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Structural and lipid peroxidation effects of lead on rat hippocampus and its attenuation by hydrogen rich water

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ABSTRACT

Despite the well-known toxicity and the efforts to control its exposure, lead still has a serious health concern, particularly in young ages. Chelation therapy cannot correct the neurocognitive effects of chronic exposure. So, there is a requirement to test different protective agents for lead intoxication. Hydrogen-rich water (HRW) has gained attraction recently as an antioxidant. Four groups of rats received sodium acetate, HRW, lead acetate (LA), or LA plus HRW for 8 weeks. Oxidative stress, histological and immunohistochemistry using p53 antibody were used to investigate the toxic effect of lead and the possible HRW protective effect in rat hippocampus. Results showed that HRW corrected the elevated malondialdehyde levels (MDA) and restore the lead-induced depletion of antioxidant enzymes; glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD). HRW does not affect the diminished nitric oxide (NO) level in the LA-treated group. Moreover, HRW reversed the LA-induced histological and immunohistochemical changes. It significantly decreased the percentage of the apoptotic index. We concluded that HRW protects the neurons against lead-induced oxidative stress and has anti-apoptotic effects without a noticeable change in NO level which already was diminished by LA.

1. Introduction

Despite the well-known toxicity, even at low levels, and efforts to control its exposure, lead still annoys health professionals, particularly in children (Khodamoradi et al., 2015). There are many sources of lead intoxication include gasoline, soil, food, water contamination, and toys (Karamian et al., 2015).

The prevention and control of lead toxicity are still away from what targeted to meet, as the efficacy of remedies of the environment is costly and questionable. In addition, chelation therapy, although its ability to decrease the immediate hazards associated with acute lead ingestion, it can't correct the cognitive behavioral changes of chronic intoxication. Moreover, the severe and multiple side effects of chelators limit its extensive use clinically (Wang et al., 2016). Therefore, there is a critical need to look for other trials that could protect our community against lead toxicity.

Reactive oxygen species are produced during the usual cell metabolism. It is rapidly eliminated by the antioxidant system. Reactive oxygen and nitrogen species are removed by both non-enzymatic (as glutathione) and enzymatic pathways (glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD). On exposure to toxic agents, the production of these reactive species increases beyond the cell capacity to deal with and start to produce detrimental effects on the cell structure (Valko et al., 2016). Brain tissue is more vulnerable to damage by oxidative stress. This liability is attributed to the copious polyunsaturated fatty acid in the cell membrane of the neuronal cells. In addition, the limited effect of the antioxidant system in the brain (Mates, 2000).

Hydrogen gas (H_2) is a colorless, odorless, and tasteless gas that has recently attracted scientists as an antioxidant (Ohta, 2015). It has been reported to have a beneficial role in the various diseases, including ischemia (Han et al., 2015), atherosclerosis (Ohsawa et al., 2008), parkinsonism (Fu et al., 2009), and autoimmune diseases (Zhao et al., 2016).

This study aimed to check if HRW has a protective effect on lead-induced neurotoxicity on the hippocampus. Antioxidant, histological and antiapoptotic effects of HRW were used as evaluating parameters.

2. Material and methods

2.1. Animals and chemicals

Eighty male adult albino rats (190 ± 20gm) were purchased from Faculty of Agriculture, Minia University. The animals were confined in plastic cages and kept under standard conventional laboratory conditions at a temperature of 22 ± 4 °C and a 12-h/12-h light/dark cycle.

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Animals were left for one week in the same experimental conditions with free access to food ad libitum for acclimatization. Rats were left in the same condition to calculate their water consumption. Rats consumed water 20 ± 3 ml/day (subtracting the initial amount from that remains at the end of the day then divided by the number of rats). Experimental procedures were performed in accordance with the guide on the care and use of laboratory animals approved by the Committee of Minia University.

Lead acetate (LA) was obtained from Sigma Chemical Company, Egypt. HRW was prepared with the help of the Department of Biochemistry, Faculty of Medicine, Minia University. The method of (Nakao et al., 2010a), was followed. In brief, the purchased hydrogen water sticks (Sigma Chemical Company, Egypt) were used to produce hydrogen. It was immersed in normal water for 6 h before use. The stick was replaced every 7 days to maintain adequate, suitable hydrogen in water (>500 μM) according to manufacturer notes. Water was changed daily for rats.

2.2. Experimental design

Eighty rats were divided into 4 groups (20 rats each) as follows: the first group received sodium acetate 50 mg/kg/day via nasogastric tube as a control group. HRW group, has a free access to HRW. Lead toxicity group was given LA at a dose of 50 mg/kg body weight/day via nasogastric tube. The fourth group received LA plus allowing animals to free access to HRW. The first and third groups were allowed access to tap water. This regimen was followed for 8 weeks.

From each group, 10 rats were used for biochemical assays, and 10 rats for histological analysis for immunohistochemical analysis.

