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RESEARCH ARTICLE

Potential effects of some antioxidants against lead toxicity

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Abstract

The aim of the present study was to investigate the impact of quercetin or zinc and their mixture against lead toxicity.Thirty five adult male albino rats were divided into five groups of seven rats in each. The first was a control group, the second and third group received lead nitrate and quercetin orally in adose of 20 and 50mg/kg b.wt /dayrespectively;thefourth group wasi.pingected with 2mg/kg b.wt/day of zinc.The last group supplemented both antioxidants (quercetin&zinc) for 2 weeks.

Lead administration showed a significant elevation in serum aspartate aminotransferase (AST),alanine aminotransferase (ALT),alkaline phosphatase (ALP) andlactate dehydrogenase (LDH) activities and in serum level of total bilirubin beside reduction in serum level of albumin .The concentration ofreduced glutathione (GSH) ,GSH/GSSG ratio and the activity ofof superoxide dismutase (SOD) were significantly decreased in the liver of rats that had received lead whileoxidized glutathione (GSSG) andmalonaldehyde (MDA) were significantly increased.

The results of the current study showed significant improvement in serum biochemical parameters beside reduction in alterations in liver antioxidant defensestatus mainly through glutathione dependent system..Itcan be concluded that best amelioration was occurred inlead-toxicated rats that received both antioxidants for 2 weeks and quercetin acts synergistically with zinc to protect and inabating some hazards of lead on liver tissue

INTRODUCTION

Exposure to toxic metals is a well-known problem in industrialized countries.Metals interfere with a number of physiological processes, including central nervous system (CNS), haematopoietic, hepatic and renal functions (Sinicropi et al,2010). Long term exposure to these metals could lead to apoptosis. Signaling components affected by metals include growth factor receptors, G-proteins, MAP kinases and transcription factors (Flora et al.,2008).Lead is a widespread and insidious environmental toxins and is known as a sever and aggressive contaminant on human and animals organisms health status.

Lead exposure mainly occurs through the respiratory and gastrointestinal systems. Absorbed Pb (whether inhaled or ingested) is stored in soft tissues. Autopsy studies of Pb-exposed humans indicate that liver tissue is the largest repository (33%) of Pb from among the soft tissues followed by kidney cortex and medulla. As environmental exposures to Pb have increased, the toxic effects of Pb on various organ systems in the body have been recognized (Lyn,2006).

Leadpoisoning produces various deleterious effects on the hematopoietic, renal, reproductive and central nervous system, mainly through increased oxidative stress. These alterations play a prominent role in disease

manifestations(Kalia& Flora, 2005). Further, many studies have reported the presence of various cellular, intracellular and molecular mechanisms behind the toxicological manifestations caused by lead in the body (Flora et al.,2012).

Quercetin (3,5,7,3–4-pentahydroxy flavone) is the major flavonoid found in the human diet.The presence of multiple hydroxyl groups in its chemical structure and conjugated electrons account for its antioxidant and metal chelating property(Skibola et al.,2000).A number of beneficial effects of quercetin on human health have been shown (Boots et al.,2008&Askari et al.,2013). Further,Indra et al.,2013 reportedthat Quercetin suppressesinflammation by reducing ERK1/2 phosphorylation and NF kappa B activation in Leptin-induced Human Umbilical Vein Endothelial Cell (HUVECs). Numerous experimental studies have proved that quercetin exhibited hepatoprotective activity (Janbaz et al.,2004) and may have preventative effects on arteriosclerosis relevant to VSMC disorders (Ishizawa et al.,2011).Also, quercetin provide anti-inflammatory and antioxidant properties (Askari et al.,2013 ;Liu et al.,2013).

Zinc (Zn) is an essential trace element with many biological roles as, for example, in hundreds of Zn-containing enzymes and thousands of Zn-finger proteins (Maret,2005). Zn also acts as a growth cofactor, immunoregulator and antioxidant with anti-inflammatory and anti-apoptotic effects (Prasad,2008). The liver is an important tissue for regulation of Zn storage and homeostasis, and Zn is clearly necessary for appropriate liver function (Stamoulis,2007).

In human Zn is an essential component of numerous proteins involved in the defense against oxidative stress. The impact of zinc (Zn) on the immune system, the ability of zinc to act as an antioxidant in order to reduce oxidative stress and the neuroprotective and neurodegenerative role of zinc in the etiology of Alzheimer's disease is also discussed (valco et al,2005**;**JomovaandValko2011).

A novel therapeutic approach to suppress oxidative stress is based on the development of dual function antioxidants comprising not only chelating, but also scavenging components.

