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Research Article

STUDY ON EFFECTS OF CHROMIUM ON ANTIOXIDANT ENZYMES IN MICE

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ABSTRACT

Chromium, naturally occurring, largest environmental toxicant that affects human health and most common pollutant; over the last few decades, it has been a concern that the fresh water consisting of massive metals can be agitated because of their campaigns primarily through the fraudulent programs of humans, industrial and domestic programs, chromium, heavy metal, primarily in oxidation states of hexavalent. Chromium obstructs the metabolic pathways and despite of all the above Chromium is an essential nutrient. Chromium adverse effects at low level is not well established. Hence, the study intended to examine the alterations of chronic exposure to chromium at low dose on antioxidant defense enzymes in mice. Exposure to chromium depleted significantly in catalyses, superoxide dismutase, and GST activity ($p < 0.0001$) in brain, liver and kidney when compared with their respective control group. The catalyses are considered one of the most important free radical scavenging enzymes, and the body's secondary protection against oxygen metabolites produced by the transformational massive metals increased activity levels of CAT, GST and SOD formation was observed experimental animals. The increase in antioxidant enzymes in Cr-treated animals indicated that one of the Cr-induced toxic effects as generated free radicals and in turn damages cell membrane.

Keywords: Mice, Chromium, Catalyse, Glutathione S Transferase, and Superoxide dismutase.

INTRODUCTION

Over the last few decades, it has been a concern that the fresh water consisting of massive metals can be agitated because of their campaigns primarily through the fraudulent programs of humans, industrial and domestic programs, chromium, heavy metal, primarily in oxidation states of hexavalent, Cr (III) and Cr (VI). Hexavalent state is predominant toxicant in the environment due to its high solubility and mobility. Chromium, naturally occurring, largest environmental toxicant that affects human health and most common pollutant[1,2].

Chromium, occurs in rocks, volcanic dust, gases, soil, plants and animals. Present in various oxidized forms rages from -2 to 6. Chromium (0), chromium (III), and chromium (VI), are three major oxidative structures of chromium. Generally, these are found at workplaces and natural environment. Chromium (0) a steel-gray solid with high melting point used to make steel and its alloys. Chromium (III) and Chromium (VI) compounds are extensively used in stainless steel production, leather tanning, paints, welding, electroplating, and pigment production. Whereas, industrially used as wood preservative[2,3]. Mainly percolates via water and food, oral intake is its route of exposure to general population. Chromium obstructs the metabolic pathways and despite of all the above Chromium is an essential nutrient[1].

Oral administration of chromium (VI) has been reported as 10-100 folds more poisonous than chromium (III). Chromium (VI) enters in to cell via SO_4^{2-} and PO_4^{2-} channels and chromium (V) and chromium (IV) forms by cellular reductant chromium (VI) glutathione, ascorbic acid, riboflavin and serum protein[4-6]. Suppressed intracellular binding to proteins could leads bio accumulation of chromium in blood and may produces Reactive Oxygen Species (ROS), finally oxidative stress takes place. Chromium (VI) extensively harmful to vital organs, tissues, including reproductive toxicity[6,7]. Chromium toxicity explored at higher doses than normal levels of drinking water and under the permissible levels[1,2].

Most of the researchers have been noted and reported on devastating impacts of chromium and mixed chromium administration with heavy metals in experimental animals and aqua system and hexavalent chromium comprehends with the possible devastating effects of heavy metals[8,1]. Trace metals such as Ni and Pb are reflected in Fe, Cu, