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Potential Protective Effect of Magnesium Supplementation on Nickel Induced Hepatotoxicity in Albino Wistar Rats

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Abstract

Nickel is considered one of the most harmful heavy metals on human health. This experimental study was designed to investigate whether magnesium supplementation has any influence on nickel-induced toxicity in albino rats. Twenty one adult rats were divided into three groups of seven animals each group. The first group was used as a control and received saline solution. The second group was administered with nickel sulfate (2.0 mg/100 g, i.p) and the third group was given both nickel sulfate (2.0 mg/100 g, i.p.) and magnesium sulfate (300 mg/kg, i.p) simultaneously on alternate days. Body weight gain was recorded regularly. After 21 consecutive days, cellular functions were evaluated by biochemical and oxidative stress markers. The nickel sulfate-treatment decreased body weight gain in rats. Nickel treatment produced also oxidative injury characterized by an increase in glucose level, enzymes markers activities, lipid profile and total proteins and bilirubin concentrations. Simultaneously, glutathione level (GSH) and catalase activity was diminished in liver tissue. These results are further substantiated with obviously changes in hepato- histoarchitecture. However, the treatment with magnesium ameliorated the previous parameters and resulted in an improvement of the histopathological lesions. Our results suggest that magnesium supplementation may be effective in reducing nickel oxidative damage.

Keywords: Hepatic Toxicity, Histopathology, Magnesium, Nickel, Oxidative Stress, Rats

1. Introduction

Potential toxicological impact of heavy metals is still a matter of investigation and our actual knowledge on the effects of contaminants on biological systems remains incomplete. Nickel (Ni) is one of the most metal pollutants and it is seriously threatening our ecosystem and human health[1]. Nickel and its compounds are released into the environment through different human activities like industries, application of phosphatic fertilizers, burning of fossil fuels, sludge water, smelting, vehicle emissions and electroplating industries[2]. Global production of nickel is approximately 70% consumed in the production of stainless steel alloys; the remaining is used in the manufacturing of other nickel alloys, catalysts, electroplating, batteries, paints, foundries, welding rods, jewelry, coinage, dental and medical implants.

After entering the body, nickel penetrates all organs and accumulates primarily in bone, liver, kidney and excreted through bile and urine. Nickel reportedly causes severe liver and kidney damage by altering several marker enzymes and ascorbate cholesterol metabolism[3]. Experimental data suggests that nickel can generate free radicals and ROS

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causing lipid peroxidation, protein oxidation and DNA damage which can lead to cytotoxicity, genotoxicity and even carcinogenesis when mutated cells can proliferate. Furthermore, it has been reported to reduce antioxidant enzymes activities like Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GSH-Px) with depletion in glutathione concentration[4-6].

Oxidative stress, which is an imbalance between free radicals production and the normal antioxidant capacity favoring a ROS (Reactive Oxygen Species) excess, has been identified as a common mechanism for cell damage. This imbalance can be amplified by aging, smoking, pollutants, diseases and some kinds of food[7]. During oxidative stress, ROS are produced mainly from the mitochondrial electron transport chain. Free radicals can be enzymatically converted to other less toxic molecules such as hydrogen peroxide and water by SOD and CAT or GSH-Px enzymes, respectively[8]. Elements are well known to play an important role in many life supporting processes.

Magnesium (Mg) is the second most abundant element in cellular systems and it is undoubtedly one of the most important bivalent ions[9]. Magnesium is involved in many physiologic pathways, including regulation of metabolic pathways, energy production, signal transduction, ion transport, antioxidative status maintaining, cell proliferation, apoptosis and angiogenesis[10]. Else, it is an important cofactor in more than 300 enzymatic reactions[10,11]. Magnesium deficiency or the displacement of Mg^{2+} by other toxic divalent metal ions leads to an increased genomic instability, as evident by inhibited DNA repair, oxidative stress, aging and carcinogenicity[12]. Accumulating evidence suggests that Mg supplementation is associated with enhance in SOD, Glutathione-S-transferase (GST) activities and decreased malondialdehyde (MDA) levels[13], inversely, dietary deficiency in magnesium was shown to development of poor metabolic control and increased free radical dependent-oxidative tissue damage[14]. Protection of cell membrane from lipid peroxidation becomes a necessity to prevent, to cure, or to delay the aforesaid pathologies. Therefore, the present study was designed to evaluate whether magnesium supplementation could have a protective effect against nickel induced oxidative stress related liver dysfunction in rats.

