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The Biological and Biochemical Study to Mitigate the Toxicity of Manganese in Experimental Animals

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Abstract

This study was conducted to investigate the effect of manganese toxicity by manganese chloride (MnCl_2) on the experimental animals and to evaluate the efficacy of rice bran and/or ascorbic acid in attenuating the deleterious effect induced by manganese chloride toxicity. For this purpose, fifty-four healthy rabbits weighing $1655 \pm 367.07\text{g}$ were divided into nine groups each of six rabbits. Group 1; rabbits fed on commercial diet and normal water served as normal control. Group 2; rabbits fed on commercial diet and received 200 mg/L of MnCl_2 in drinking water. Group 3; rabbits received MnCl_2 in drinking water (200 mg/L) and fed on commercial diet supplemented with 200 g dried powder of rice bran added to 1 kg diet as a source for phytic acid. Group 4; rabbits fed on commercial diet and received 200 mg/L MnCl_2 in drinking water and orally administered with ascorbic acid (30 mg/kg body weight daily). Group 5; rabbits received MnCl_2 in drinking water (200 mg/L) and supplemented with rice bran (200 g/kg diet) and orally administered ascorbic acid (30 mg/kg body weight daily) by intragastric tube. Group 6; Rabbits were fed commercial diet and injected intravitreally with MnCl_2 (25 μl twice/week). Group 7; Rabbits were injected intravitreally with MnCl_2 and fed rice bran (200 g/kg diet). Group 8; Rabbits were injected intravitreally with MnCl_2 and were orally administered ascorbic acid (30 mg/kg body weight daily) by intragastric tube. Group 9; Rabbits were

injected intravitreally with MnCl_2 and fed rice bran (200 g /kg diet) and were orally administered ascorbic acid (30 mg/kg body weight daily) by intragastric tube. Results showed that MnCl_2 intoxication significantly reduced haemoglobin (Hb) concentration and serum iron with a significant increase in total iron binding capacity. Also, it induced a significant increase in malondialdehyde (MDA) level accompanied by a significant decrease in reduced glutathione (GSH) concentration and superoxide dismutase (SOD) activity. Moreover, MnCl_2 intoxication caused a significant increase in serum alanine transaminase (ALT), aspartate transaminase (AST) activities. Also, serum urea and creatinine significantly elevated in MnCl_2 intoxicated group. An improvement was noticed in these altered parameters after oral administration of rice bran and/or ascorbic acid. Histopathological examination showed retinal detachment in oral MnCl_2 intoxicated group and destruction of retinal layers in injected MnCl_2 group. On the other hand, oral administration with rice bran + ascorbic acid prevented retinal detachment and have protective effect on retina due to the strong chelating power of rice bran and antioxidant properties of ascorbic acid.

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List of Abbreviations

ALT	Alanine aminotransferase
ARVO	The association for research in vision and ophthalmology
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BBB	Blood brain barrier
CAT	Catalase
CNS	Central nervous system
CSF	Cerebrospinal fluid
D.F	Dilution factor
DMT-1	Divalent metal ion transporter -1
DNA	Deoxyribonucleic acid
DTNB	Dithio-bis-2-nitrobenzoic acid
EFSA	European Food Safety Authority
ER	Endoplasmic reticulum
ETC	Electron transport chain
FDA	Food and Drug Administration
Fe	Iron
GI	Gastrointestinal
GPx	Glutathione peroxidase
GS	Glutamine synthetase
GSH	Reduced glutathione
Hb	Hemoglobin
Hcl	Hydrochloric acid
HIV-1	Human immunodeficiency virus
IOP	Intraocular pressure
IP6	Inositol hexaphosphate
MANEB	Mn ethylenebis-dithiocarbamate
MDA	Malondialdehyde
MEMRI	Manganese-enhanced magnetic resonance image
MMT	Methylcyclopentadienyl manganese tricarbonyl

Mn	Manganese
MnCl ₂	Manganese chloride
Mn-SOD	Mn superoxide dismutase
Na ₂ EDTA	disodium ethylene di amine tetra acetic acid
NAC	N-acetyl cysteine
NAS	National Academy of Science
NK	Natural killer cells
NRC	National Research Council
O.D	Optical density
OZ	Oryzanol
PD	Parkinson's disease
P-R	Pulse rate
RB	Rice bran
RBE	Rice bran extract
RBO	Rice bran oil
RGCs	Retinal ganglion cells
ROS	Reactive oxygen species
SEPO	Schirmer strips
SOD	Superoxide dismutase activity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TIBC	Total iron binding capacity
USEPA	United States Environmental Protection Agency
WHO	World health organization

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Introduction

Manganese, one of the essential elements for the living body, is required for protein, lipid and carbohydrate metabolism and for the normal function and development of the brain. Manganese (Mn) is an essential nutrient for intracellular activities; it acts as a cofactor for a variety of enzymes, including arginase, pyruvate carboxylase, glutamine synthetase (GS) and Mn superoxide dismutase (Mn-SOD). Through these metalloproteins, Mn plays an important role in development, digestion, reproduction, antioxidant defense, energy production, immune response and regulation of neuronal activities (*Chtourou et al., 2011 and Chen et al., 2018*).

