Effect of Phytic Acid and/or Ascorbic Acid to Mitigate Manganese Toxicity in Experimental Animals

Tahany E. Kholief*, Anhar, M. Gomaa**, Enas Ali Kamel*; and Samar Lotfy Mahmoud*

*Biochemistry and Nutrition Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
** Biochemistry Department, Research Institute of Ophthalmology, Cairo, Egypt.

Abstract
This study was conducted to investigate the effect of manganese toxicity by manganese chloride (MnCl₂) on the experimental animals and to evaluate the efficacy of phytic acid and/or ascorbic acid in attenuating the deleterious effect induced by manganese toxicity. For this purpose, thirty healthy rabbits weighing 1655±367.07g were divided into five 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 MnCl₂ in drinking water. Group 3; rabbits received MnCl₂ in drinking water (200mg/L) and fed on commercial diet supplemented with phytic acid (20 g /kg diet). Group 4; rabbits fed on commercial diet and received 200 mg/L MnCl₂ in drinking water and orally administrated with ascorbic acid (30mg/kg body weight daily). Group 5; rabbits received MnCl₂ in drinking water (200mg/L) and fed phytic acid (20 g /kg diet) and orally administrated ascorbic acid (30mg/kg body weight daily) by intragastric tube. Results showed that MnCl₂ 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, MnCl₂ intoxication caused a significant increase in serum alanine transaminase (ALT), aspartate transaminase (AST) activities. Also, serum urea and creatinine significantly elevated in MnCl₂ intoxicated group. An improvement was noticed in these altered parameters after oral administration of phytic acid and/or ascorbic acid.

Key words: Manganese toxicity; phytic acid; ascorbic acids; oxidative stress.

Introduction
Manganese is an essential element in human diet. Manganese is required for the regulation of reproduction, carbohydrate and lipid metabolism, bone development, wound healing, prevention of sterility and normal brain function. Manganese acts as a cofactor with many enzymes throughout the body as arginase and pyruvate carboxylase, also as a cofactor for superoxide dismutase, which is critical for prevention of oxidative stress (Jankovic, 2005).

Manganese intoxication, often termed "Manganism" is related to long-term high level occupational exposure to the metal or its inorganic compounds. The primary source of manganese-induced intoxication in human is by means of occupational exposure in miners, smelters, welders and dry-cell battery employees (Bowler et al., 2006). Manganese toxicity has also been reported by ingestion in patients receiving long-term parenteral nutrition (Dickerson, 2001). Also, patients with chronic liver dysfunction or renal failure, as a result of their inability for elimination and clearance of manganese from blood (Ikeda et al., 2000).
Human study further confirms the oxidative damage among welders exposed to airborne Mn (Li et al., 2004). Manganese toxicity induced neurodegenerative diseases have been demonstrated (Bowman et al., 2011), but little is known concerning the adverse effects of the element on the eye.

Reduction in manganese toxicity, other than reduced exposure, may be aided by supplementing the antagonistic nutrients. High dietary levels of fiber, phytate, ascorbic acid, iron, phosphorus, and calcium can limit the oral bioavailability and retention of manganese (NAS, 2001). Thus, Mn toxicity and severity can be reduced by cellular antioxidants. The beneficial role of antioxidant vitamins and other dietary factors in improving manganese toxicity remains unclear.

The present study aims to investigate the effects of manganese toxicity on the retina and to evaluate the role of phytic acid and ascorbic acid in attenuating the deleterious effect caused by manganese toxicity.

Materials and Methods:

Chemicals:
All chemicals were purchased from Sigma Chemical Co (USA). An extra pure analar form of Manganese chloride (MnCl₂) molecular weight 197.91 g, from (Prolabo 12, Rue Pelee * 75-PARIS X1) was used for experimental model of manganese toxicity.

