Effect of selenium and vitamin E on some physiological parameters of male albino rats subjected to drink polluted water contain mixture of heavy metals

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Abstract: Toxic heavy metals in water, air and soil are global problems that are a growing threat to humanity. Heavy metals pollution of surface water can create health risks. The current study aimed to demonstrate (1) the alterations in biochemical parameters, free radicals and enzyme activities induced by mixture of heavy metals in serum of male albino rats, and (2) the role of selenium and vitamin E in alleviating the negative effects of these heavy metals. Method: Ten rats per group were assigned to one of six treatment groups: group I served as control which received standard diet, group II received in drinking water mixture of heavy metals alone, group III received Se alone (0.5 Na\textsubscript{2}SeO\textsubscript{3} mg/kg of diet), group IV received both mixture of heavy metals and Se (0.5 mg/kg of diet), group V received vitamin E alone at a dose of 50 IU/kg body weight, group VI received both mixture of heavy metals and vitamin E (50 IU/kg body weight). Evaluations were made for some tumor markers, enzyme activities and biochemical parameters. Results showed that heavy metals resulted into significant increase (P<0.05) in (AFP), (GGT) and (ALAT), (ASAT), (ALP), and (LDH) and blood urea, serum creatinine, and serum uric acid. On the other hand VE or Se in combination with mixture of heavy metals partially or totally alleviated its toxic effects on the studied parameters. Conclusion: VE and Se have beneficial effects and could be able to antagonize heavy metals toxicity.

Keywords: Heavy metals, Tumor marker, Liver enzymes, Kidney functions

1. Introduction

Environmental pollution is the contamination of the ecosystem that causes instability, disorder, harm or discomfort to the physical systems or living organisms. Environmental factors have important links with infectious as well as non-infectious diseases of both acute and chronic nature. Global burden of disease attributable to selected sources of environment like water sanitation and hygiene, urban outdoor and indoor pollution, occupational carcinogens, noise and airborne particulates has been assessed to be 8–9%, measured either in terms of mortality or disability adjusted life years (DALYs) (Ezzati et al., 2002).

Metal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation (Jaishankar et al., 2014).

Vitamin E is an important component in human diet and considered the most effective liposoluble antioxidant found in the biological system. It is composed of various subfamilies of which tocopherols and tocotrienols are the most studied. The structural difference between the two subfamilies is that tocotrienols possess three double bonds in their isoprenoid side chain and this structural difference results in differences in their efficacy and potency as antioxidants (Musalmah et al., 2002).

Selenium was recognized as an essential trace element within a relatively low concentration range and its physiological role was established when it was shown to be one of the glutathione peroxidase (GPx) components. Selenium deficiency is usually associated with increased lipid peroxidation which alters the integrity of cell membranes and consequently, affects cell functions (El-Sharakya et al., 2007).

The current study aimed aimed to illustrate the protective role of selenium and vitamin E referred to its antioxidant defense system in controlling these chronic toxic effects.

2. Material and Methods

2.1. Chemicals

2.1.1. Selenium

Sodium selenite was obtained from Sigma Company, Egypt. Na\textsubscript{2}SeO\textsubscript{3} (pure white powder, 25gm package) was prepared by dissolving 0.1 mg into one liter of distilled water.

2.1.2. Vitamin E

It is the ester of acetic acid and tocopherol (vitamin E) Good N natural vitamin E, the commercial form of α- tocopheryl acetate, was procured as a gelatinous capsule with a gel volume of
1ml, with a concentration of 1000mg/ml (Monsen, 2000).

2.2. Grouping of animals and treatment

This study was carried out on 60 adult male healthy Albino rats (170±10 gram at the beginning of experiment). Each 5 rats were placed in a separate cage to avoid stress resulting from isolation or overcrowding. Rats were divided randomly into 6 groups, 10 rats per each group. Na$_2$SeO$_3$ and vitamin E were administered orally by Gavage's tube. Tap water was used in all groups. The period of the experiment was 2 months.