2. Material and Methods

2.1 Chemicals

Nickel sulfate ($NiSO_4$), magnesium sulfate ($MgSO_4$) and all other chemicals used in the experiment were procured from Sigma Chemical Corporation (St. Louis, MO).

2.2 Animals

Male normal albino (Wistar) rats of 7 weeks of age with a body weight of 150-200 g were supplied by Pasteur Institute Algiers. Rats were housed in plastic cages and located in air-conditioned room (humidity was around 70%, temperature was 22 ± 2^{0}C and 12 h light/dark cycle), and fed with standard laboratory stock diet (ONAB factory, Annaba, Algeria) and water ad-libitum for one week.

2.3 Experimental Design

The experimental procedures were carried out according to the guidelines for the care and use of animals approved by the National Institute of Health and the Ethics Committee of our institution (PNR-ANDRS 8/u23/332). The acclimatized animals were divided into three groups of seven animals each. Group 1 served as a control and received saline solution. Group 2 rats were administered hydrated nickel sulfate (2.0 mg/100 g b.wt.; intraperitonially, i.p.)[4] and Group 3 was given both nickel sulfate (2.0 mg/100 g b.wt.; i.p.) and magnesium sulfate (300 mg/kg b.wt.; i.p.)[13] simultaneously on alternate day and total duration of exposure period were 21 days. Body weight gain was recorded regularly. The animals sacrifice was carried out in the morning by decapitation to avoid effect of stress. Blood was transferred into ice cold centrifuged tubes and a portion taken for whole-blood glucose analysis, which was performed promptly after decapitation. The remaining blood was centrifuged for 15 min at 2200g then serum samples were stored at -20^{0}C for biochemical analysis.

2.4 Assessment of Biochemical Markers

The serum was used for estimating the biochemical parameters viz., glucose, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT), Alkaline Phosphatase (ALP), bilirubin, Lactate Dehydrogenase (LDH), total protein, total cholesterol, triglycerides and total lipid by using standard assay kit method (Spinreact, Spain).

2.5 Assessment of Antioxidant Parameters

Hepatic tissues of rats were homogenized (10%) in phosphate buffer (pH 7.4) with a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 10 000 g for 15 min at 4^{0}C. The resulting supernatants were used to determine catalase (CAT) activity and reduced Glutathione (GSH) level.

2.5.1 Estimation of Reduced GSH Levels

Reduced GSH was estimated by the method of Jollow *et al.*[15] based on the reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) with compounds containing sulfhydryl groups and

the development of yellow color. The maximum absorbance was recorded at 412 nm. Total GSH content was expressed as nmol/mg protein.

2.5.2 *Catalase Activity*

Catalase (CAT) activity was assayed by the method of Aebi[16]. Enzymatic reaction is initiated by adding an aliquot of 20 ml of the homogenised tissue and the substrate (H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of micromoles of H_2O_2 consumed per minute per milligram of total protein.

2.6 Protein Estimation

The protein contents of various samples were determined by the method of Bradford[17], using bovine serum albumin as a standard.

2.7 Histopathological Studies

A portion of liver was fixed in 10% formalin for at least 24 h and then embedded in paraffin. Sections of 5 µm in thickness were cut, deparaffinized, dehydrated and stained with hematoxylin and eosin dye according to method of Hould[18].

2.8 Statistical Analysis

Data are presented as mean values ± SE and the statistical analyses of the data were performed by one-way Analysis of Variance (ANOVA) followed by Student's t-test. A probability value of $p<0.05$ was considered to denote a statistically significant difference.

3. Results

3.1 Effects of Treatments on Body Weight, Absolute and Relative Liver Weights

During the course of present investigation, rats treated with nickel sulphate shown significantly ($p<0.01$) less weight gain as compared to control group rats. Otherwise, it was observed that liver absolute and relative weights of rats treated with nickel were significantly increased as compared to controls. Conversely, in nickel sulfate + magnesium sulfate treated rats, there was no significant change compared to the control group (Table 1). No death was observed in any group during the experimental period.