Diet:
A commercial diet from The Animal House of Research Institute of Ophthalmology was used as basal diet. The commercial diet consists mainly of not more than 64% carbohydrates, not less than 21% protein, not less than 6% fat, not less than 3% fiber, and not less than 6% of vitamins and minerals mix, methionine and choline chloride (National Research Council, 1995).

Experimental animal design:
Thirty New Zealand housed rabbits were purchased and randomly individually in polyethylene cages. The experimental protocol was approved by the local ethical committee that applies ARVO (The Association for Research in Vision and Ophthalmology) statements for using animals in ophthalmic and vision research. Rabbits were divided into five groups each group contain six rabbits. The groups were classified as follows:

Group 1; rabbits fed on commercial diet and normal water served as normal control.

Group 2; rabbits fed on commercial diet and received MnCl₂ in drinking water (200mg/L) served as +ve control.

Group 3; rabbits received MnCl₂ in drinking water (200mg/L) and fed on phytic acid (20 g /kg diet).

Group 4; rabbits fed on commercial diet and received 200 mg/L MnCl₂ in drinking water and orally administrated with ascorbic acid (30mg/kg body weight daily).

Group 5; rabbits received MnCl₂ in drinking water (200mg/L) and fed on phytic acid (20 g /kg diet). and orally administrated ascorbic acid (30mg/kg body weight daily) by intragastric tube.
At the end of experiment, after 8 weeks, all rabbits were fasted overnight, anesthetized, and blood was collected from eye canthus (Retro-orbital blood collection). Blood samples were collected using two separated tubes. The first tube contained ethylene diamine tetra acetic acid (EDTA) and the second tube was used to separate serum by centrifuging at 3500 r.p.m for 10 minutes.

**Preparation of phytic acid**

The rice bran was purchased from the agricultural research center then, mixed with water and dried as possible, protected from direct sunlight, and then added to the commercial diet (20%) used as pellets. Two hundred g of dried powder of rice bran added to 1 kg diet (each 100 g of rice bran contain 10 g phytic acid according to the method described by **Mohamed et al. (1986)**).

**Preparation of ascorbic acid solution:**

Thirty mg of dried powder of ascorbic acid/kg body weight was dissolved in water and given orally daily with intragastric tube (30mg/kg body weight) (**Oliveira et al., 2003**).

**Induction of MnCl₂ toxicity:**

Pure form of Manganese chloride (MnCl₂) was used for induction in experimental animal model of manganese toxicity. Manganese was prepared in drinking water (200mg/L) as described by **Ishizuka et al. (1991)**.

**Biochemical measurements**

Manganese was determined in serum by atomic absorption spectroscopy, using a graphite furnace with a temperature ramp controller (**Sohler et al. 1979**). Hemoglobin was determined according to the colorimetric method described by **Drabkins and Austin (1957)**. Iron was determined according to the colorimetric method described by **Dreux (1977)**. Total iron binding capacity (TIBC) was determined according to the colorimetric method described by **Piccardi et al. (1972)**. Reduced glutathione (GSH) was determined according to the colorimetric method described by **Beutler et al. (1963)**. The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol according to the method described by **Marklund and Marklund (1974)**. Malondialdehyde (MDA) was determined in serum according to the method described by **Mihara and Uchiyama (1978)**. AST and ALT activities were determined according to the colorimetric method described by **Reitman and Frankel (1957)**. Urea was determined according to the colorimetric method described by **Fawcett and Scott (1960)**. Creatinine was determined according to the colorimetric method described by **Schirmeister (1964)**.

**Statistical analysis:**

Data were presented as the mean ± standard deviation (SD) compare between multiple groups, the analysis of variance (ANOVA), mean and standard deviation were descriptive measures of data. Least significant difference (LSD) multiple comparison test was then carried out and employed using a commercially available software package (SPSS- 10 for
windows, SPSS Inc, Chicago, IL, USA). The results were considered to be significant at $P \leq 0.05$.

