tissue accumulation. Epidemiological and experimental studies have suggested that exposure to high concentrations of $MnCl_2$ is associated with its accumulation in major organs, particularly the liver **(Thomsen et al., 2004),** and brain **(Liccione and Maines, 1988)**. Excessive accumulation of $MnCl_2$ in the brain leads to the development of a syndrome called manganism **(Aschner et al., 2007; Normandin and Hazell, 2002)**, and accumulation in the liver causes damage to hepatocytes, resulting in reduced excretion of Mn **(Madejczyk et al., 2009)**.

In our study $MnCl_2$ challenged group exhibited significantly increased levels of both serum and brain ammonia in contrary to control group, and this was in agreement with **(Kosenko et al., 1997; Norenberg, 2003)**

who mentioned that ROS participates as a critical factor in the mechanism of hepatoencephalopathy production, especially in hyper-ammonemic conditions.

(Abraham and Maria, 2009) mentioned that the pathogenic role of portosystemic shunts and elevated blood ammonia concentrations are part of the hepatic encephalopathy syndrome as in normal subjects, intestinal ammonia, produced from nitrogen products, is taken up by the liver and metabolized to urea. Damaged livers do not accomplish this step adequately; then, portosystemic shunts are created and ammonia is sent to the systemic circulation and finally reaches the brain.

Exposure to $MnCl_2$ in our experiment was accompanied by excessive ROS production which in turn induces the oxidation of membrane polyunsaturated fatty acids, yielding a multitude of lipid peroxidation products **(Halliwell and Gutteridge, 1990),** as there was an increase in serum, liver and brain MDA levels, which indicated increased oxidative stress and lipid peroxidation. The observed elevation in lipid peroxidation can be attributed to the increased level of free radicals **(Lavie et al., 2004)**, in addition, oxidative stress associated with lipid peroxidation has been implicated in the patho-physiological mechanisms underlying several neurodegenerative brain disorders **(de la Monte et al., 2000)**, suggesting that oxidative stress is a potential contributor to the cellular injury associated with $MnCl_2$.

Butyrylcholinesterase (BchE) is enzyme that catalyses the hydrolysis of the neurotransmitter acetylcholine, a key process in the regulation of the cholinergic system **(Darvesh S. et al., 2003).** A recent study has shown that BchE can promptly and apparently substitute AchE in maintaining the structural and functional integrity of central cholinergic pathways **(Mesulam MM et al., 2002)**. Then, the inhibition of BchE might result in deleterious peripheral cholinergic effects. Moreover, as BchE is located mainly in plasma and has a lower specificity than acetylcholine esterase (AchE), its physiological role can be considered broader than that of AchE.

Referring to neurotransmitters, our present study reported a significant inhibition of serum, liver and brain BchE activity which observed following lengthy period of exposure to $MnCl_2$ in rats, and this may be attributed to that BchE prevents the hydrolysis of Ach, leading to accumulation of Ach in the synaptic cleft and over stimulation of muscarinic and nicotinic Ach receptors. Decreased activity of BchE, the enzyme responsible for Ach hydrolysis, was shown to be associated with increased oxidative stress **(Milatovic et al., 2006)**, corroborating other reports on the ability of $MnCl_2$ to increase oxidative stress **(Hamai and Bondy, 2004)**.

Such observation came in disagreement with **(Amin et al., 2011)** who reported a high BchE activity in $MnCl_2$ treated rats, which in turn decrease the vasodialting effect of acetylcholine via its hydrolysis causing vasoconstriction of brain blood vessels rendering it susceptible to dysfunction.