At the end of the experiment, animals were sacrificed under ether anesthesia, and hippocampal tissue was homogenized in cold sodium phosphate buffer (pH 7.4) containing 1 mmol/L ethylene diamine tetra-acetic acid. The homogenates were then centrifuged at 9000 *g* for 15 min at 4 °C. The supernatants were separated and used for oxidative stress analysis.

Oxidative stress biomarkers in hippocampal tissues were assessed by the established procedures in the literature. Malondialdehyde (MDA) levels were determined as an indicator of lipid peroxidation by thiobarbituric acid method as previously described by (Ohkawa et al., 1979). Glutathione reductase(GR), superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) were measured according to (McGowan, 1989), (Lu et al., 2013), (Aebi, 1984), and (Green et al., 1982) respectively.

2.3. Histopathological analysis

Biopsies from hippocampus tissue samples were dehydrated and embedded in paraffin using the routine histological procedures. Serial cross-sections of 6 μm were prepared. The sections were mounted and stained with hematoxylin-eosin. To ensure an accurate assessment of histopathological changes of the studied groups, random coding for different slides was ascertained. An observer evaluated the slides blind to the coding system. For comparison between groups, the following findings were assessed; laminar organization, the density of packed cells, vaculations, and degenerated neurons. A different grade was given for each of the 4 findings. Grade (0) for normal condition, (1) for mild changes, (2) for moderate changes, and (3) for severe changes. The mean of the scores was calculated by calculating the sum of the scores for an observation and divided by the total number of examined animals (Dodd et al., 2012).

2.4. Immunohistochemical staining

The steps and methods used in immunohistochemical staining were the same followed by (Hsu et al., 1981):

Biopsies of hippocampal tissue were cut on slides (6 μ thickness) with poly-1-lysine and left for 12 h at 56 C for fixation. Deparaffinization was done using Xylene. Then, washing in 3% H_2O_2 in distilled water for 30 min to inhibit endogenous oxygenase enzymes to be followed by another wash with phosphate-buffered saline (PBS). Blocking the nonspecific binding of antibodies was done by incubation of the sections in normal coat saline and then rinsed with PBS. Last, incubation of the sections with p53 antibodies overnight at 4 C.

Apoptotic index (AI) is a semi-quantitative method that could be used for evaluation both the toxic effect of LA and the protective role of HRW. AI was determined by counting at least 1000 cells per slide subdivided into 10 fields chosen randomly at ×400 magnification. P53 stained nucleus appears dark brown (Xu et al., 2007). After randomly coding the slides, two observers blind to the coding system calculated the AI. The mean value was obtained for the 2 readings. AI was obtained using the following formula:

$$AI = \frac{\text{Number of p53 labeled cells}}{\textit{total number of cells}} \times 100$$

The data were statistically analyzed using SPSS Version 22 software. All data were expressed as means ± standard errors. Two way ANOVA was used to assess the effect of HRW, LA and their interaction on different tested parameters. A t-test was done to compare between rats received HRW or not and rats exposed to LA or not. Differences at $p < 0.05$ were considered significant.

3. Results

As it is shown in Table (Table 1), MDA did not differ materially in rat hippocampus tissue that received HRW or tap water as long as the LA was not given. However, the mean MDA was higher (5.33 ± 0.24 nmol/g protein) in rats received LA and tap water than in rats treated with LA plus HRW (2.37 ± 0.21 nmol/g protein). HRW, LA and their interaction significantly affected the MDA level (Table 2). By t-test, MDA decreased significantly in the hippocampus of rats dank HRW in comparing with their levels in rats drank tap water. In addition, LA increased MDA in rats' hippocampus in comparing with those were not exposed to LA (Table 3).

Enzymatic pathways of oxidative stress were diminished markedly in rats' hippocampus received LA and drank tap water while in case of HRW (instead of tap water), these enzymes increased to reach the

Table 1
Descriptive statistics of oxidative stress markers in hippocampal tissue of rats treated with tab or hydrogen rich water with or without lead acetate.

	Rats did not receive LA		Rats received LA	
	Tab water (10 rats)	HRW (10 rats)	Tab water (10 rats)	HRW (10 rats)
MDA(nmol/g protein)	2.23 ± 0.18	2.21 ± 0.24	5.33 ± 0.25	2.37 ± 0.21
GR(μg/100 mg tissue weight)	62.72 ± 2.47	61.7 ± 5.03	46.1 ± 2.13	60.8 ± 2.34
SOD(U/mg protein)	11.08 ± 1.19	11.34 ± 0.97	6.48 ± 0.42	10.54 ± 1.21
CAT(U/g protein)	41.91 ± 3.42	39.64 ± 4.34	31.2.54	42.27 ± 1.67
NO(nmol/g protein)	62.8 ± 2.97	65.2 ± 1.23	44 ± 3.19	42.2 ± 5.2

HRW: hydrogen rich water, LA: lead acetate, MDA: malondialdehyde, GR: glutathione reductase, SOD: superoxide dismutase, CAT: catalase, NO: nitric oxide. Data are expressed as mean ± SD.