Material and methods

The experiments were carried out with thirty five maleadult albino rats (Sprague Dawley strain) weighing 200 ± 5 g. The animals were obtainedfrom Helwan Laboratory Farms of Egyptian Organization for Vaccine and Biological preparations. Theanimalsacclimatized for two weeks prior the experiments. They were fed to appetite on standard laboratory animal rodent feed according to NRC (1977) and water was available for animalsad libitum. They were housed in a well ventilated animal housekept under standard managerial and environmental conditions(12 h light/dark cycles at 25 ± 2 C).The protocol of this study was approved by the Department of Zoology Council, Women's College, Ain Shams University, Egypt, which has an ethical authority.

Animals were divided into five groups ofseven rats in each.Thefirst rats group served as control.The second one has treated orally with 20 mg/kg body weight/day of lead nitrate for 2 weeks (Chougule et al.,2005).The third group was orally received 50 mg/kg body weight/day ofquercetin for the same intervals (Tieppoea al.,2007).The fourth group was interaperitonealy injected with 2mg/kg body weight/day of zinc chlorid(Goulart et al.,2001).The last group(fifth group) was received both quercetin and zinc by the same previous routs for 2 weeks.All chemicals were purchased fromSigma Chem. Co., St. Louis, MO, USA.

At the end of experimental period and after overnight fasting, animals were scarified the blood samples were collected from each rat. Blood samples were centrifuged for 10 minutes at 3000 rpm within an hour of the blood collection and the sera were obtained. Sera were separated and divided into considerable aliquots to avoid the effects of repeated thawing and freezing. All specimens of sera were stored at -20°C until use.

Estimation of biochemical parameters

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined kinetically according to Bergmeyer et al. (1978) however, the activity of serum alkaline phosphatase (ALP) was determined kinetically according toHaussament (1977) using commercial kits (BioAssay Systems,USA). The activities of serum lactate dehydrogenase (LDH) were determined kinetically according to Weisshaar et al. (1975). The kit was purchased from BioVision Incorporated, Milpitas, USA.

The level of total bilirubin was assayed colourimetricallyaccording to Fossatl et al. (1989) by one-step protocol with stable combined reagents and using a commercial kit from DiaChem, Ltd., Budapest, Hungary. Moreover, the level of serum albumin was determined using commercial kit purchased from DiaChem, Ltd., Budapest, Hungary according to the method of Doumas et al. (1971). A green colour is produced upon the reaction of serum albumin with bromocresol green at pH 4.2 in succinate buffer and in the presence of Brij-35.

Determination of liver enzymes antioxidants status

The content of liver reduced glutathione (GSH) and oxidized glutathione (GSSG) were estimated according to the methods of Baker et al. (1990) and Pastore et al. (2001) respectively by the aid of ELISA technique and using commercial kits purchased from IBL, Gesellschaft, Hamburg (Germany).

The activity of superoxide dismutase (SOD) was measured by ELISA technique using commercial kits purchased from IBL, Gesellschaft, Hamburg (Germany) according to the methods of Oyanagui (1984).

Analysis of malondehyde in liver tissue

Determination of malonaldehyde (MDA) in the liver tissue was performed to estimate the extent of lipid peroxidation in the damaged liver. At the end of the experiment, livers were removed and frozen at -20°C until assay. On the day of analysis, after thawing, liver samples were washed in ice-cold 20 mMTris-HCl, pH 7.4 and blotted on absorbent paper. Each sample was then minced in ice-cold 20 mMTris-HCl, pH 7.4 containing 1 mg/ml butylatedhydroxytoluene and homogenised in a 1:10 (w/v) ratio with an Ultra-Turrax homogenizer. After centrifugation for 10 min at 3000 g and 4°C, the clear supernatant was used for biochemical assay. Analysis was performed with a colorimetric commercial kit (Lipid peroxidation assay kit, Oxis, USA) according to Pedeson et al. (1990).

Statistic analysis

The comparison between the effects of different antioxidant nutrients on biochemical parameters recorded herein were statistically analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range tests as described by Snedecor and Cochran (1982).

