Research Article

Effect of the Joint Supplementation of Vitamin E and Selenium on Chronic Silver Induced Liver Injury in Male (Wistar) Albino Rats

Mouna Gueroui, Zine Kechrid*
Applied Biochemistry and Microbiology Laboratory, Dept of Biochemistry, Science faculty, Badji Mokhtar University, BP 12 Sidi Amar, Annaba, Algeria.
*Corresponding author's E-mail: kechridzine@yahoo.fr

ABSTRACT

The aim of this investigation was to determine the protective effect of vitamin E and selenium on chronic exposure to silver (Ag) induced liver injury in rats. Thirty male albino (Wistar) rats were divided into five groups of six each: the first group used as control group; group II given silver as silver nitrate (AgNO₃)(20mg/l) in their drinking water;group III given vitamin E (400mg/kg) of diet and AgNO₃; group IV given both AgNO₃ and selenium (1mg/l); group V given AgNO₃, vitamin E and selenium. All groups were treated for three months. In Ag-intoxicated rats, an increase in lactate dehydrogenase (LDH) activity and cholesterol level, a decrease in total protein and alkaline phosphatase (ALP) activity were noticed. Moreover, the results also revealed that Ag ions affected antioxidant defense system by decreasing hepatic superoxide dismutase (SOD) activity and increasing vitamin E concentration in liver, in addition, histological study showed some hepatic tissue alterations. Treatment with vitamin E and/or selenium exhibited a defensive role on the toxic effects of silver on the parameters mentioned previously. In conclusion, selenium and vitamin E act as powerful antioxidants which may protect body against silver induced harmful effects.

Keywords: silver, vitamin E, selenium, oxidative stress, liver injury, Wistar rats.

INTRODUCTION

Silver occurs naturally in the earth's crust and is found in soil, fresh and sea water, and the air.[1,2] It has antibacterial and antifungal activities and has been used as an additive in wound dressing catheters, bone cements, dental devices, hygiene textiles, photographic industry, jewelry, silverware, deodorant sprays, and other consumer products.[3] Silver is not an acknowledged trace element in the human body and fulfills no physiological or biochemical role in any tissue.[4] The most common health effect associated with prolonged exposure to silver ions is the development of irreversible pigmentation of the skin (argyria) or the eyes (argyrosis).[3,5] Silver nitrate is absorbed through the lung and mucous membranes.[6] Its ion is transported in blood and bound to macroglobulin and albumin; most accumulation has been reported to occur in mammalian liver, skin, and fur.[7,8] The cellular pathology and the molecular mechanisms involved in silver toxicity in mammalian cells are largely unknown. Similar to other transition metals, Ag⁺ has been shown to affect the cellular oxidative status.[9] The role of both, endogenous and exogenous antioxidants in alleviating harmful effects associated with heavy metals exposure has been demonstrated in several experimental systems.[10] Within this context, selenium is an essential nutrient for humans, animals and bacteria. It is important for many cellular processes because it is a component of several seleno proteins and seleno enzymes with essential biological functions.[11] However, protective mechanisms of selenium vary from organ to organ.[12] This trace element is known to antagonize the toxicity of silver ions which show chemical and morphological signs similar to those of selenium deficiency.[13] Vitamin E is regarded as the most important lipid soluble biological antioxidant.[14] In addition to preventing free radical – initiated lipid peroxidation damage, increasing evidence indicates that vitamin E may exert its biological functions in relation to its membrane localization property.[15] Deficiency of vitamin E has been shown to impair the tolerance of rats to the presence of silver ions in the diet or drinking water.[15,16] Therefore, this study was carried out to elucidate the possible changes in the levels of some biochemical hepatic parameters, lipid peroxidation, antioxidants and histopathology examination in experimental rats exposed to silver and treated with vitamin E and/or selenium.

MATERIALS AND METHODS

Chemicals

Silver nitrate (AgNO₃), sodium selenite (Na₂SeO₃), vitamin E (α-tocopherol), CDNB (1-chloro-2.4 dinitrobenzene), DTNB (5,5'dithio-bis-(2-nitrobenzoic acid), GSH (reduced glutathione), GSSG (oxidized glutathione) and epinephrine were obtained from sigma Chemical Co (St Louis, USA), bathophenanthroline and NADPH (nicotinamide adenine dinucleotide phosphate reduced form) were purchased from TCI (Tokyo Chemical industry co., LTD, Tokyo, Japan), all other chemicals used in the experiment were of analytical grade.