**Results and Discussion:**

Results in table (1) showed that oral administration with MnCl$_2$ induced a significant increase in serum manganese level as compared to normal control group. The results of Woolf *et al.* (2002) are similar to the results of the current study who found abnormal verbal and visual memory function with elevated serum manganese concentrations when induced high concentration of manganese in drinking water.

Despite its essentiality, high concentrations of Mn$^{2+}$ is neurotoxin *(Crossgrove and Zheng, 2004 and Olanow, 2004)*. More recent reports suggest that aspects of the disease may occur in individuals exposed to Mn from environmental sources *(Lucchini et al., 2014)*. Manganese in drinking water was also another route of manganese toxicity *(ATSDR, 2000)*. Phytic acid consumption provides protection against heavy metals toxicity through metal chelation and antioxidant properties *(Vikas et al., 2010)*. Vitamin C is an important free radical scavenger that may be also used to reduce manganese toxicity.

In the current study, Coadministration of phytic acid with MnCl$_2$ showed a significant decrease in serum manganese level as compared to MnCl$_2$ group. This is similar to the results of Lonnerdal *et al.* (1999) who found that phytic acid can reduce manganese bioavailability and reduce Mn level in blood. Results also showed that coadministration of ascorbic acid either alone or in combination with phytic acid significantly reduced the elevated manganese levels as compared to MnCl$_2$ group ($p<0.05$), but serum manganese level still recording significant increase in ascorbic acid treated group when compared to normal control group.

As shown in table (1), Hb concentration in MnCl$_2$ group showed statistically significant decrease as compared to normal control group. There was significant increase in Hb concentration in all groups treated with oral phytic acid and/or ascorbic acid as compared to MnCl$_2$ group. Animal and human studies have confirmed that manganese absorption is inversely associated with Hb concentration *(Santos-Burgoa *et al.*, 2001). The level of Mn in blood was higher in patients with iron deficient anemia and iron therapy increased Hb levels and diminished blood Mn in the same patients *(Kim *et al.*, 2005). In another study, Montes *et al.* (2008) found a negative correlation between blood Mn and hemoglobin.

Fredric *et al.* (2011) reported that plasma vitamin C is positively associated with higher hemoglobin level. The result of this study confirmed that, vitamin C could play a major role to utilize iron and achieve a better hemoglobin response during iron deficiency. In addition to the antioxidant effect of ascorbic acid in decreasing bad effect of free radicals from manganese exposure, and better utilization of iron due to improved of iron absorption pathway rather than manganese.

The mean values ± SD of serum iron and total iron binding capacity (TIBC) levels in all groups are shown in table (1). There was a significant decrease in serum iron in MnCl$_2$ intoxicated group accompanied with a significant increase of TIBC level as compared to
normal control. *Erikson et al.* (2002) suggested that an inverse relationship exists between iron and manganese concentrations. *Erikson et al.* (2004) showed that iron deficiency causes manganese accumulation compared to control animals. *Smith et al.* (2013) showed partial correlation between blood manganese and iron deficiency with the increase of manganese concentrations in biological materials. Serum TIBC is low-normal or decreased in association with inflammatory disorders and increased in iron-deficient humans (*Ottenjann et al.*, 2006). A slight increase in serum TIBC was reported in an experimental study of diet-induced iron deficiency anemia (*Fry and Kirk*, 2006). The results of the current study revealed that iron deficiency and increased TIBC are believed to be associated with manganese toxicity which induced diminished plasma iron. This may be attributed to manganese-enhanced intracellular distribution of iron therefore iron can be used as a biomarker to toxicity. This study suggests that an inverse relationship exists between iron and manganese.

In the current study, results showed a significant decrease in serum iron of phytic acid treated group. While oral ascorbic acid caused significant increase in serum iron level as compared to MnCl$_2$ group with no significant difference when compared with normal control group (table 1). This is similar to the study of *Porres et al.* (1999) who indicated that high levels of dietary phytate reduce iron level.