The first group was control group (group 1); rats of this group were received normal drinking water orally once daily. Group 2 received polluted water from Bahr El-baqar and selected the highest concentrations of heavy metals which was (Pb, Cd, Cu, and Fe) in their drinking water as follows: (0.08 mg/l Pb, 0.006 mg/l Cd, 3.9 mg/l Cu and 0.8 mg/l Fe) daily for 60 days. Group 3 was selenium group; received normal drinking water and treated with selenium at a dose of 0.5mg/kg body weight, daily for 60 days as control. Group 4 was received polluted water which contains heavy metals (Pb, Cd, Cu, and Fe) in their drinking water and protected with selenium at a dose of 0.5mg/kg body weight, daily for 60 days. Group 5 was vitamin E group; received normal drinking water and treated with vitamin E dissolved in crude olive oil at a dose of 35mg/kg body weight, daily for 60 days. Group 6 was received polluted water which contains heavy metals (Pb, Cd, Cu, and Fe) in their drinking water and treated with vitamin E dissolved in crude olive oil at a dose of 35mg/kg body weight, daily for 60 days.

Doses of Na$_2$SO$_3$ and vitamin E in mg/Kg body weight were calculated according to the animal’s BW before treatment. The selenium dose (0.5 mg/kg of diet) used in our experiment and in other findings gave high protection against stress conditions in several tissues (Ognjanovic et al., 2008 and Ben Amara et al., 2009). On the other hand, the vitamin E dose was selected based on the clinical application and on results from previous studies on human and experimental animals (Al-Attar, 2011b).

At the end of experiment, blood samples were taken from orbital venous plexus under total anesthesia with diethyl ether. The blood samples were collected and put into chilled non heparinized tube, which were leaved for clotting then centrifuged at 3000 r.p.m for 10 minutes to prepare serum. The sera were frozen at -20 °C for the following biochemical measurements.

Serum α -Feto Protein (AFP) is determined according to the method of Engall (1980). Serum Gamma Glutamate Transaminase (γ-GT) is determined according to the method of Rosalki et al. (1971) using a commercial kit derived from Randox. Serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assayed by the method of Schumann and Klaue (2003). ALP activity was estimated according to the method of Belfield and Goldberg (1971). Lactate dehydrogenase (LDH) activity was assayed using commercial kit from (Spinreact, Spain) according to the method of Young and Friedman (2001). Concentrations of urea, creatinine and uric acid were determined by the methods of Chaney and Marbach (1962); Heinegård and Tiderstörm (1973) and Fossati (1980), respectively.

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 20.7 for windows, and data were expressed as percentages. Significance was assumed when probabilities (P) were less than 0.05.

3. Results

Treatment with heavy metals significantly increased (p<0.05) serum α- feto protein (AFP) (+ 78.05%) and GGT (+ 366.6%) activities as in table (1) and Figs. (1, 2) as compared to the control. On the other hand, the presence of either selenium or vitamin E with heavy metals caused a significant decrease (p<0.05) in the elevated serum α- feto protein (AFP) and GGT due to heavy metals treatment. As seen in table (2) and Figs. (3, 4, 5 & 6), the levels of serum ALAT (+ 76.2%), ASAT (+ 29.1%), ALP (+ 30%) and LDH (+ 13.23%) were significantly increased (p<0.05) in rats treated with polluted water containing mixture of heavy metals compared with control. Insignificant alterations in the levels of these enzymes were observed in rats treated with only selenium or vitamin E. The data obtained revealed significant decrease (p<0.05) in alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and alkaline phosphatase (ALP) activities in polluted water + selenium treated group when compared to polluted water treated group as in table (3) and Figs. (3, 4 & 5). While rats treated with polluted water + vitamin E showed significant decrease in alanine aminotransferase (ALAT) only as seen in table (2) and Fig. (3).