Experimental animals

All experiments were performed with 'Wistar' male rats weighing about 260-280g, which were purchased from Pasteur Institute, Algiers, Algeria. The animals were kept under good ventilation and were maintained on standard diet and water throughout the experimental period. They were kept at 22±2°C with the 12 h light/dark cycle and

40% humidity. All animal experiments were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution. After two weeks of adaptation, thirty rats were randomly divided into five groups of six each: group I fed standard diet and used as control group; group II received AgNO$_3$ in their drinking water (20 mg/l); group III given both AgNO$_3$ in drinking water (20 mg/l) and standard diet enriched with vitamin E at a dose of 400 mg/kg diet; group IV received AgNO$_3$ (20 mg/l) and selenium (1 mg/l) in drinking water; group V given AgNO$_3$, vitamin E and selenium. During the course of treatment, body weight gain, food intake and water consumption were recorded regularly. The doses of AgNO$_3$ and the period of treatment were basically selected on previous study of U.S. Environmental Protection Agency (EPA).[7] Vitamin E and selenium doses were also chosen on the clinical application and on results from previous investigations of Kim[17] and Qingzhi[18] respectively. The treatments of rats continued for a period of three months. At the end of the experiment, animals were sacrificed by cervical decapitation without anaesthesia to avoid animals stress. At the time of sacrifice, blood was transferred into non-heparinised tubes. Serum was obtained by centrifugation of the blood at 3000 rpm and then quickly frozen at -20 °C for biochemical analysis. Liver samples were rapidly excised, rinsed in ice cold saline [0.9% (w/v) NaCl]. Then, one lobe of liver was homogenized in a twice volume of ice cold TBS (50 mM TRIS, 150 mMNaCl, pH 7.4), the homogenates were centrifuged at 10.000g for 15 min at 4°C, and the resultant supernatant was frozen at -20°C for oxidative parameters analysis. The other lobe of liver was fixed in formol solution and used for histological studies.

Biochemical analysis

Serum biochemical markers: Transaminases (alanine transaminase: ALT, aspartate transaminase: AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholesterol, triglycerides and total proteins were assessed using Spinreact Laboratory Spain diagnostic kits using spectrophotometer (Jenway 6505, Jenway LTD, UK). The references were as follow: AST-1001161, ALT-1001171, ALP-1001131, LDH-1001260, triglycerides-100131, cholesterol-1001091, total proteins-1001291.

Determination of oxidative parameters

Lipid peroxidation level

Lipid peroxidation as evidenced by formation of thiobarbituric acid reacting substances (TBARS), were measured by the method of Esterbauer and Cheeseman.[19]

Two hundred fifty microliters of tissue homogenate were added to 1.5 ml of 1% phosphoric acid (pH 2.0) and 1 ml of 0.6 % of TBA in air light tubes and heated for 45-60 min in a boiling water bath. After cooling, MDA (malondialdehyde)-TBA was extracted with 2.5 ml of butanol. Organic phase was separated by centrifugation

for 5 min at 2000g and measured at 532 nm. A 99% TBARS are MDA, so TBARS concentration of the samples were calculated using the extinction coefficient of MDA at $1.56 \times 10^5 M^{-1} cm^{-1}$. Lipid peroxidation is expressed as nmol TBARS/mg prot.

Estimation of enzymatic antioxidants

The specific activity of liver superoxide dismutase (SOD) was determined according to the method described by Misra and Fridonich.[20] Ten micro liters of tissue homogenate were added to 970 µl of EDTA – Sodium carbonate buffer (0.05M) at pH10.2. The reaction was started by adding 20 µl of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min. A unit of SOD is defined as the amount of enzyme that inhibits by 50% the speed of oxidation of epinephrine and the results were expressed as U/mg protein.

Glutathione peroxidase (GSH-Px) catalyzes the reduction of hydroperoxides by utilizing GSH as a reductant. Determination of tissue GSH-Px activity was carried out according to the method of Flohe and Gunzler.[21] The reaction mixture contained 0.2 ml of TBS (pH 7.4), 0.4 ml of GSH (0, 1mM), 0.2 ml of homogenate was added and allowed to equilibrate for 5min at 25°C. The reaction was initiated by adding 0.2 ml of H$_2$O$_2$ (1.3 mM), reaction was terminated by addition of 1 ml of 1% TCA. Tubes were centrifuged at 1500 g for 5min and the supernatant was collected. To 0.48 ml of resultant supernatant, 2.2 ml of TBS (pH 7.4) and 0.32 ml of DTNB (1.0mM) were added. After mixing, absorbance was recorded at 412 nm and the specific activity of this enzyme is expressed as µmole GSH/mg protein.

Glutathione -S- transferase (GST) activity of tissues was measured spectrophotometrically by the method of Habig[22] using CDNB as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340nm. The activity of GST was expressed in terms of mmol CDNB-GSH conjugate formed/min/mg protein.

Glutathione reductase (GR) activity was based on the method of Goldberg and Spooner.[23] The enzymatic activity was assayed photometrically by measuring, NADPH consumption.