Table 1. Body weight, absolute and relative liver weights of control and treated rats

Parameters	Control	Ni	Ni + Mg
Initial body weight (g)	195.42 ± 6.57	196.42 ± 5.20	202.42 ± 4.00
Body weight gain (g)	56.85 ± 5.09	17.85 ± 2.65**	45 ± 3.45#
Absolute liver weight (g)	2.47 ± 0.09	2.78 ± 0.08*	2.56 ± 0.09
Relative liver weight (g/100 g BW)	13.05 ± 0.26	14.14 ± 0.26*	13.31 ± 0.27

Values are expressed as mean ± SE of 7 rats in each group.
p values: *<0.05, **<0.01, compared with control group.
p values: #<0.001, compared with Ni group.

3.2 Effects of Treatments on Blood Biochemical Parameters

The results of serum biochemical markers are shown in Table 2. Treatment of rats with 2 mg/ 100 g bw. of nickel significantly elevated the concentration of whole blood glucose ($p<0.001$), hepatic enzymes activities (GOT, GPT, ALP and LDH), total bilirubin ($p<0.01$) and protein ($p<0.05$) levels and lipid profile (cholesterol, TG and total lipid) in comparison with the control group. However, the combination of magnesium with nickel has restored partially the tested parameters towards the value of the control group rats. In addition, there were a significant ($p<0.001$) reduced of GPT activity in combination treated rats when compared to nickel group II.

3.3 Effects on GSH Contents and CAT Activity

GSH constitutes the first line of defense against free radicals. The toxicity of nickel sulfate significantly decreased ($p<0.01$) the hepatic GSH level and CAT activity in the nickel-treated animals compared to the normal control group (Figure 1). Administration of magnesium significantly increased ($p<0.05$) the GSH levels as compared to Ni-treated group. Moreover, no significant differences in the above-mentioned oxidative stress parameters between Ni+Mg- treated group and the control rats.

Table 2. Changes in biochelmical parameters of control and treated rats

Parameters	Control	Ni	Ni + Mg
Glucose (g/L)	1.25 ± 0.11	2.14 ± 0.12***	1.30 ± 0.15
GOT (U/L)	49.95 ± 2.7	61.16 ± 3.65*	54.64 ± 2.96
GPT (U/L)	16.54 ± 0.75	26.97 ± 0.77***	19.65 ± 0.89*,###
ALP (U/L)	107.76 ± 5.59	143.90 ± 9.38**	129.6 ± 11.04
LDH (U/L)	930.51 ± 54.57	1165.1 ± 53.24**	1002.56 ± 52.9#
Total bilirubin (mg/dL)	0.63 ± 0.08	1.16 ± 0.09**	0.90 ± 0.09*
Cholesterol (mg/dL)	79.18 ± 3.51	99.57 ± 5.74**	81.46 ± 4.54#
Triglycerides (mg/dL)	88.18 ± 3.74	113.31 ± 4.16***	99.53 ± 3.62*#
Total lipid (mg/dL)	324.34 ± 10.92	441.38 ± 42.02*	391.95 ± 34.82
Total protein (g/dL)	6.63 ± 0.28	7.75 ± 0.30*	6.57 ± 0.70*

Values are expressed as mean ± SE of 7 rats in each group.
p values: *<0.05, **<0.01, ***<0.001 compared with control group.
p values: #<0.001, ###<0.001 compared with Ni group.