There was significant alteration in renal function in rats exposed to polluted water containing heavy metals in comparison to control and other treated groups as indicated by significant increases of blood urea (+ 46.6%), creatinine (+ 84.3%) and uric acid (+ 46.6%) concentrations as in table (3) and Figs. (7, 8 & 9). The levels of all studied parameters were not significantly altered in rats treated with only selenium or vitamin E compared to control rats.
Table 1. Fetoprotein alpha (AFP) (ng/ml) and γ-Glutamate (U/L) concentrations in rats drinking polluted water protected or non with selenium or V.E for 60 days (mean ± SE) in different treated groups of experiments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment Groups</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Polluted water</td>
</tr>
<tr>
<td>(AFP) Mean ± SE</td>
<td>0.65 ± 0.05[a]</td>
<td>1.36 ± 0.04[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(GGT) Mean ± SE</td>
<td>1.5 ± 0.4[a]</td>
<td>7 ± 0.4[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Values are expressed as means ± SE; n = 10 for each treatment group. Superscript letters indicate significant differences between groups. The groups with the same letter mean there is no significance difference and which have different letter mean there is a significance change. %: Percent of changes from control values. P.W: Polluted water. Se: Selenium. V.E: Vitamin E.

Table 2. Serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities (U/L) in rats drinking polluted water protected or non with selenium or V.E for 60 days (mean ± SE) in different treated groups of experiments

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Controls</td>
<td>Polluted water</td>
</tr>
<tr>
<td>ALAT (U/L) Mean ± SE</td>
<td>10.1 ±1.3[a]</td>
<td>17.8 ± 1.5[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>76.2</td>
</tr>
<tr>
<td>ASAT (U/L) Mean ± SE</td>
<td>8.6±0.45[ac]</td>
<td>11.1 ± 0.4[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>29.1</td>
</tr>
<tr>
<td>ALP (U/L) Mean ± SE</td>
<td>151.2±14.6[ac]</td>
<td>196.5 ± 5.2[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>LDH (U/L) Mean ± SE</td>
<td>2951.2±181.3[ac]</td>
<td>3341.8 ± 20.1[bc]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>13.23</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 10 for each treatment group. Superscript letters indicate significant differences between groups. The groups with the same letter mean there is no significance difference and which have different letter mean there is a significance change. %: Percent of changes from control values. P.W: Polluted water. Se: Selenium. V.E: Vitamin E.

Table 3. Serum urea, creatinine and uric acid (mg/dl) concentrations in rats drinking polluted water protected or non with selenium or V.E for 60 days (mean ± SE) in different treated groups of experiments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment Groups</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Polluted water</td>
</tr>
<tr>
<td>Urea (mg/dl) Mean ± SE</td>
<td>31.55 ± 2.95[a]</td>
<td>52.64 ± 3.68[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>66.8</td>
</tr>
<tr>
<td>Creatinine (mg/dl) Mean ± SE</td>
<td>0.83 ± 0.04[ac]</td>
<td>1.53 ± 0.1[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>84.3</td>
</tr>
<tr>
<td>Uric acid (mg/dl) Mean ± SE</td>
<td>4.29 ± 0.23[a]</td>
<td>6.29 ± 0.1[b]</td>
</tr>
<tr>
<td>% of change</td>
<td>-</td>
<td>46.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 10 for each treatment group. Superscript letters indicate significant differences between groups. The groups with the same letter mean there is no significance difference and which have different letter mean there is a significance change. %: Percent of changes from control values. P.W: Polluted water. Se: Selenium. V.E: Vitamin E.
Figure 1. Serum concentrations of AFP (ng/ml) in different treated groups of albino rats

Figure 2. Serum concentrations of Gamma Glutamyl transferase (γ-GT) in different treated groups of albino rats

Figure 3. Serum activity of alanine aminotransferase (ALAT) activity (U/L) in different treated groups of albino rats

Figure 4. Serum activity of aspartate aminotransferase (ASAT) activity (U/L) in different treated groups of albino rats

Figure 5. Serum activity of alkaline phosphatase (ALP) activity (U/L) in different treated groups of albino rats

Figure 6. Serum activity of lactate dehydrogenase (LDH) activity (U/L) in different treated groups of albino rats

Figure 7. Serum concentrations of urea (mg/dl) in different treated groups of albino rats

Figure 8. Serum concentrations of creatinine (mg/dl) in different treated groups of albino rats
4. Discussion

It is well known that heavy metals are widely distributed in the environment and some of them can cause physiological, biochemical and histological disorders. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances. The physiological influence of metals on organisms, humans and animals is conditioned by the nature of metal, by the type of compounds and by their amount. Moreover, different scientific studies indicated that the degree of toxic manifestation of different metals depends on dose, duration, route of administration and other physiological factors, especially nutrition (Roy Chowdhury, 2009).