In the presence of GSSG and NADPH, GR reduces GSSG and oxidizes NADPH, resulting in a decrease of absorbance at 340 nm.

Quantification was based on the molar extinction cœfficient of 6.22 mM^{-1}cm^{-1} of NADPH, one unit of GR was defined as the amount of enzyme that reduced 1 µmol GSSG (corresponding to the consumption of 1 µmol of NADPH) per minute at 25°C. The GR activities were expressed as one unit per milligram protein.

Non-enzymatic antioxidants measurement

GSH concentration was performed with the method described by Ellman[24] based on the development of a

yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 ml of tissue homogenate was added to 0.2 ml of 0.25 % sulphosalylic acid and tubes were centrifuged at 2500g for 15 min. Supernatant (0.5ml) was mixed with 0.025 ml of 0.01 M DTNB and 1ml TBS (pH 7.4).

Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg prot.

Vitamin E estimated by the method of Desai,[25] to 1ml of tissue homogenate, 1 ml of ethanol and 3 ml of petroleum ether were added, shaken rapidly and centrifuged at 4000 rpm for 10 min, 2 ml of supernatant was evaporated to dryness at 80℃, to that added 0.2 ml of bathophenanthroline, 0.2 ml of ferric chloride and 0.2ml of phosphoric acid kept in dark for 5 min and then complete with 3 ml ethanol.

The color developed was read at 530 nm, vitamin E levels were expressed as mg vitamin E/mg protein.

Protein determination

The protein content of tissue samples were measured by the method of Bradford[26] using bovine serum albumin as a standard.

Histological studies

For histological examination, liver was dissected and immediately fixed in formalin solution for 24 h, processed by using a graded ethanol series, and then embedded in paraffin. The paraffin sections were cut into 5 μm thick slices and stained with hematoxylin and eosin for light microscopic examination by Hould.[27] The sections were then viewed and photographed.

Statistical analysis

All the results were expressed as mean values ± SEM. Comparisons between the groups were performed by one-way ANOVA followed by student's t-test. Differences were considered significant at $p \leq 0.05$.

RESULTS

Effect of treatments on body weight, food intake, water consumption, absolute and relative liver weight

AgNO$_3$ dose (20 mg/l) did not affect clinical appearance, all animals survived until the termination of the study. It was observed that AgNO$_3$ had no effect on body weight gain and relative liver weight, food intake and water consumption (Table 1). Meanwhile, AgNO$_3$+Se treated group showed significant decrease ($p \leq 0.05$) in relative liver weights as compared to AgNO$_3$ exposed animals.

Table 1: Body weight gain, relative liver weight, food intake and water consumption of control and experimental rats

Treatments	Control Mean ± SEM	AgNO$_3$ Mean ± SEM	AgNO$_3$+vit E Mean ± SEM	AgNO$_3$ + Se Mean ± SEM	AgNO$_3$+ vit E+ Se Mean ± SEM
Body weight gain (g)	88.57±32.63	97.14±57.21	75.72±51.12	97.14±18.42	66.71±47.97
Relative liver weight (%)	3.37±0.37	3.44± 0.22	3.21±0.09	3.17±0.17 [a]	3.42± 0.46
Food intake (g/rat/day)	21.22±2.02	23.5±4.21	24.58± 3.12	25.47± 1.69	22.5±3.55
Water consumption (ml/rat/day)	32.46±4.02	32.92±6.46	32.38±4.05 [k]	31.66±5.45	30.05±4.62 [a]

n = 6 (n: number of animals in each group); [a] $p \leq 0.05$: significantly different from AgNO$_3$; [k] $p \leq 0.01$: statistical difference between AgNO$_3$ + vit E and AgNO$_3$ + vit E + Se

Table 2: Changes in serum biochemical parameters (AST, ALT, ALP, LDH, cholesterol, triglyceride and total protein) of control and experimental rats

Treatments	Control Mean± SEM	AgNO$_3$ Mean±SEM	AgNO$_3$+vit E Mean± SEM	AgNO$_3$ + Se Mean± SEM	AgNO$_3$+ vit E+ Se Mean± SEM
AST (U/L)	56.99±12.94	72.01±16.86	66.20±21.50	64.17±18.34	72.92±10.24
ALT (U/L)	34.22±16.57	36.70±16.74	43.07±14.59	36.55±9.12	45.28±11.15
ALP (U/L)	93,06±28,65	58,85±17,73 [*]	77±5,16	101,7±34,85 [b]	83,6±28,14
LDH (U/L)	481.2114.3	654.9±141.9 [*]	436.9±156.1	443.8±160.7	370.2±141.0 [b]
Cholesterol (mmol/l)	1.86±0.62	3.12± 0.62 [**]	1.26±±0.46 [c]	1.64± 0.65 [b]	1.32±0.31 [c]
Triglycerides (mmol/l)	1.11±0.35	1.18±0.65	1.23± 0.13	1.52±0.4	1.26±0.4
Total protein(g/dl)	7.28±1.68	4.78±1.06 [**]	5.86± 0.66 [a]	7.80±1.01 [b k]	5.20±.41

n = 6 (n: number of animals in each group); [*] $p \leq 0.05$, [**] $p \leq 0.01$: significantly different from control group,