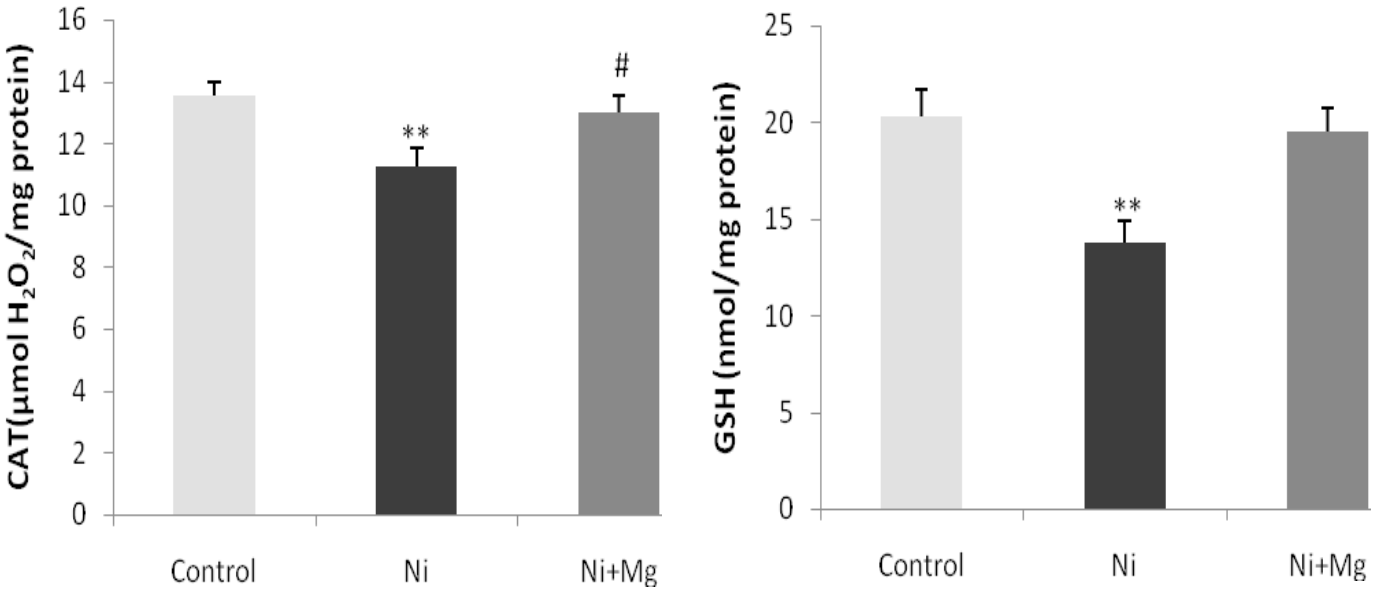


Figure 1. CAT activity and reduced GSH level in liver of control, nickel alone and Ni + Mg combination-treated rats. Values are expressed as mean ± SEM of 7 rats in each group. p values: **<0.01 compared with control group. p values: #<0.001 compared with Ni group.

3.4 Histopathological Observations

Next, to confirm the above findings, the histopathology of liver was examined. Microscopic examination of liver section of control rats (Figure 2a) shows normal hepatocytes cells which were well separated from sinusoids and uniformly stained. In contrast to histological examination of the liver tissues of the untreated control, marked Hepatocyte Cytolysis (HC) and Vacuolization (V) were noticed in nickel treated rats (Figure 2b). In Ni and Mg treated animals, most of the hepatocytes appeared with normal architecture (Figure 2c).