Results&Discussion

Pb is a widespread environmental hazard, and the hepatotoxic effects of Pb are a major public health concern. The liver, via the portal vein, is the first organ exposed to enterally absorbed nutrients and other xenobiotics. The liver is composed of highly active metabolic tissue containing a huge complement of detoxification machinery referred to as phase I and phase II enzyme systems that ideally serve to guard other physiological systems from the toxic effects of xenobiotic compounds.Earlier studies reported alterations in hepatic xenobiotic metabolism, cholesterol metabolism, liver cell proliferation, and DNA synthesis indicative of Pb-induced hepatic hyperplasia (US, EPA,1986).
The current study indicated a significant ($P<0.05$) increment in activities of serum aspartate aminotransferase (AST) ,alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in lead- toxicated animals (Table 1).These results are in harmony with the data obtained by Chougule et al. (2005) and Shalan et al.(2005).The authours suggested that The alteration in enzymes level may be due to the damage dysfunction of the related tissues.

Liver cell damage is characterized by a rise in plasma enzymes such as AST, ALT, ALP and ACP level (Aniagu et al.,2005;Adaramoye et al.,2008).Bersenyi et al.(2003) reported that heavy metals are responsible to increase the level of both enzymes (ALT & AST). Along with ALT & AST enzymes, ALP and ACP are also used as marker enzymes for liver function and integrity(Jens and Hanne,2002;Adaramoye et al.,2008). ALT activity is only found in increased level of heavy metal toxicity, toxic and musculardystrophy. Lead exposure is ableto disturb the lipid-bilayer order of the membrane structure of related organ system(Sharmaet al., 2012).

Total protein level is a frequent parameter of metal poisoning in any living organism. Albumin is the protein with the highest concentration in plasma. The present study showed that serum level of bilirubin (total as well as direct)was significantly($p<0.05$) increased in rats exposed to lead, but on the other hand decreased the level of serum albumin(Bandhu et al.,2006). These results may be attributed to the great demands and cellular damage that occurred in the tissues of lead-toxicated rats and lead toxicity maybe possible cause of

protein and albumin breakdown. Bilirubin is a breakdown product of hemoglobin. Bilirubin formed in the reticulo-endothelial system is transported to the liver bound albumin. In the liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin. Conjugated bilirubin is excreted via the bilary system into the intestine where it is metabolized. Total bilirubin is elevated in obstructive conditions of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies(Sharma et al .,2012).Also,Chadegani et al.(2011)concluded that lead reacts with chromatin components even at very low concentrations (<0.3 mM) induce chromatin aggregation through histone–DNA cross-links suggesting higher toxicity of lead nitrate on rat liver soluble chromatin and histone proteins.

Lead administrationinduces overproduction of reactive oxygen species (ROS) and depletes the cellular antioxidantcapacitythat represented by a significantalternation in the peroxidative process following lead nitrate toxicity(Aykin-Burnset al.,2003).
In the current experimentasignificant ($P<0.05$) depletion in reduced glutathione content(GSH)accompanied by asignificant ($P<0.05$) elevation in oxidized glutathione (GSSG)with a marked reduction in Liver reduced/oxidized (GSH/GSSG)glutathione ratio was noticed (Table 2).

Recent studies have shown that lead reduced the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effects of lead (Gurer andErcal, 2000;Patrick, 2006; Flora et al.,2008;Malekirad et al.,2010).

Table 1 antioxidant potential of quercetin or/and zinc on serum biochemical parameters of lead-toxicated rats.

Parameters \ Groups		Normal control group	Pb toxic group	Pb toxic Treated with Quercetin	Pb toxic Treated with $ZnCl_2$	Pb toxic Treated with Mixture
AST (U/L))	Mean±SE	110.69 ± 1.27^{A}	189.56 ± 2.11^{B}	148.92 ± 1.73^{C}	160.07 ± 1.82^{D}	131.43 ± 1.52^{E}
ALT (U/L)	Mean±SE	24.83 ± 0.457^{A}	80.17 ± 0.962^{B}	47.35 ± 0.607^{C}	53.06 ± 0.581^{D}	30.01 ± 0.574^{E}
ALP (U/L)	Mean±SE	20.89 ± 0.461^{A}	60.58 ± 0.813^{B}	35.82 ± 0.589^{C}	41.72 ± 0.614^{D}	30.68 ± 0.523^{E}
LDH (U/L)	Mean±SE	227.81 ± 3.769^{A}	429.32 ± 6.527^{B}	330.11 ± 4.859^{C}	372.67 ± 5.101^{D}	272.49 ± 4.043^{E}
Bil. (mg/dL)	Mean±SE	0.46 ± 0.008^{A}	0.93 ± 0.013^{B}	0.65 ± 0.010^{C}	0.72 ± 0.011^{D}	0.53 ± 0.009^{E}
Alb mg/dL)	Mean±SE	4.17 ± 0.106^{A}	3.29 ± 0.082^{B}	3.61 ± 0.091^{C}	3.59 ± 0.090^{C}	3.88 ± 0.099^{D}

Values are expressed as means ± S.E ,n=7 rats.