Regarding to the increase in uric acid content in the liver and brain tissue in $MnCl_2$ treated rats as shown in was an indication of an increase in purine metabolism that remains targeted by free radicals that produced from $MnCl_2$. These results were confirmed by the increase in percentage of DNA fragmentation and chromosomal aberrations. Interestly, elevated uric acid in serum was thought to determine whether increased levels of uric acid are associated with the presence of silent brain infarction (SBI) **(Heo and Lee, 2010).**

Glutathione (GSH), glutathione transferase (GST), catalase (CAT) and superoxide dismutase (SOD) have been commonly used as a biomarker of oxidative stress **(Erikson et al., 2004)**. Our results revealed significant reduction in GSH, GST, CAT and SOD levels in liver and brain in $MnCl_2$ exposed rats **(Marreilha dos Santos et al., 2011)** which indicate liver and brain dysfunction.

It was provoked that using of *G. biloba* in our study was just a perfect choice as it reveals a significant protective effect against $MnCl_2$ toxicity, the cellular mechanisms underlying the multiple effects of *G. biloba* can be attributed to the different constituents of the extract, which may act independently or synergistically. The most important constituents of *G. biloba* extract that contribute to its pharmacological effects include flavone glycosides (quercetin, kaempferol, and isorhamnetin) and terpene lactones (ginkgolides and bilobalide) **(Mahadevan and Park, 2008)**. The study of the underlying principle behind the therapeutic action of G. biloba on chronic ailments, such as neurodegenerative diseases, has focused on its antioxidant properties.

The two proposed mechanisms of the antioxidant action are (1) directly scavenging free radicals and (2) indirectly inhibiting the formation of free radicals. G. biloba can scavenge reactive oxygen (ROS), such as hydroxyl radicals (OH•), peroxyl radical (ROO•), superoxide anion radical (O2−), nitric oxide radical (NO•) and hydrogen peroxide (H2O2) **(Louajri et al., 2001)**. *G. biloba* can also enhance activities of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione s-transferase, and catalase thereby indirectly contributing as an antioxidant **(Atmaca et al., 2005; DeFeudis and Drieu, 2004)**, and improves cell viability, a fact that was reflected in our results in the form of increased GSH levels and GST, SOD and CAT activities, these are protective enzymes that

function against ROS-induced uncoupling of oxidative phosphorylation, and thereby *G. biloba* increases ATP levels and regulates energy metabolism **(Tendi et al., 2002)**.

Previous studies have shown that, *G. biloba* has a regulatory effect on the activities of brain acetylcholine (Ach), and it is able to increase the rate of Ach turnover and to stimulate binding activity of ligands to the muscarinic receptors **(Smith and Luo, 2003)**. Furthermore, it can reverse the beta-amyloid-inhibited Ach release, a fact that is indicated by a direct interaction of *G. biloba* on the cholinergic nerve terminals **(Lee et al., 2004)**. In addition, when administered in doses that exhibit antiamnesic activity, *G. biloba* was found to retain the expression and activity acetyl cholinesterase (AchE) in a dose-dependent manner, a fact that enhances cholinergic transmission **(Gong et al., 2006)**. These outcomes suggest that *G. biloba* may elicit its antiamnesic effect by enhancing central cholinergic transmission.

In the current study *G. biloba* protected rat liver and brain against DNA damage induced by $MnCl_2$ exposure **(Zhang et al., 2000)**. In addition, ginkgolide B and bilobalide, the main constituents of *G. biloba*, inhibit apoptosis and prevent DNA fragmentation induced by hydroxyl radical and hydrogen peroxide induced by $MnCl_2$ **(Ahlemeyer and Krieglstein, 2003).**

In addition to its direct protective effect against ROS and induced DNA damage, *G. biloba* can stimulate the DNA repair machinery via protection against the ROS inhibitory effects and direct stimulation to DNA repair, in our results, *G. biloba* decreased both liver and brain chromosomal aberration levels. These findings show the ability of it to protect against oxidative DNA damage not only centrally but also in the peripheral tissues in animals exposed to $MnCl_2$, a fact that suggests its usefulness in the protection against central and peripheral morbidities that may result from chronic exposure to $MnCl_2$ **(Marques et al., 2011).**

There is evidence that the aqueous extracts of the leaves of R. officinalis L. have a large number of pharmacological **(Nabekuraa et al., 2010; Pereira et al., 2005)** properties, including hepatoprotective **(Sotelo-Félix et al., 2002)** and antioxidant activities. **(Bakirel et al., 2008)** concluded that *R. officinalis* water extract and its antioxidant compounds inhibit lipid peroxidations and free radicals generation in vitro and in vivo and this was in agreement with our finding in which the level of MDA in serum, brain and liver tissues was significantly reduced confirming inhibition of lipid peroxidation.