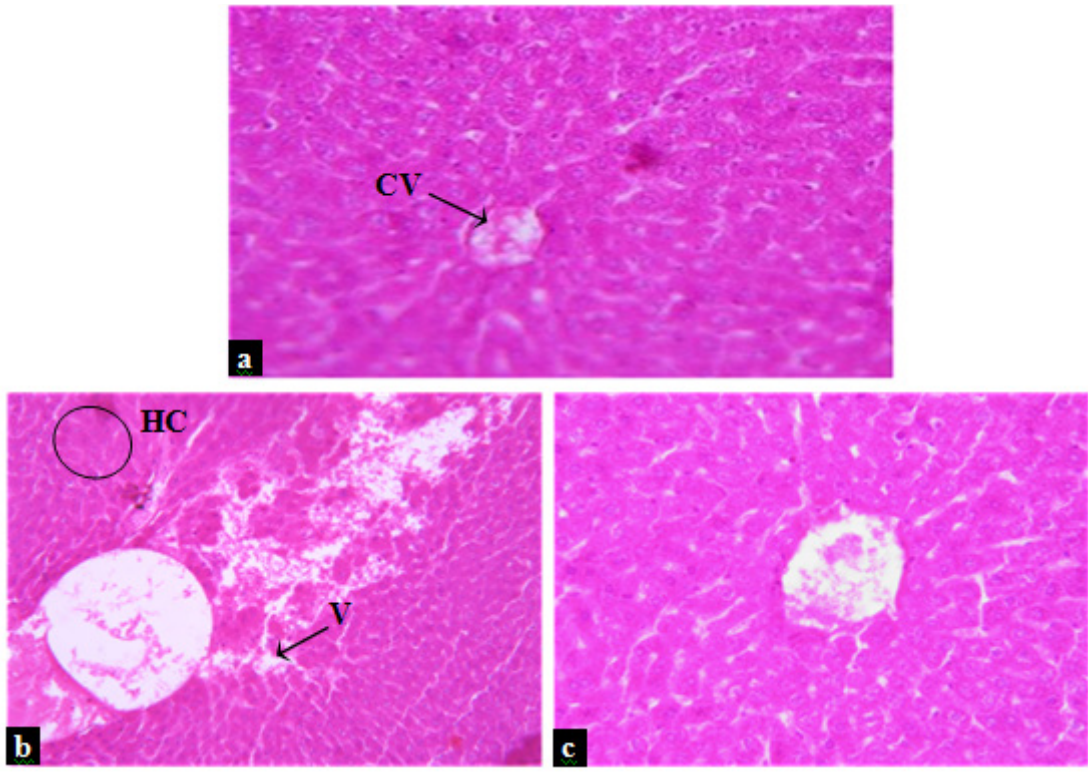


Figure 2. Photomicrograph of H and E stained sections of liver (200×) from a controls **(a)** showing normal histological structures, nickel sulfate **(b)** induced histopathological changes and nickel sulfate with magnesium sulfate groups **(c)** showing improvement in the liver architecture treated rats. Arrows: CV, centrilobular vein; HC, hypatocyte cytolysis; V, vacuolization.

4. Discussion

Liver is the most important organ and it is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body. Although viral infection is one of the main causes of hepatic injury. Xenobiotics, hepatotoxins, environmental pollutants and chronic alcohol ingestions can also cause severe liver injury[19]. Nickel compounds are hazardous to both human health and the living environment.

In the present study, nickel sulphate group animals gain less weight than the control group rats. This is a good agreement with some previously published reports[20,21]. A significant reduction in body weight (10% or more) has also been observed in rats after being treated with nickel chloride at 8.6 mg Ni/kg/day for 91 days by gavage[22]. The decrease in body weight might be due to the overall increased degeneration of lipids and proteins as a result of nickel toxicity[23]. In other hand, elevation in blood glucose, recorded in the present study, is a common result of nickel toxicity and is usually linked with inhibition of insulin release from Langerhans'islets[24] or blocked to glucose utilization by cells even in the presence of elevated concentrations of insulin[25] or due to glycogen breakdown and supply of glucose production from other non carbohydrate sources such as proteins[26]. An increase in serum glucose concentrations has been reported by Das and Das[27] in male rats exposed to nickel chloride for 28 days. It has been further found that a single-dose injection of nickel chloride (4.5 mg Ni/Kg b.wt.) significantly increased serum glucose concentration in rabbits along with histopathogical changes in pancreatic cells[28]. However there is unchanged of blood glucose concentration in animals treated with both metals nickel and magnesium. It is probably as a result of the hypoglycaemic effect of magnesium sulphate by decreasing systemic glucose accumulation, diminishing nickel binding to biomolecules, improving insulin secretion and action[29] and/or protects the enzymes and ATP involved in glucose metabolism against inactivation caused by this metal[25].

In nickel treated rats, the activities of serum GOT, GPT, LDH, ALP and serum bilirubin concentration were significantly increased, compared to their normal levels indicate liver damage. It could be attributed due to the

hepatic damage resulting in increased release and leakage out of these enzymes and bilirubin substance from the liver cytosol into the blood stream which gives an indication of hepatotoxic effect of this metal[30]. Furthermore, it has been found that liver was necrotized in heavy metals exposition[31]. In this study we also found a significant rise in serum total protein in nickel group as compared to the control group. This again indicates that synthesized proteins were liberated as a result of cytolysis and to other pathological changes in the liver tissue, associated with progression of the toxicity condition, and then the excessive accumulation of amino acids (glutamic and alanine) in serum of affected animals. These excessive amino acids are converted to ketonic bodies (α keto glutaric and pyruvate) for which the enzyme GOT and GPT are needed, and confirm the result of high concentration of blood glucose[32].