[a] $p \leq 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$: significantly different from AgNO$_3$; [k] $p \leq 0.01$: statistical difference between AgNO$_3$ + Se and AgNO$_3$ + vit E + Se

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
178

Effect of treatments on biochemical parameters

As seen from table 2, AgNO$_3$induced a significant increase (p ≤ 0.05) in LDH activity, a significant decrease (p ≤ 0.05) in ALP activity, a highly significant increase (p ≤ 0.01) in cholesterol and a highly significant decrease (p ≤ 0.01) in total proteins compared to the corresponding control values. Supplementation of vitamin E and/or Se to AgNO$_3$-treated group restored the levels of ALP, total protein, LDH and cholesterol, the two later parameters presented a notable decreases(LDH: p ≤ 0.01, AgNO$_3$ + vit E+ Se and cholesterol: p ≤ 0.001, AgNO$_3$+ vit E, AgNO$_3$+ vit E + Se, p ≤ 0.01, AgNO$_3$+ Se) compared to metal exposed animals, while an amelioration in ALP activity (p ≤ 0.01, AgNO$_3$+ Se), and total protein concentration (p ≤ 0.05, AgNO$_3$ + vit E, p ≤ 0.01, AgNO$_3$ + Se) compared to AgNO$_3$ group.

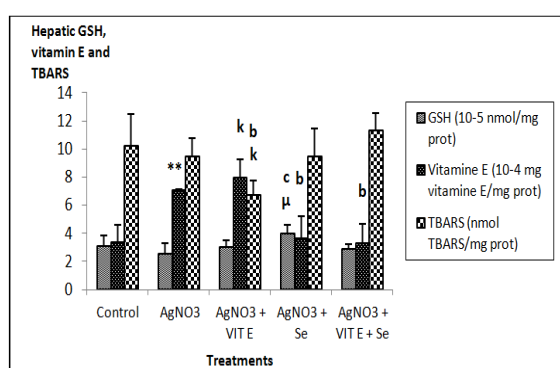## Effect of treatments on oxidative stress parameters

Figure 1: Reduced glutathione (GSH), vitamin E and lipid peroxidation levels in liver tissue of control and experimental rats

Each value is a mean ± SEM; n = 6; **p ≤ 0.01: significantly different from control group; ^{b}p ≤ 0.01, ^{c}p ≤ 0.001: significantly different from AgNO$_3$,; $^\mu$p ≤ 0.05, ^{k}p ≤ 0.01: statistical difference between AgNO$_3$ + vit E, AgNO$_3$ + Se and AgNO$_3$ + vit E + Se

Exposure to AgNO$_3$ did not result significant variation in TBARS level in liver tissue compared to control group.

However, AgNO$_3$+ vit E treated group caused a highly significant decrease (p ≤ 0.01) in hepatic TBARS concentrations when compared to AgNO$_3$ animals (Figure 1).

Liver GSH content was not affected in Ag-exposure, while, a very highly significant increase(p ≤ 0.001) in Se supplemented group was observed in comparison with AgNO$_3$ animals.

Liver vitamin E concentration was increased in AgNO$_3$ treated group compared to controls (p ≤ 0.01), this concentration was highly significant decreased (p ≤ 0.01) in AgNO$_3$ + Se and AgNO$_3$ + vit E + Se treated groups compared to AgNO$_3$ group.

Data on GSH-Px, GST, GR and SOD activities are presented in figure 2 and figure 3.

Liver GR, GSH-Px and GST activities were not altered under the pro-oxidant AgNO$_3$.

However, hepatic SOD activity was decreased (p ≤ 0.05) in AgNO$_3$ group compared to the control group.
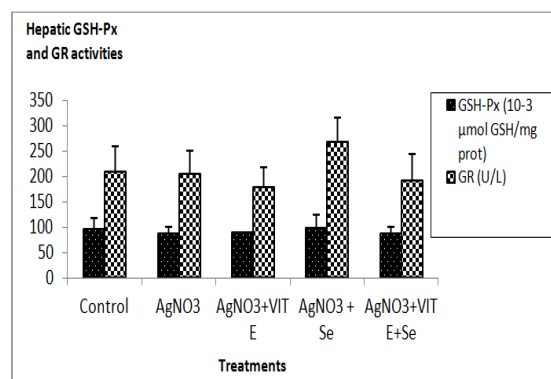