The active compounds of *R. officinalis* extracts are mainly phenolic compounds, which belong to three groups: phenolic diterpenes, flavonoids and phenolic acids **(Mulinaccia et al., 2011)**. Carnosic acid which is the major phenolic diterpene present in RM leaves and carnosol, both of which have been suggested to account for over 90% of the antioxidant properties of *R. officinalis* extract ,and rosmarinic acid, rosmanol, a hydroxyl cinnamic acid ester, are the main antioxidant compounds present in RM **(Al Sheyab et al ., 2012)**.

Several biological activities of some of these compounds of *R. officinalis* extracts are probably linked to their ability to reduce the oxidative damage caused by free radicals over cellular elements like DNA, proteins or membrane phospholipids. It is generally assumed that these antioxidant molecules from *R. officinalis* may act as free radical scavengers but additionally might play a role by regulating the activity and/or expression of certain enzymatic systems implicated in relevant physiological processes like apoptosis, or xenobiotic-metabolizing enzymes in liver **(Offord et al., 2002; Fawcett et al., 2002)** Consequently, the effects of *R. officinalis* on impaired Ach-stimulated vasodilation was through restoring the brain GSH, CAT, GST, SOD level and decrease brain MDA compared to the $MnCl_2$ treated rats. This support the hypothesis that oxidative stress contributes to brain and liver dysfunction, and this findings came in agreement with ours as there was a significant induction of antioxidant enzymes (GSH, GST, CAT and SOD).

In this study, inclusion of *R. officinalis* extract provided an anti-lipoperoxidant activity, as it reduced the formation of MDA, DNA fragmentation, chromosomal aberrations, and uric acid and ammonia levels, thus confirming the anti-encephalohepatotoxic action of *R. officinalis* extract **(Fahim et al., 1999; Posadas et al., 2009)**.

Conflict of interest statement:

None of the authors has any financial interest or conflict of interest related to the manuscript.

CONCLUSION

Manganese is an essential nutrient, which can be harmful at higher doses or accumulations. As Mn toxicity can cause a number of functional problems, such as manganism, it is imperative that we understand the mechanisms of damage to attempt to prevent or counter them.

In conclusion, $MnCl_2$ accumulates specifically in the mitochondrial matrix, where it increases the rate of free radicals production leading to initiation of the apoptotic processes. It must be recalled that the presence of oxidative stress in liver and brain without adequate defenses aggravates the hepatoencephalopathic condition.

In our study, treatment with *G. biloba* and *R. officinalis* was significantly reversed the deleterious effects of $MnCl_2$. Although they are widely exerted, there are only few reports available analyzing the molecular events establishing these therapeutic attributes to heavy metals especially manganese, so our study is of significantly important.

Because of the ease of administration and low cost and low toxicity of *G. biloba* and *R. officinalis*, it is of potential use in the therapy of manganese toxicity.

Future research endeavors should be aimed not only at pinpointing the mechanisms underlying the damage but also at identifying biomarkers for exposure and treatment strategies.

REFFERENCES

Abraham Lemberg; Maria Alejandra Fernández (2009). Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress. Annals of Hepatology. 8(2): April-June: 95-102.

Aebi, H. (1984): Catalase in vitro. Methods Enzymol 105: 121-126.

Ahlemeyer, B., Krieglstein, J., (2003). Neuroprotective effects of Ginkgo biloba extract. Cell. Mol. Life Sci. 60 (9): 1779–1792.