A, B, C, D, E = means bearing different superscripts within the same row that differ significantly ($P< 0.05$).

Reduced glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine) that contains a free thiol group. GSH is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water.When cells are exposed to increased levels of oxidative stress, GSSG will accumulate and the ratio of GSH to GSSG will decrease. Therefore, the determination of the GSH/GSSG ratio and the quantification of GSSG are useful indicators of oxidative stress in cells and tissues(Anderson, 1996).On the other hand, the redox inactive metals, such as cadmium (Cd), arsenic (As) and lead (Pb) show their toxic effects via bonding to sulphydryl groups of proteins and depletion of glutathione (Jomova and valko ,2011).

Table 2 Antioxidant potential of quercetin or/and zinc on liver biomarker parameters of lead-toxicated rats.

Parameters \ Groups		Normal control group	Pb toxic group	Pb toxic Treated with Quercetin	Pb toxic Treated with $ZnCl_2$	Pb toxic Treated with Mixture
GSH (µM/mg)	Mean±SE	3.569±0.193^{A}	1.986±0.106^{B}	2.897±0.141^{C}	2.482±0.129^{D}	3.115±0.171^{B}
GSSG (µM/mg)	Mean±SE	0.273±0.0053^{A}	0.298±0.0061^{B}	0.281±0.0057^{C}	0.283±0.0059^{C}	0.271±0.0055^{A}
GSH/GSSG ratio	Mean±SE	11.073±0.648^{A}	4.664±0.297^{B}	8.310±0.485^{C}	6.770±0.403^{D}	9.494±0.572^{E}
SOD (NU/mg (protein /30	Mean±SE	7.577±0.381^{A}	4.489± 0.176^{B}	6.174± 0.239^{C}	5.977± 0.211^{C}	7.596±0.285^{A}
MDA (µM/mg	Mean±SE	0.126±0.014^{A}	0.324±0.037^{B}	0.217±0.028^{C}	0.254±0.033^{D}	0.181±0.021^{E}

Values are expressed as means ± S.E, n=7 rats.

A, B, C, D, E = means bearing different superscripts within the same row that differ significantly (P< 0.05).

In the current investigation lead was found to be responsible for highmalonaldehyde (MDA) formation after the 14 days of lead administration. On the contrary, levels of superoxiddismutase (SOD) was found to significantly (P<0.05) decreased as compared to control rats.MDA elevated values may be due to free radical generated by lead also react with unsaturated lipid generating hydroproxides, which in turn can induce changes in the lipid bilayer thereby altering the membrane permeability and induced lipid peroxidation. This observation is in conformation with Flora et al.(2008) andMalekirad et al.(2010).Modulation of cellular thiols for protection against reactive oxygen species (ROS) has been used as a therapeutic strategy against lead poisoning. N-acetylcysteine, α-lipoic acid, vitamin E, quercetin and a few herbal extracts show prophylaxis against the majority of leadmediated injury in both in vitro and in vivo studies (Flora et al.,2012).

Quercetin, a strong antioxidant and radical scavenger, is the representative natural flavonoid molecule abundant in fruits and vegetables. Several studies have indicated an important role for quercetin in fighting the deleterious effects of reactive oxygen species and in the inhibition of redox-sensitive signaling pathways, including NF-κB, in several diseases (Moreira et al.,2004;Dias et al.,2005;Liu et al.,2012). In previous research, quercetin increased the genomic stability in rats with HPS, probably due to its antioxidant properties (Tieppo et al.,2007).Moreover,quercetin have many protection against various diseases such as osteoporosis, certain forms of cancer, pulmonary and cardiovascular diseases (Ishizawa et al.,2011) but also against aging. Especially the ability of

quercetin to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical is suggested to be involved in these possible beneficial health effects (boots et al.,2008).

In the current study,quercetin corrected the rise in serum enzyme values (AST,ALT,ALPand LDH), total bilirubin and partially prevented the decrease in serumalbumin.Also, quercetinTreatment resulted in a significant ($p<0.05$) preservation of the activities of antioxidant enzymes (SOD),deplet the elevation in GSSGand MDAand ameliorate the reduction inGSH concentration table(1&2).The obtained data in agreement with Peres et al. (2000) who observed that Quercetin reduces liver oxidative damage, ductular proliferation and fibrosis in biliary-obstructed rats and it might be a useful agent to preserve liver function in patients with biliary obstruction.Moreover,These results indicate that quercetin exhibited hepatoprotectiveactivity possibly through multiple mechanisms (Janbaz et al.,2004).Also,These data may be attributed to the powerful of quercetin which decrease oxidative stress via stabilizes the reactive oxygen species by reacting with the reactive compound of the radical due to the high reactivity of the hydroxyl group of quercetin (Sanchez et al.,2006).