4.1. Tumor markers

4.1.1. Serum alpha-fetoprotein (AFP) activity

Regarding the results of tumor markers AFP, it showed significant increase in polluted water and polluted water + selenium and polluted water + vitamin E treated groups as in table (1) and fig. (1) when compared to the corresponding values in control group and treated groups. These results may be due to the hepatic necrosis.

Mizejewski et al. (1990) found that low doses of nickel and copper were associated with elevated AFP levels in amniotic fluid in 15 – 17 day pregnant animals, while maternal serum AFP levels mostly remained unchanged. Decreased concentrations of maternal serum AFP occurred with increased doses of copper and lead in contrast to elevated concentrations of AFP in amniotic fluid. Futhermore, there was an increase in fetal wastage when higher doses of copper and lead were administered.

4.1.2. Serum Gamma Glutamyl transferases level (γ-GT)

Regarding to the results of tumor marker GGT, it showed significant increase in polluted water and polluted water + selenium treated groups as in table (1) and fig. (2) when compared to the corresponding values in control group. This agreement with Al-Attar (2011a) who found that the mixture of heavy metals (Pb, Hg, Cd, and Cu) induced significant increases of plasma GGT these indicated impaired liver function and Adeyemi et al. (2009) who showed increased in gamma glutamyl transpeptidase activities which considered as an index of hepatobiliary dysfunction (Green and Flamm, 2002).

The data obtained revealed significant decrease in Gamma Glutamyl transferases level (γ-GT) in polluted water + vitamin E treated group when compared to the corresponding values in polluted water treated group due to antioxidant activity of vitamin E, this results comes in agreement with Al-Attar (2011a).

4.1.3. Liver enzymes

Liver function tests are helpful screening tools to detect hepatic dysfunction and are further used to categorize hepatic dysfunctions, to estimate the severity of hepatic disease, and for the follow-up of liver diseases. Since liver performs a variety of functions, no single test is sufficient alone to provide complete estimate of function of liver (Kim, 2008).

The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal vein from the digestive system, and liver function can be detrimentally altered by injury resulting from acute or chronic exposure to toxicants (Al-Attar, 2011a).

The present study revealed significant increase in the activity of ALAT in serum of polluted water and polluted water + selenium and polluted water + vitamin E treated groups while the activity of ASAT and ALP showed significant increase in polluted water and polluted water + vitamin E treated groups while LDH activity showed significant increase in polluted water treated group only as in table (2) and fig. (3, 4, 5, 6) when compared to corresponding values in control group. These results may be due to the hepatic necrosis.

These results are in agreement with Al-Attar (2011a) who showed that the mixture of heavy metals (Pb, Hg, Cd, and Cu) induced significant increases of plasma ALAT, ASAT, ALP and GGT and these indicated impaired liver function. El-Demerdash et al (2004) who recorded increase in plasma ASAT and ALAT activities in rats treated with CdCl₂; this may be due to a large number of cellular processes including its replacement of zinc in many vital enzymatic reactions and these are in agreement with the findings of Rana et al. (1996).

Similar observations were reported in many experimental investigations on animals exposed to Pb (Bersenyi et al., 2003; Shalan et al., 2005; Garg et
There are many scientific researchers showed that intoxication with Pb, Hg, Cd and Cu caused several hepatic damages in experimental animals (Aburto et al., 2001; Jihen et al., 2008; Cavusoglu et al., 2009; Haleagrarahar et al., 2010; Oguz et al., 2010 and Jabeen and Chaudry, 2011).

The result of alkaline phosphatase which showed significant increase in polluted water and polluted water + vitamin E treated groups comes in contrast with Rana et al. (1996); El-Demerdash and Elagamy (1999) and El-Demerdash et al. (2004). Rana et al. (1996) showed that the decrease in ALP activity may be due to changes in the permeability of plasma membrane in addition to changes in the balance between synthesis and degradation of enzyme.

The data obtained revealed significant decrease in (ALAT) activity in polluted water + selenium and polluted water + vitamin E treated groups while the activity of ASAT and ALP showed significant decrease in polluted water + selenium group when compared to polluted water treated group.