Figure 2: Glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities in liver tissue of control and experimental rats
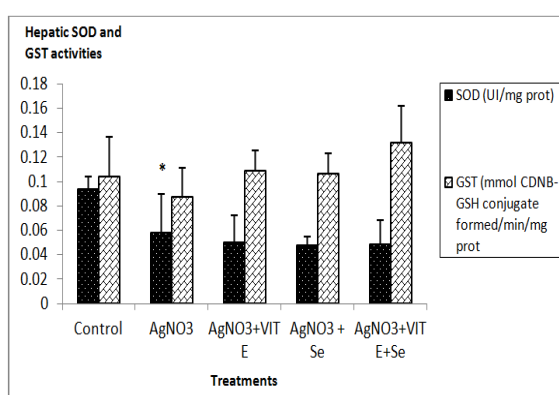

Figure 3: Superoxide dismutase (SOD) and glutathione transferase (GST) activities in liver tissue of control and experimental rats

Each value is a mean ± SEM; n = 6; *p ≤ 0.05: Significantly different from control group

Histological results

Microscopic examinations of the liver sections after 3 months from the control group showed normal architecture (Figure 4.A1.A2). However, histopathological examination of liver from rats treated with silver revealed the absence of radial arrangement of hepatocytes with vascular congestion; sinusoidal dilatation was observed along with erythrocyte accumulation, congestion and enlargement in portal veins (Figure 4.B1.B2).

In Vitamin E supplementation, the normal arrangement of hepatocytes was restored with normal sinusoidal spaces though congestion was seen in around central vein (Figure 5.C). In AgNO$_3$ + Se treated group, no venal congestion was observed, but enlargement of sinusoids and irregular histological structures were still presented (Figure 5.D). The co-administered of vitamin E and Se showed that the lamellar pattern of hepatocytes was restored in comparison with metal exposed rats, though, venal erythrocytes accumulation and congestions were seen as shown in Fig 5.E1.E2.

DISCUSSION

In the present study, bodyweight gain and relative liver weight of animals were not affected by AgNO$_3$. Such an observation does not agree with some previous studies where growth rates were retarded or bodyweight gain was decreased following oral administration of silver ions,[28,29] even though few studies reported that in a range of oral investigations, silver administration did not affect body weight.[30,31] Also no appreciable changes were observed in diet and water consumption in rats exposed to AgNO$_3$. Simultaneously, the treatment with selenium and/or vitamin E did not result any variations in body weight gain and food intake except for a lower consumption of water in AgNO$_3$ + vit E + Se treated-group which could be a spurious finding and not an indication of toxicity effects. Liver plays a central role in the detoxification process faces the threat of maximum exposure to xenobiotics and their metabolic by product.[32] Serum enzymes including AST, ALT, LDH and ALP are used in the evaluation of hepatic damage. Silver exposure caused an increase in LDH and a decrease in ALP activities in serum of rats comparing to controls. Increased levels of LDH could be attributed to liver disease, myocardial infarction or muscular dystrophy,[33] the ALP is an enzyme which is presented in rat with high proportion in intestine, a decreased serum ALP may be due to hypothyroidism, hypoparathyroidism, malnutrition and/or gastrointestinal disease.[34] Thus, several studies demonstrated that silver ions had an effect on the gastrointestinal tract.[35,36] Similarly to these results, some reports found an alteration in blood serum enzymes activities following oral administration of ionic silver or silver nanoparticles.[7,37,38] Our investigation showed also that chronic silver administration led to an increase in blood cholesterol and a decline in serum total protein.[28] Hypercholesterolemia might be resulted from hypothyroidism, hyper lipoproteinemia and/or liver toxicty.[39,40] However, the decrease in serum total protein of Ag-treated rats might be due to changes in proteins synthesis and/or metabolism.[41] Meanwhile, the findings showed that animals treated with vitamin E and /or selenium presented lower blood LDH and cholesterol and higher ALP and total protein values than those of metal exposed animals, a findings which have also been observed by other researchers.[13,14]

ROS are a group of short-lived reactive oxidants, including the superoxide radical($O_2^{\circ-}$), hydroxyl radical (OH°), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). ROS can be generated directly or indirectly inside cells, and oxidative stress results from an imbalance between ROS-generation and cellular defensive functions, including those of antioxidant enzymes and antioxidants. Oxidative stress engenders many problems in cells, such as protein oxidation, DNA damage, and lipid peroxidation.[42] Silver has been suggested to induce reactive oxygen species generation,[10,42] in the current study, no significant variations in liver TBARS levels as biomarkers of lipid peroxidation were remarked in metal exposed group, a