Al Sheyab, F.M., N. Abuharfeil, L. Salloum, R Bani Hani and D.S. Awad (2012). The effect of rosemary (Rosmarinus officinalis. L) plant extracts on the immune response and lipid profile in mice. Journal of Biology and Life Science, 3(1): 1-22.

Amin, K.A., Kamel, H. H. and Abd Eltawab, M. A. (2011). The relation of high fat diet, metabolic disturbances and brain oxidative dysfunction: modulation by hydroxy citric acid. Lipids in Health and Disease. 10:74.

Arushanian, E.B., Beˇıer, E.V., (2008). Ginkgo biloba as a cognitive enhancer. Eksp. Klin. Farmakol. 71 (4): 57–63.

Aschner M, Guilarte TR, Schneider JS, Zheng W. (2007). Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol. 221:131–47.

Atmaca, M., Tezcan, E., Kuloglu, M., Ustundag, B., Kirtas, O., (2005). The effect of extract of Ginkgo biloba addition to olanzapine on therapeutic effect and antioxidant enzyme levels in patients with schizophrenia. Psychiatry Clin. Neurosci. 59 (6): 652–656.

Au C, Benedetto A, Aschner M. (2008) Manganese transport in eukaryotes: the role of DMT1. Neurotoxicology. 29: 569–76.

Bakirel, T., Bakirel, U., Keles, O.U., Ülgen, S.G., Yardibi, H., (2008). In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. Journal of Ethnopharmacology. 116: 64–73.

Beutler, E.; Duran, O. and Kelly, B. (1963): Improved method for the determination of blood glutathione. J. of lab. And clinic. Med., 61: 882.

Burton, N.C., Guilarte, T.R., (2009). Manganese neurotoxicity: lessons learned from longitudinal studies in nonhuman primates. Environ. Health Perspect. 117: 325–332.

Chen MT, Cheng GW, Lin CC, Chen BH, Huang YL. (2006). Effects of acute manganese chloride exposure on lipid peroxidation and alteration of trace metals in rat brain. Biol Trace Elem Res;110:163–78.

Chtourou Y, Fetoui H, Sefi M, Trabelsi K, Barkallah M, Boudawara T, et al. Silymarin (2010), a natural antioxidant, protects cerebral cortex against manganese-induced neurotoxicity in adult rats. Biometals. 23:985–96.

Crossgrove, J., Zheng, W., (2004). Manganese toxicity upon overexposure. NMR Biomed. 17 (8): 544–553.

Darvesh S, Hopkins DA, Geula C. (2003). Neurobiology of butyrylcholinesterase. Nat Rev Neurosci. 17:131–8.

De la Monte, S.M., Neely, T.R., Cannon, J., Wands, J.R., (2000). Oxidative stress and hypoxia-like injury cause Alzheimer-type molecular abnormalities in central nervous system neurons. Cell. Mol. Life Sci. 57 (10): 1471–1481.

DeFeudis, F.V, Drieu, K., (2004). "Stress-alleviating" and "vigilance-enhancing" actions of Ginkgo biloba extract (EGb 761). Drug Dev. Res. 62: 1–25.

Dos Santos AP, Milatovic D, Au C, Yin Z, Batoreu MC, Aschner M (2010). Rat brain endothelial cells are a target of manganese toxicity. Brain Res;1326:152–61.

Dukhande, V.V., Malthankar-Phatak, G.H., Hugus, J.J., Daniels, C.K., Lai, J.C., (2006). Manganese- induced neurotoxicity is differentially enhanced by glutathione depletion in astrocytoma and neuroblastoma cells. Neurochem. Res. 31: 1349–1357.

Erikson, K.M., Dorman, D.C., Lash, L.H., Dobson, A.W., Aschner, M., (2004). Airborne manganese exposure differentially affects end points of oxidative stress in an age and sex-dependent manner. Biol. Trace Elem. Res. 100 (1): 49–62.

Fahim, F., A. Esmat, H. Fadel and K. Hassan, (1999). Allied studies on the effect of Rosemarinus officinalis L. on experimental hepatotoxicity and mutagenesis. International Journal of Food Sciences and Nutrition, 50: 413-427.