The hepatoprotective effect of vitamin E may be due to its antioxidant activity. These results are in agreement with Al-Attar (2011a) who reported that vitamin E reversed Pb, Hg, Cd and Cu-induced liver injury and the levels of plasma ALAT, ASAT, ALP and GGT were statistically decreased in mice treated with the mixture of heavy metals plus vitamin E compared with heavy metals treated group.

These results are also in agreement with Rao et al. (2006); Ahmadi zaledeh and Baghpa (2008); Osfor et al. (2010) and Sajitha et al. (2010) who reported that administration of vitamin E declined the histopathological and biochemical alterations induced by Pb intoxication in female Sprague-Dawley albino rats.

41.4. Kidney function tests

Diseases of the kidney have two consequences; the first is failure to retain substances such as protein, amino acids, sugar, water and ions. The second consequence is failure to excrete substances such as urea, creatinine and waste products (Oloyede et al., 2003).

The present work showed a significant increase in blood urea and serum uric acid concentrations in polluted water and polluted water + selenium and polluted water + vitamin E treated groups while creatinine concentration showed significant increase in polluted water treated group only when compared with control group as in (Table 3) and (Fig. 7,8,9) after 60 days; increase in blood urea is known to be correlated with an increased protein catabolism in mammals and/or conversion of ammonia to urea as a result of increased synthesis of ornithine enzyme involved in urea production (Harper et al., 1979).

These results are in agreement with Adeyemi et al. (2009) who showed that the presence of lead in water might have caused impairment of the brush border epithelial cells and making them impermeable to urea and creatinine thereby causing their elevated levels in the blood. The overall effect of this may be impaired kidney function. Also in agreement with Osfor et al. (2010) and these findings were supported by those of Hanafy and Soltan (2004). These results are also in agreement with Al-Attar (2011b) who demonstrated that mice chronically intoxicated with a mixture of some heavy metals display a pronounced impairment in kidney function which is confirmed by the enhancement of plasma creatinine, urea and uric acid levels.

Several studies demonstrated a significant enhancement of blood urea, creatinine and uric acid concentrations in experimental animals intoxicated with lead, mercury, cadmium, copper and other heavy metals (Brzoska et al., 2003; Odigie et al., 2004; Chen et al., 2006; Goran et al., 2008; Al-Madani et al., 2009; Saxena et al., 2009 and Missoun et al., 2010).

The present study showed that, the administration of vitamin E and selenium (g/kg b.w) significantly improves blood urea, creatinine and uric acid levels (Table 3) in polluted water + vitamin E and polluted water + selenium when compared with polluted water treated group which is significantly decreased.

The improvement in blood urea, creatinine and uric acid is may be attributed to ameliorative effect of vitamin E and selenium which are known to reduce oxidative radical-induced reactions.

These results are in agreement with El-Demerdash et al. (2004) who reported that Vitamin E and Selenium alone caused a significant decrease in levels of urea and creatinine as compared with Al intoxicated group and this may be due to ability of sodium selenite to maintain a functional renal state in the case of Al intoxication (Rudenko et al. 1998). Hanafy and Soltan (2004) concluded that the combined exposure to a mixture of vitamin E and examined heavy metals can diminish blood urea and serum creatinine levels.

These results are in agreement with Agrawal et al. (2010) and Osfor et al. (2010) who studied the effect of both pre- and post-treatment of vitamin E on
Hg induced acute toxicity in rats. As Hg is nephrotoxic and neurotoxic, it is interesting to note that post-treatment of vitamin E showed more protection in the kidney compared to pre-treatment.

5. Conclusion

It could be concluded that heavy metals have a hazardous adverse effects on general health of people exposed to copper, lead, cadmium and iron. This research led to an important conclusion which is the protective role of selenium against cadmium induced renal impairment. This protective role of selenium comes from the fact that selenium is a well-known potent antioxidant. This study therefore suggests that vitamin E may be a useful preventive agent against the effect of the studied heavy metals at least partly due to its antioxidant properties.

6. Recommendations

From this study, it is clear that selenium and vitamin E have a protective role against heavy metals induced toxicity, so it is recommended to be used regularly especially for populations at high risk.

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