result which could be correlated with any significant changes in antioxidant enzymes activities such as GSH-Px, GR and GST, except a significant decrease in hepatic SOD activity. Moreover, estimation of non- enzymatic antioxidants showed an increase in hepatic vitamin E concentration. Thus, the unchangeable TBARS levels could be explained by the rapidly elimination of silver, whether administered orally or parenterally from liver into the bile (about 70% - 90% of administered dose within 24 hours).[30,43] In addition Wijnhoven[44] have hypothesized that the toxic effects of silver are proportional to free silver ions which could be associated with the dose of intoxicant used in experiment. The decrease of SOD activity in AgNO$_3$ intoxicated animals may be owed to the consumption of this enzyme in converting the $O_2^{\circ-}$ to H_2O. In other words, Park[42] found that AgNO$_3$ increased the production of superoxide anion radicals within mitochondria. Thus, it might be due to the reaction with SH groups of enzymes belonging to the respiratory chain. Whereas, the augmentation of hepatic vitamin E level might be induced by silver ions like some transition metal as cadmium which increases the concentration of vitamin E in the liver.[45] Animals treated with vitamin E and/or Se presented important differences in cellular oxidative status by reduction of lipid peroxidation and an increase of GSH content. Thus, these results have been accentuated by some investigators who demonstrated the powerful antioxidant role of both vitamin E and Selenium.[46] In other words, selenium is an essential constituent of glutathione peroxidase, which catalyzes the degradation of hydrogen peroxide and organic hydroperoxide and vitamin E is the major lipid-soluble antioxidant which interacts directly with a variety of oxygen radicals, including superoxide. It is known to accumulate in the inner mitochondrial membranes, where it protects them against respiratory oxidative stress.[46] Liver toxicity as also evaluated by histopathology included venal enlargement and congestion, venal erythrocytes accumulation, vascular congestion, sinusoidal dilatation and irregular hepatocytes arrangement, but neither inflammatory cell infiltration nor necrosis or apoptosis was observed.[29]
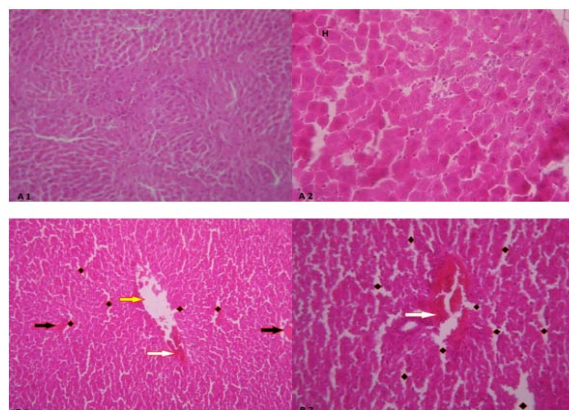

Figure 4: Effect of silver (Ag) on histological damage in the liver. Control (A1×250, A2×400), treated with Ag

(B1×250, B2×400). Optic microscopy section were stained using the haematoxylin-eosin method

H: hepatocytes, ▭⟶ congestion of veins,

▭⟶ dilatation of veins, ◼⟶ vascular congestion ◆ dilatation of sinusoids

Presumably, silver cytotoxicity was not mediated by the concurrent lipid peroxidation,[9] while treatment with antioxidants was seen to diminish hepatic parenchymal alterations compared to silver treated group. Therefore, these findings are consistent with some toxicological studies which reported that deficiency of dietary vitamin E and selenium induced a number of lesions including erythrocyte hemolysis, myodegeneration, liver necrosis and kidney degeneration in rats depending upon the degree of depletion.[13,15]
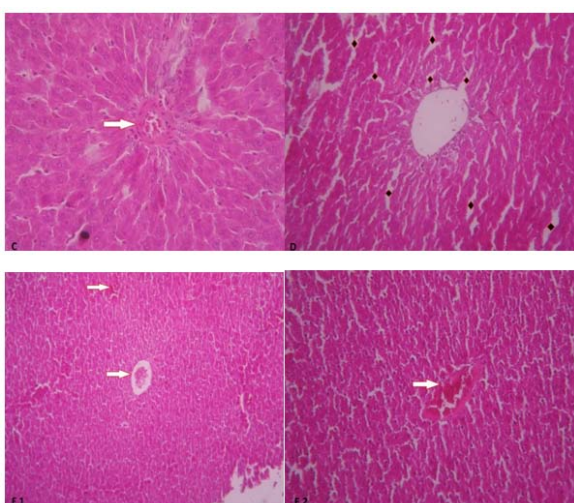

Figure 5. Effect of vitamin E and/or selenium (Se) administered with silver (Ag) on histological damage in the liver. Ag-vit E (C×250), Ag-Se (D×250), and Ag-vit E-Se (E1×100, E2×250). Optic microscopy section were stained using the haematoxylin-eosin method. Optic microscopy section were stained using the haematoxylin-eosin method

▭⟶ Congestion of veins, ◆ dilatation of sinusoids

CONCLUSION

The present data showed that silver intoxication altered serum blood enzymes such as LDH and ALP activities, induced a hypercholesterolemia, disturbed antioxidant system especially hepatic SOD activity and vitamin E content and caused parenchymal hepatic disorders, an effects which could be more toxic in high doses or in long term exposure. However oral administration of selenium or vitamin E attenuated the adverse effects of this metal, it interacts with selenium resulted in the formation of silver selenide deposits in the liver which may be considered a silver detoxification process; also vitamin E is characterized by preventing metal influences.