Fawcett, Bordayo, Jackson, Liu, Peterson, Svitak, Frey 2nd, Brain Res. (2002). 950: 10–20.

Gong, Q.H., Wu, Q., Huang, X.N., Sun, A.S., Nie, J., Shi, J.S., (2006). Protective effect of Ginkgo biloba leaf extract on learning and memory deficit induced by aluminum in model rats. Chin. J. Integr. Med. 12 (1): 37–41.

Golub MS, Hogrefe CE, Germann SL, Tran TT, Beard JL, Crinella FM, et al. (2005) Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese. Neurotoxicol Teratol; 27: 615–27.

Habig, W.; Pabst, M. and Jakoby, W. (1974): The first enzymatic step in mercapturic acid formationJ. Biol. Chem., 249: 7130- 7139.

Halliwell, B., Gutteridge, J., (1990). Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 186: 1–85.

Haloui, M., Louedec, L., Michel, J.B., Lyoussi, B., (2000). Experimental diuretic effects of Rosmarinus officinalis and Centaurium erythraea. Journal of Ethnopharmacology 71: 465–472.

Hamai, D., Bondy, S., (2004). Oxidative basis of manganese neurotoxicity. Ann. N. Y. Acad. Sci. 1012: 129–141.

Heo, S.H. and Lee, S.H. (2010): High levels of serum uric acid are associated with silent brain infarction. J Neurol Sci, 297(1-2): 6-10

Huang P, Chen C, Wang H, Li G, Jing H, Han Y, et al. (2011) Manganese effects in the liver following subacute or subchronic manganese chloride exposure in rats. Ecotoxicol Environ Saf;74:615–22.

Jiao, J., Qi, Y., Fu, J., Zhou, Z., (2008). Manganese-induced single strand breaks of mitochondrial DNA in vitro and in vivo. Environmental Toxicology and Pharmacology 26: 123–127.

Jover R, Rodrigo R, Felipo V, Insausti R, Sa´ez-Valero J, Garc´ıa-Ayllo´n MS,et al. (2006): Brain edema and inflammatory activation in bile duct ligated rats with diet-induced hyperammonemia: a model of hepatic encephalopathy in cirrhosis. Hepatology; 43: 1257–66.

Kelly, J.P., Kaufman, D.W., Kelley, K., Rosenberg, L., Anderson, T.E., Mitchell, A.A., (2005). Recent trends in use of herbal and other natural products. Arch. Intern. Med. 165: 281–286.

Kosenko E, Kaminsky Y, Kaminsky A, Valencia M, Lee L, Hermenegildo C, et al (1997). Superoxide production and antioxidant enzymes in ammonia intoxication in rats. Free Radic Res; 27: 637-644.

Lavie, L., Vishnevsky, A., Lavie, P., (2004). Evidence for lipid peroxidation in obstructive sleep apnea. Sleep 27: 123–128.

Lee, T.F., Chen, C.F., Wang, L.C., (2004). Effect of ginkgolides on beta-amyloidsuppressed acetylocholine release from rat hippocampal slices. Phytother. Res. 18 (7): 556–560.

Lian Wang , YingBai , BoWang , HaoCui , HaoWu , Jin-RuLv , YongMei , Jin-Song Zhang , ShengLiu , Lian-WenQi , YanChen. (2013) Suppression of experimental abdominal aortic aneurysms in the mice by treatment with Ginkgo biloba extract(EGb761) . Journal of Ethnopharmacology150: 308–315

Liapi C, Zarros A, Galanopoulou P, Theocharis S, Skandali N, Al-Humadi H, et al. (2008) Effects of short-term exposure to manganese on the adult rat brain antioxidant status and the activities of acetylcholinesterase, (Na,K)-ATPase and Mg-ATPase: modulation
by L-cysteine. Basic Clin Pharmacol Toxicol;103:171–5.