Dietary consumption is the primary route of Mn intake for majority of people. Drinking water contains Mn levels ranging from 1 µg/L up to 2 mg/L depending on the locations and contamination. Drinking water, Mn-rich vegetables, nuts, vitamins, supplements and infant formula are the major food source of Mn. Although Mn is required for various physiological activities, accumulation of excessive Mn in human body results in toxicity (*Frisbie et al., 2002 and Roth, 2006*).

Mn toxicity may be encountered upon overexposure to this metal. Excessive Mn tends to accumulate in the liver, pancreas, bone, kidney and brain, which is the major target of Mn intoxication. Also, Hepatic cirrhosis, polycythemia, hypermanganesemia, dystonia and Parkinsonism-like

symptoms have been reported in patients with Mn poisoning. Molecular mechanisms of Mn toxicity include oxidative stress which occurs when the antioxidant defense system is overwhelmed by the production of reactive oxygen species that induce lipid peroxidation and protein oxidation, hence potentially causing cell death (*Chen et al., 2018*).

Mn overexposure can also impair cardiovascular function, causing abnormal electrocardiogram, increased heartbeat, shorter pulse rate (P-R) interval and lower diastolic blood pressure, liver impairments with elevated transaminases and unconjugated hyperbilirubinemia (*Jiang and Zheng, 2005* and *Tuschl et al., 2012*).

Mn is widely used in various industrial settings. In manufacture, Mn is incorporated in production of batteries, steel, ceramics, cosmetics, fireworks, leather and glass. In energy consumption, Mn used as gasoline additive in the form of methylcyclopentadienyl Mn tricarbonyl (MMT) (*ATSDR, 2008 and 2012*).

Every day people have contact with Mn through environmental, occupational and medical exposure routes. Inhalation of airborne Mn is the major exposure route in occupational Mn intoxication. Industrial workers, especially miners, smelters and welders, breathe in a significant amount of Mn-containing fume and dust, thus the adult population are with the highest risk for Mn-induced toxicity. Inhaled Mn is absorbed in the lung and enters the circulation. It can be rapidly transported to the olfactory bulb and enter the brain

bypassing the liver and blood brain barrier (BBB) (*Bowler et al., 2011 and Chen et al., 2015*).

The present study focused on the deleterious effect of MnCl_2 toxicity either orally or intravitreally injected in the eye. Also, the protective effect of rice bran and/or ascorbic acid against MnCl_2 toxicity was studied. To our knowledge, this is the first work which showed that rice bran and/or ascorbic acid protects body cells and eye against Mn-induced ROS generation and glutathione depletion by increasing the antioxidant capacity.

In the current study, rabbits were used as the experimental model, which was different from mouse models used in prior studies (*Sun et al., 2011; 2012 and Lin et al., 2014*). The rabbit model is more suitable for studying the progress of disease over time in humans and provides better access to the ocular surface, making evaluation of the signs of damage more reliable (*Schrader et al., 2008*). In addition, mouse and rat eyes are too small to provide enough aqueous and vitreous humor samples for intraocular Mn^{2+} concentration analysis.

Rice bran is a rich source of essential nutrients like carbohydrates, proteins, fats, vitamins, fibers and antioxidants. Also, it contains micronutrients like tocotrienols, oryzanol, tocopherols and phytosterols which comprises vitamin E and exhibit significant antioxidant activity and possesses several health benefits. Due to its beneficial nutritive and biological effects, it can be incorporated into functional foods. The

nutritional composition of rice bran confirms its potential for the development of food products (*Sharma et al., 2015*).

Phytic acid (known as inositol hexaphosphate, IP6, or phytate when in salt form) is an organic acid extracted from rice bran. Rice bran due to its high content of phytic acid is used to reduce manganese toxicity. Phytic acid binds to metals strongly because of its strong chelating effect. Moreover, phytic acid shows antioxidant action and prevention of color degradation. The most outstanding feature of phytic acid is its strong metal chelating function (*Saad et al., 2011*)

Ascorbic acid (vitamin C) is a micronutrient affecting nuclear cataract progression. Vitamin C intake has long been studied in relation to age-related cataract. A significant concentration of ascorbate is present in the aqueous humor that bathes the lens and may reduce oxidation products in the lens, thus reducing oxidative stress (*Shui et al., 2009 and Beebe et al., 2010*).

The protective effect of rice bran and/or ascorbic acid against oxidative stress induced by $MnCl_2$ toxicity was studied by assessing biochemical parameters, in order to evaluate enzymatic antioxidant defenses, including reduced glutathione, superoxide dismutase and oxidative stress marker, including lipid peroxidation (MDA).