In brief an equilibrate alimentation; rich in antioxidants can prevent silver toxicity.

REFERENCES

1. Hiriart-Baer VP, Fortin C, Lee DY, Campbell PGC, Toxicity of silver to two freshwater algae, Chlamydamonasminhardtii and Pseudokirchneriellasubcapitata, grown under continuous culture conditions: influence of thiosulphate, AquaticToxicology, 78, 2006, 136-148.

2. Wan AT, Conyers RAJ, Coombs CJ, Masterton JP, Determination of silver in blood, urine and tissues of volunteers and burn patients, Clin. Chem, 37, 1991, 1683.

3. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA, Effect of silver on burn wound infection control and healing: Review of the literature, Burns, 33, 2007, 139-148.

4. Lansdown ABG, Silver 2: Toxicity in mammals and how its products aid wound repair, J. Wound Care, 11, 2002, 173-177.

5. Lansdown ABG, Silver in health care: antimicrobial effects and safety in use, Curr. Prob. Dermatol, 33, 2006, 17-34.

6. WHO, Silver. Guidelines for Drinking water Quality, Vol.2, Geneva, 1996, 338-343.

7. EPA, Ambient water quality criteria for silver, Washington, DC, EPA, 440/5-80-071, 1980.

8. Saeki K, Nakjimi M, Loughlen T, Calkins DC, Baba N, Kiyota M, Tatsukawa R, Accumulation of silver in the liver of three species of pinnipeds, Environ. Pollut, 112, 2001, 19-25.

9. Baldi C, Minoia C, Nucci AD, Capodaglio E, Manzo L, Effects of silver in isolated rat hepatocytes, Toxicology Letters, 41, 1988, 261-268.

10. SerafinMünoz AH, Wrobel K, Gutierrez Corona JF, Wrobel K, The protective effect of selenium inorganic forms against cadmium and silver toxicity in mycelia of Pleurotusostreatus, Mycological research I II., 2007, 626-632.

11. Letavayová L, Vlasáková D, Spallholz JE, Brozmanová J, Chovanec M, Toxicity and mutagenicity of selenium compounds in Saccharomyces cerevisiae, Mutat Res FundamMolMech Mutagen, 638, 2008, 1-10.

12. Shamberger RJ, Biochemistry of selenium, New York: Plenum Press, 1983.

13. Chow CK, Effects of dietary vitamin E and selenium on rats: pyruvate kinase, glutathione peroxidase and oxidative damage, Nutrition Research, 10, 1990, 183-194.

14. Chow CK, Vitamin E and blood, World Rev Nutr Dietetics, 45, 1985, 133-166.

15. Ganther HE, Interaction of vitamin E and selenium with mercury and silver, Annals New York Academy of Sciences, 1980, 212-226.

16. Diplock AT, Green J, Bunyan J, McHale D, Muthy IR, Vitamin E and stress. 3, The metabolism of D-α-tocopherol in the rat under dietary stress with silver, Brit. J. Nutr, 21, 1967, 115-125.

17. Kim KR, Kim JK, Rhee SJ, Effects of vitamin E on arachidonic acid cascade in platelets and aorta of acute cadmium-poisoned rats, Nutrition, 21, 2001, 657-665.

18. Qingzhi W, Kaixun H, Huibi X, Effects of long-term selenium deficiency on glutathione peroxidase and thioredoxin

reductase activities and expressions in rat aorta, Inorganic Biochemistry, 94(4), 2003, 301-306.

19. Esterbauer H, Cheeseman K, Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynenal, Enzymology, 186, 1990, 407-421.

20. Misra HP, Fridovich I, Anal. Biochem, 79, 1977, 553-560.

21. Flohe L, Gunzler WA, Analysis of glutathione peroxidase, Methods Enzymol, 105, 1984, 114-21.

22. Habig WH, Pabst MJ, Jakoby WB, Glutathione S-transferase, the first enzymatic step in mercapturic acid formation, J. Biol. Chm, 249, 1974, 7130-7139.

23. Goldberg DM, Spooner RJ, In: Bergmeyen HV, Ed Methods of enzymatic analysis, 3rded, Vol 3, VerlogChemie, Deerfield Beach. FI, 1983, 258-265.