Liccione JJ, Maines MD (1988). Selective vulnerability of glutathione metabolism and cellular defense mechanisms in rat striatum to manganese. J Pharmacol Exp Ther;247:156–61.

Louajri, A., Harraga, S., Godot, V., Toubin, G., Kantelip, J.P., Magnin, P., (2001). The effect of Ginkgo biloba extract on free radical production in hypoxic rats. Biol. Pharm. Bull. 24 (6): 710–712.

Madejczyk MS, Boyer JL, Ballatori N. (2009) Hepatic uptake and biliary excretion of manganese in the little skate, Leucoraja erinacea. Comp Biochem Physiol C Toxicol Pharmacol;149:566–71.

Mahadevan, S., Park, Y., (2008). Multifaceted therapeutic benefits of Ginkgo biloba L.: chemistry, efficacy, safety, and uses. Journal of Food Science 73: 14–19.

Malecki, E.A., (2001). Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res. Bull. 55 (2): 225–228.

Marques, F., Azevedo, F., Johansson, B., Oliveira, R., (2011). Stimulation of DNA repair in Saccharomyces cerevisiae by Ginkgo biloba leaf extract. Food Chem. Toxicol. 49 (6): 1361–1366.

Marreilha dos Santos, A.P., Lopes Santos, M., Batoreu, M.C., Aschner, M., (2011). Prolactin is a peripheral marker of manganese neurotoxicity. Brain Res. 1382: 282–290.

Martins, E.N., Pessano, N.T.C., Leal, L., Roos, D. H., Folmer, V., Puntel, G O., Rocha, J B T, Aschner, M, Ávilaa, D. S and Puntel, R. L. (2012): Protective effect of Melissa officinalis aqueous extract against Mn-induced oxidative stress in chronically exposed mice. Brain Research Bulletin., 87: 74–79

Mesulam MM, Guillozet A, Shaw P, Levey A, Duysen EG, Lockridge O. (2002) Acethylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyse acetylcholine. Neuroscience;110:627–39.

Milatovic D, Gupta RC, Yu Y, Zaja-Milatovic S, Aschner M. (2011) Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury. Toxicol Appl Pharmacol . doi: 10.1016/ j.taap. 06.001.

Milatovic D, Gupta RC, Aschner M. (2006) Anticholinesterase toxicity and oxidative stress. Sci World J ;6:295–310.

Mulinaccia, N., Innocentia, M., Bellumoria, M., Giaccherinia, C., Martinib, V., Michelozzib, M., (2009). The Scientific Foundation for Herbal Medicinal Products, second ed. European on Phytotherapy Scientific Cooperative, pp. 429–436.

Mulinaccia, N., Innocentia, M., Bellumoria, M., Giaccherinia, C., Martinib, V., Michelozzib, M., (2011). Storage method, drying processes and extraction procedures strongly affect the phenolic fraction of rosemary leaves: an HPLC/ DAD/MS study. Talanta 85: 167–176.

Muthuviveganandavel, V.; Muthuraman, P.; Muthu S. and Srikumar K. (2011): Individual and combined biochemical and histological effect of Cypermethrin and Carbendazim in male albino rats. Journal of Applied Pharmaceutical Science 01 (09): 121-129.

Nabekuraa, T., Yamakia, T., Hiroia, T., Uenoa, K., Kitagawab, S., (2010). Inhibition of anticancer drug efflux transporter P-glycoprotein by rosemary phytochemicals. Pharmacological Research 61: 259–263.

Nagpure, N.S., Kumar, R., Kushwaha, B., Singh, P.J. and Srivastava, S.K. (2007). Genotoxicity Assessment in Fishes –A Practical Approach. National Bureau of fish genetic resource. Lucknow India. p. 63

Nishikimi, M.; Rao, N. and Yogi, K. (1972): The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research . Common. 46: 849– 854.

Norenberg MD. (2003) Oxidative and nitrosative stress in ammonia neurotoxicity. Hepatology; 37: 245-248.