The normalization of enzymes activities, total protein and bilirubin concentrations following magnesium supplementation to Ni intoxicated rats, well be observed. Magnesium was shown to inhibit nickel toxicity in several microorganismes[25]. This protection may be attributed to a simple competition between nickel and magnesium ions for common intracellular counterparts because these divalent cations resemble each other in terms of their physicochemical properties[33].

Oxidative stress, which is an imbalance between ROS production and the antioxidant systems favouring an excess ROS, has been identified as a common mechanism for cell damage. During oxidative stress, ROS are produced mainly from the mitochondrial electron transport chain. To minimize the damage induced by ROS, free radicals can be transformed to other less toxic molecules, for example, the superoxide anion is enzymatically converted into hydrogen peroxide by Superoxide Dismutase (SOD) and hydrogen peroxide enzymatically converted into water by catalase or glutathione peroxidase enzymes[8]. Divalent nickel enhances lipid peroxidation at all DNA bases by either in vitro or in vivo systems[34,35]. The incubation of Ni with cysteine in the presence of oligopeptides and an oxygen environment produces hydroxyl radicals, which then react with cysteine and generate a carbon-centered alkyl radical and, therefore, free radicals from lipid hydroxyperoxides[36]. Hence Das *et al.*[3] deduced that Ni toxicity lies on free radical generation in a complex manner from Ni – thiol complexes, singlet oxygen or lipid hydroxyperoxides.

The diminution of glutathione level in nickel-treated rats may be as a result of oxidative stress, which has been occurred, in nickel toxicity. Otherwise, the reduced of antioxidant production was due to the increased oxygen metabolites and the elevated free radicals, which cause a decrease in the activity of the anti-oxidant defense system[37]. It has been demonstrated that nickel induced oxidative stress by depleting glutathione and oxidatively activating various transcription factors[38]. In Group 3 (nickel sulphate + magnesium sulfate), the significant improvement of the glutathione level was noticed when compared with that of Group 2. Magnesium is a ubiquitous intracellular cation and it activates many steps in the cellular metabolic pathways. Overall magnesium is important for more than 300 different enzyme systems, among them enzyme for glutathione synthesis (γ-glutamylcysteine synthetase and glutathione synthetase). Various investigations show that magnesium deficiency induces a decrease in cellular GSH which renders the tissue susceptible to oxidative damage[39]. Li *et al.*[40] concluded that Mg may stimulate de novo GSH biosynthesis. In addition the decreased activity of hepatic CAT in nickel treated animals, suggests that there is an interaction between the accumulated free radicals and the active amino acids of this enzyme. The present study agree with the results Das *et al.*[41] who have proved that treatment of rats with nickel sulfate for 20 days leads to an increase of plasma MDA and LPO concentration with a decrease in GSH content, and SOD, CAT, and GSH-Px activities. Supplementation of magnesium to the rats exhibited mitigation effect in the liver. Magnesium deficiency is known to induce an oxidative stress[42], to increasing susceptibility to peroxidation in various organs such as heart, liver, skeletal muscle and testes[43]. Conversely, magnesium supplementation was found to decrease malondialdehyde (MDA) levels and increase Superoxide Dismutase (SOD) and glutathione-S-transferase (GST) activities in liver of diabetic rats[43]. Olatunji and Soladoye[14] were also found that supplementation of magnesium led to a further increase in plasma L-ascorbate concentrations. This improvement could be explained by a sparing-effect of magnesium on L-ascorbic acid by preventing its utilization or enhancing its synthesis.

5. Conclusion

Based on the present findings, it may be concluded that hepatotoxicity which was induced by nickel through free radical generation leading to oxidative stress, was effectively reduced by co-treatment with magnesium. Further studies are required to understand the detailed mechanisms of potential protection being afforded by this element.

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