24. Ellman GL, Tissue sulfhydryl groups, Arch BiochemBiophys, 82, 1959, 70-7.

25. Desai ID, Vitamin E analysis method for animal tissue, Methods Enzymol, 105, 1984, 138-143.

26. Bradford M, A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein binding, Anal Biochem, 72, 1976, 248-54.

27. Hould R, Techniques d'histopathologie et de cytopathologie, Ed Maloine, 19-21, 1984, 225–227.

28. Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH, Chang HK, Lee JH, Oh KH, Kelman BJ, Hwang IK, Yul J, Subchronic oral toxicity of silver nanoparticles, Part FibreToxicol, 7, 2010, 20.

29. Hadrup N, Loschner K, Bergström A, Wilcks A, Gao X, Yogel U, Frandsen HL, Larsen EH, Lam HR, Mortensen A, Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats, Arch Toxicol, 86, 2012, 543-551.

30. Van Der Zande M, Vanderbriel RJ, Van Doren E, Kramer E, Rivera ZH, Serrano-Rojero CS, Gremmer ER, Mast.J, Peters RJ.B, Hollman PCH, Hendriksen PJM, Marvin HJP, Peijnenburg ACM, Bouwmeester H, Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure, American Chemical Society, 6(8), 2012, 7427-7442.

31. Espinosa-Cristobal LF, Matrinez-Castanon GA, Loyola-Rodriguez JP, Patino-Marin N, Reyes-Macias JF, Vargas-Morales JM, Ruiz F, Toxicity, distribution and accumulation of silver nanoparticles in Wistar rats, J. Nanopart. Res, 15(6), 2013, 1-12.

32. Khan SM, Sobti RC, Kataria L, Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract, Clin.Chim.Acta, 358, 2005, 131-138.

33. Burtis CA, Ashwood ER, Tietz textbook of clinical chemistry, Eds Philadelphia. W.B. Saunders Company, 3rded, 1999.

34. Evans GO, O'Brien PJ, Watterson CL, Animal clinical chemistry, Taylor & Francis Group, Boca Raton, 2009.

35. Loschner K, Hadrup N, Qvortrup K, Larsen A, Gao X, Vogel U, Martensen A, Lam HR, Larsen EH, Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate, Part FibreToxicol, 8, 2011, 18.

36. Shahare B, Yashpal M, Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice, Toxicol. Mech. Methods, 23, 2013, 161-167.

37. Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD, Choi BS, Lim R, Chang HK, Chung YH, Kwon IH, Jeong J, Han BS, Yu IJ, Twenty-eight-day oral toxicity, genotoxicity, and gender related tissue distribution of silver nanoparticles in Sprague Dawley rats, InhalToxicol, 20(6), 2008, 575-583.

38. Park EJ, Bae E, Yi J, Kim Y, Choi K, Lee SH, Yoon J, Lee BC, Park K, Repeated dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles, Environ. Toxicol.Pharmacol, 30, 2010, 162-168.

39. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH, Antimicrobial effects of silver nanoparticles, Nanomedicine, 3, 2007, 95-101.

40. Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH ChungYH, Chang HK, Lee JH, Cho MH, KelmanBJ, Yu IJ, Subchronic inhalation toxicity of silver nanoparticles, ToxicolSci, 108(2), 2009, 452-61.

41. Das KK, Dasgupta S, Effect of nickel on testicularnucleicacid concentrations of rats on protein restriction, Biol. Trace. Elem. Res, 73(2), 2000, 175-180.

42. Park HJ, Kim JY, Ki J, Lee JH, Hahn JS, Gu MB, Yoon J, Silver-ion-mediated reactive species generation affecting bactericidal activity, Water Research. 43, 2009, 1027-1032.

43. Eisler R, Silver hazards to fish, wildlife, and invertebrate: a synaptic review. Biological Review, U.S. Department of the interior Washington, DC, No. 32, 1996.

44. Wijnhoven SWP, Peljnenburg WJGM, Herbets CA, Hagens WI, Oomen AG, Heugens EHW, Moszek B, Bisschops J, Gosens I, Dc Meent BR, Dekkers S, De Jong WH, Zijverden MV, Sips AJAM, Geertsma RE, Nanosilver : A review of available data and knowledge gaps in human and environmental risk assessment, Nanotoxicology, 3, 2009, 109-138.

45. Ognjanovic BI, Pavlovic SZ, Maletic SD, Zikic RV, Stajn AS, Radojicic RM, Saicic ZS, Petrovic VM, Protective influence of Vitamin E on Antioxidant defense system in the Blood of Rats Treated with Cadmium, Physiol. Res, 52, 2003, 563-570.

46. Djurasevic SF, Djordjevic J, Jasnic N, Lakic I, Vujovic P, Cvijic G, The influence of vitamin E supplementation on the oxidative status of rat interscapular brown adipose tissue, Arch. Biol. Sci., 62(4), 2010, 993-1003.