Normandin, L., Hazell, A.S., (2002).Manganese neurotoxicity: an update of pathophysiologic mechanisms. Metab. Brain Dis. 17: 375–387.

Offord, Gautier, Avanti, Scaletta, Runge, Kramer, Applegate, (2002) Free Radic. Biol. Med. 32: 1293–1303.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem., 95:351- 358.

Paradones E, Illera A, Peckham D, Stunz L and Ashman F (1993): Regulation of apoptosis in vitro in mature spleen T cell. J Immunol; 151: 3521-3529.

Pereira, P., Tysca, D., Oliveira, P., Bruma, L.F.S., Picada, J.N., Ardenghi, P., (2005). Neurobehavioral and genotoxic aspects of rosmarinic acid. Pharmacological Research 52: 199–203.

Posadas, S.J., V. Caz, C. Largo, De la B. Gndara, B. Matallanas, G. Reglero and E. De Miguel, (2009). Protective effect of supercritical fluid rosemary extract, Rosmarinus officinalis on antioxidants of major organs of aged rats. Experimental Gerontology, 44: 383-389.

Reaney SH, Smith DR. (2005) Manganese oxidation state mediates toxicity in PC12 cells. Toxicol Appl Pharmacol; 205:271–81.

Reyes, galindo, garcía, segura-peña, caruso-neves, eblen-zajjur, proverbio, marín, proverbio. Ouabain-Insensitive,(2009) Na+-Stimulated ATPase of Several Rat Tissues: Activity during a 24 h Period. Physiol. Res. 58: 693-699.

Roth JA, Horbinski C, Higgins D, Lein P, Garrick MD.(2002) Mechanisms of manganese-induced rat pheochromocytoma (PC12) cell death and cell differentiation. Neurotoxicology;23:147–57.

Sa´ez-Valero J, Tornel PL, Mun˜oz-Delgado E, Vidal CJ (1993): Amphiphilic and hydrophilic forms of acetyl- and butyrylcholinesterase in human brain. J Neurosci Res 35:678–689.

Saleem, S., Zhuang, H., Biswal, S., Christen, Y., Dore, S., (2008). Ginkgo biloba extract neuroprotective action is dependent on heme oxygenase 1 in ischemic reperfusion brain injury. Stroke 39 (12): 3389–3396.

Smith, J.V., Luo, Y., (2003). Elevation of oxidative free radicals in Alzheimer's disease models can be attenuated by Ginkgo biloba extract EGb 761. J. Alzheimers Dis. 5: 287–300.

Sotelo-Félix, J.I., Martinez-Fong, D., Muriel, P., Santillán, R.L., Castillo, D., Yahuaca, P., (2002). Evaluation of the effectiveness of Rosmarinus officinalis (Lamiaceae) in the alleviation of carbon tetrachloride-induced acute hepatotoxicity in the rat. Journal of Ethnopharmacology 81: 145–154.

Taylor MD, Erikson KM, Dobson AW, Fitsanakis VA, Dorman DC, Aschner M. (2006) Effects of inhaled manganese on biomarkers of oxidative stress in the rat brain. Neurotoxicology; 27:788–97.

Tendi, E.A., Bosetti, F., Dasgupta, S.F., Stella, A.M., Drieu, K., Rapoport, S.I., (2002). Ginkgo biloba extracts EGb 761 and bilobalide increase NADH dehydrogenase mRNA level and mitochondrial respiratory control ratio in PC12 cells. Neurochem. Res. 27: 319–323.

Thomsen HS, Svendsen O, Klastrup S. (2004) Increased manganese concentration in the liver after oral intake. Acad Radiol;11:38–44.

Zhang, W.R., Hayashi, T., Kitagawa, H., Sasaki, C., Sakai, K., Warita, H., et al., (2000). Protective effect of ginkgo extract on rat brain with transient middle cerebral artery occlusion. Neurol. Res. 22 (5): 517–521.

Zheng W, Fu SX, Dydak U, Cowan DM. (2011) Biomarkers of manganese intoxication. Neurotoxicology;32:1–8.