There was a significant increase of TIBC level in MnCl$_2$ and oral ascorbic acid groups as compared to normal control group (p<0.05). Furthermore, in oral phytic acid and oral phytic acid + ascorbic acid groups, the data showed no significant differences in TIBC levels as compared to normal control group or to MnCl$_2$ group.

The current study shows that manganese alters glutathione level and SOD activity, by decreasing their levels accompanied with increasing MDA level in MnCl$_2$ intoxicated group as compared to normal control group as shown in table (2). This may be attributed to manganese toxicity that increase reactive oxygen species, and subsequent oxidative damage, within cells. Human study further confirms the oxidative damage among welders exposed to airborne Mn (*Li et al.*, 2004). Also, Mn may be accumulated and cause an inflammation leading to increase in oxidative stress in the retina.

In oral phytic acid or ascorbic acid groups, the present data showed that, GSH level was significantly increased when compared with MnCl$_2$ group. While, there was a significant increase in SOD activity in ascorbic acid treated groups either alone or in combination with phytic acid. On the other hand, treatment with phytic acid and/or ascorbic acid significantly decreased MDA to near the normal level. *Al-Fatlawi and Al-Shammari* (2017) reported that Phytic acid treatment decreased MDA formation. Phytic acid leads to the significant prevention of membrane damage with no significant difference in the MDA level between control and phytic acid treated group. Also, phytic acid treatment increased GSH level significantly and restores SOD activity after their depletion with toxicity.

Ascorbic acid potentiates the activities of free radical scavengers, superoxide dismutase, and glutathione reductase thereby preventing lipid peroxidation in the blood and retina of the
treated groups and led to decreased inflammation. This is due to the antioxidant effect of ascorbic acid and its ability to scavenge free radicals resulted from oral MnCl₂ toxicity. *Adikwu and Deo* (2013) confirmed that ascorbic acid decreased lipid peroxidation either directly or indirectly by regenerating vitamin E.

Results in table (3) showed that Mn-intoxication induced a significant increase in serum ALT and AST activities. On the other hand, treatment with phytic acid and/or ascorbic acid significantly decreased the activities of ALT and AST in serum. Results also demonstrated that Mn-intoxication significantly increased serum urea and creatinine levels as compared to normal control group. Treatment with phytic acid either alone or in combination with ascorbic acid significantly reduced these altered levels. Treatment with phytic acid significantly effective in the normalization of AST and ALT (*Al-Fatlawi and Al-Shammari*, 2017).

The data of the present study showed significant improvement in different parameters of liver and kidney functions (table 3) in ascorbic acid treated group because ascorbic acid could prevent meaningful changes in the urea or creatinine level of the group exposed to manganese chloride. Kidney and liver protective properties of vitamin C is attributed to its antioxidant effect and it can prevent increased AST, ALT, urea and creatinine which causes oxidative damage induced by Mn toxicity. Vitamin C normalized levels of serum alanine aminotransferase, aspartate aminotransferase, urea and creatinine in intoxicated animals. The effects of antioxidants as a protective agent on body organs have been approved by *Sajadi et al.* (2008) and *Saleem et al.* (2012).