Quercetin also ameliorated liver injury and reduced the expression of hepatic endothelin-1 and HO-1 in untreated cirrhotic rats,suggesting protection by free radical scavenging (Tieppo et al., 2009).Moreover,Liu et al.(2013) showed that quercetin significantly prevented Pb-induced hepatotoxicity,markedly decreased Pb contents in blood and liver.Western blot analysis showed that Pb-induced ER stress in rat liver was significantly inhibited by quercetin.Quercetin markedly suppressed Pb-induced oxidative stress, decreased reactive oxygen species (ROS) production and increased the total antioxidant capacity in rat livers. Additionally, quercetin dramatically increased Phosphoinositide-3-kinase (PI3K) and phosphorylated protein kinase B (PKB/Akt) levels in liver rats.

Regarding zinc treatment to lead-intoxicatedrats,a significant ($p<0.05$) regulation in the altered activities of serum AST,ALT,ALP,LDH and bilirubin was observed.Additionally,serum albumin exhibited a significant($p<0.05$)elevation in zinc treated group.Moreover, zinc treatment to these animals resulted in an elevation in the levels of GSH, GSH/GSSG ratio and SOD as well as a significant ($p<0.05$) reduction in the levels of GSSG and MDA.Hence, These results showed that zinc has the potential in alleviating the toxic effects of lead in rat liver because of its property to induce metallothionein (S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels of oxygen reactive species(Sidhu et al.,2004 ;Vij, 2009).

A lead–zinc interaction has been observed. Zinc (Zn) is essential for cellular membrane integrity and metabolism as a central part of over 300 enzymes and proteins (Cengiz et al.,2004 ;Ahamed et al.,2007;Malekirad et al.,2010)). Similar to Se, Zn has been shown to possess antioxidant properties caused by its requirement for superoxide dismutase (SOD) activity. Therefore, Zn not only reduces lead-induced oxidative stress but also competes with lead for similar binding sites. Competitive binding to metallothionein-like transport protein in the rat duodenum suggests the ability of Zn to reduce lead absorption(Afridi et al.,2011).

Zinc administration at non-toxic levels protects against chemically induced acute and chronic liver injury in experimental animals. Zn treatment reduces the hepatotoxicity produced by a wide variety of diverse agents such as carbon tetrachloride ,bromobenzene ,thioacetamide (Song andChen 2003), ethanol (Zhou et al., 2005),insecticide,such as chlorpyrifos(Goel et al., 2005) , D-galactosamine, endotoxin, D-galactosamine plus lipopolysaccharides or tumour necrosis factor (TNF)-α (Zhouet al.,2007). Zn pretreatment also decreases liver injury produced by drug overdose, such as acetaminophen ,salicylate and cisplatin (Liao et al.,2008). Zn is also well known to protect against hepatotoxicity of metals, such as cadmium, mercury (Afonne et al.,2000), arsenic, nickel (Sidhu et al.,2004) ,copper (Gonzalez et al.,2005) and lead (Sidhu et al., 2005;Bandhu et al.,2006**;**JomovaandValko2011).

In experimental animals, Liu et al.(2009) demonstrated that non-toxic, hepatoprotective levels of Zn evoked a consistent pattern of gene expressions, including dramatic upregulation of MT, modest activation of Nrf2- and acute-response-related genes, and modest suppression of metabolic enzymes. Also Hepatoprotective levels of Zn treatment in rats or mice induced a pattern of hepatic gene expression changes, the most consistent change of which was increased MT expression at both the transcript and the protein levels.

By reviewing table (1&2), the best amelioration results was obtained in all studied parameters of toxicated rats with lead nitrate which treated by both agents(quercetin& zinc). These data may be attributed to the synergistic effects of both antioxidants by improving their pharmacodynamics and pharmacokinetics properties.For this reason, this combination might represent a treatment option for lead toxicity.

Conclusion

The current data could concluded that best amelioration was occurred in leadtoxicated rats that received both antioxidants which may protect and alleviate the destructive effects induced in hepatocytes in response of continuous exposure to lead particulary when individuals dealing with this toxic metal in their daily life.

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