Ascorbic acid has also an important role in protecting the kidney (*Djeffal et al.*, 2011) and *Ememghorashy et al.* (2012). Recently, *Kasnaviyeh et al.* (2017) suggested that vitamin C can enhance the activity of antioxidant enzymes in the kidney tissue and causes the decrease of urea and creatinine. This study suggested that vitamin C had protective effect on liver enzymes by return of AST and ALT activities to normal levels in oral MnCl₂ group when administered with ascorbic acid.
Table 1. The mean ± SD values of serum manganese level (ppb), hemoglobin (g/dl), Iron (µg/dl) & Total iron binding capacity (µg/dl) in different experimental groups.
Different letters mean significant in the same column.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mn (ppb)</th>
<th>Hb (g/dl)</th>
<th>Iron (µg/dl)</th>
<th>TIBC (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Mean ± SD</td>
<td>14.20 ± 0.34a</td>
<td>13.85 ± 0.41a</td>
<td>10.78 ± 2.11ab</td>
</tr>
<tr>
<td>Oral MnCl2</td>
<td>Mean ± SD</td>
<td>33.97 ± 1.31c</td>
<td>34.91 ± 2.76c</td>
<td>25.59 ± 3.39c</td>
</tr>
<tr>
<td>Oral phytic acid</td>
<td>Mean ± SD</td>
<td>43.72 ± 5.57a</td>
<td>9.60 ± 1.62bc</td>
<td>77.03 ± 7.17c</td>
</tr>
<tr>
<td>Oral ascorbic acid</td>
<td>Mean ± SD</td>
<td>43.91 ± 7.25a</td>
<td>0.97 ± 2.86a</td>
<td>61.64 ± 7.35</td>
</tr>
<tr>
<td>Oral phytic acid + ascorbic acid</td>
<td>Mean ± SD</td>
<td>32.07 ± 8.83b</td>
<td>8.73 ± 0.84b</td>
<td>94.00 ± 23.40b</td>
</tr>
</tbody>
</table>

Table 2. The mean ± SD values of reduced glutathione (mg/dl), super oxide dismutase activity (U/ml) & malondialdehyde (nmol/ml) in different experimental groups:
Different letters mean significant in the same column.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GSH mg/dl</th>
<th>SOD U/ml</th>
<th>MDA nmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Mean ± SD</td>
<td>84.17 ± 1.26a</td>
<td>424.65 ± 3.57a</td>
</tr>
<tr>
<td>Oral MnCl2</td>
<td>Mean ± SD</td>
<td>66.90 ± 4.37c</td>
<td>388.81 ± 2.00bc</td>
</tr>
<tr>
<td>Oral phytic acid</td>
<td>Mean ± SD</td>
<td>83.64 ± 2.29a</td>
<td>404.74 ± 41.78bc</td>
</tr>
<tr>
<td>Oral ascorbic acid</td>
<td>Mean ± SD</td>
<td>84.43 ± 1.55a</td>
<td>421.14 ± 5.11ab</td>
</tr>
<tr>
<td>Oral phytic acid + ascorbic acid</td>
<td>Mean ± SD</td>
<td>79.68 ± 3.97b</td>
<td>421.19 ± 1.26ab</td>
</tr>
</tbody>
</table>
Table 3. The mean ± SD values of aspartate aminotransferase (U/ml), alanine aminotransferase (U/ml) activities, urea (mg/dl) & creatinine (mg/dl) in different experimental groups:

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral phytic acid</td>
<td>38.33 ± 5.04c</td>
</tr>
<tr>
<td>ascorbic acid +</td>
<td>40.03 ± 2.41c</td>
</tr>
<tr>
<td>Oral phytic acid</td>
<td>37.91 ± 2.51c</td>
</tr>
<tr>
<td>ascorbic acid</td>
<td>1.72 ± 0.37c</td>
</tr>
</tbody>
</table>

Different letters mean significant in the same column.

**Conclusion:**
Phytic acid has a powerful chelating property on manganese, thus protecting from exposure to high concentration of this metal. While ascorbic acid effectively scavenges single oxygen, superoxide & hydroxyl radicals. It is also confirmed to be an excellent source of electrons, therefore electrons to free radicals switching off their activity. This observation may be valuable to reduce any possible toxicity related to the contamination or exposure to Mn when administered with ascorbic or phytic acids.

**Acknowledgement**
I deeply acknowledge the help and support of Biochemistry Department, Research Institute of Ophthalmology, Cairo, Egypt.

**References:**

Adikwu E and Deo O. Hepatoprotective Effect of Vitamin C (Ascorbic Acid). Department of Pharmacology and Toxicology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria, College of Health Technology, Otuogidi-Ogbia, Nigeria. 4(1): 84-92 (2013).


adult male rat exposed to formaldehyde. Department of Medical Science, Emergency Medicine Management Research Center, University of Medical Sciences, Tehran, Iran. 118-121 (2017).


من خص البحث باللغة العربية

تأثير كلاً من حمض الفيتامك و/أو الأسكوريك لحد من سمية المنجنيز في حيوانات التجارب

تهاني السيد خليف، أنهار محمد جمعه، إيناس علي كامل، سمر لطفي محمود

قسم الكيمياء الحيوية والتغذية.
المنجنيز عنصر أساسي في غذاء الإنسان. وهو مطلوب لتنظيم عملية الكارتر، التمثيل الغذائي للكربوهيدرات والدهون، التخسيس، التمثيل الغذائي للكربوهيدرات والدهون، في جميع أنحاء الجسم. النتائج التي أظهرت عليه (منجانزم) مرتبط بتعرض لجرعات عالية من المعدن أو مركباته غير العضوية. المصدر الأساسي للمنجنيز في الجسم أظهر هذا في تطور علاج أنماط العمل والصياغة ومعالجة اللمحة، وعمل صناعة البطاريات والخادمة. أيضا، مرضي الشكل الكلي والكلى نتيجة لعدم قدرة الأجسام على التخلص من المنجنيز. الントسم بالمنجنيز مرتبط بتدمير الأعصاب، ولكن دراسات قليلة أشارت إلى تأثيره على العين. أجريت الدراسة على 120 من النوع النيوزيلندي وقسمت الأرانب إلى 5 مجموعات واستمرت التجربة لمدة 8 أسابيع. وتم استخدام كلوريد المنجنيز لحدث السمية. المجموعات عباره عن (1) المجموعة الضابطة تناولت الغذاء والماء الأساسي، (2) مجموعة تناولت كلوريد المنجنيز بجرعات عالية في مياه الشرب، (3) مجموعة تناولت الردة كمصدر غني بحمض الفيتامين مع كلوريد المنجنيز في مياه الشرب، (4) مجموعة تناولت كل من حمض الفيتامين وحمض الأسكوربيك مع كلوريد المنجنيز في مياه الشرب، و (5) مجموعة تناولت كل من حمض الفيتامين وحمض الأسكوربيك مع كلوريد المنجنيز في مياه الشرب. وقد أظهرت النتائج أن تناول كلوريد المنجنيز في مياه الشرب أدى إلى حدوث زيادة مغذوية في مستوى المنجنيز في السيرم، نقص مغذوي في قياسات الدم من تركيز الهيموجلوبين، مستوى الحديد في السيرم، تركيز الجلوتاثيون المختزل، نشاط إنزيم السوبر أكسيد ديميتويرات مع وجود زيادة مغذوية في مستوى المنجنيز وهيئة. هذا بالإضافة إلى حدوث زيادة مغذوية في نسبة التحلل الناقل للأميني، AST & ALT. وأظهرت المجموعات المختزنة م_datos معلوماتية إصدار التحلل الناقل للأميني. وجد أيضا أن إعطاء كلوريد المنجنيز بجرعات عالية أحدث خلل في وظائف الكلي وذلك عن طريق إرتفاع مستوى البوليا ونتريتين مقارنة بالمجموعة الضابطة. وأظهرت المجموعات، 3، 4، 5 والتي تم معالجتها بحمض الفيتامين و/أو الأسكوربيك تحسن ملحوظ في كل التحاليل المقاسة. وخلصت النتائج إلى أن تكون حمض الفيتامين يعمل على ربط المنجنيز وحمض الأسكوربيك يعمل كمضاد للأكسدة والالتهابات، قد على مدى مسيرة المنجنيز.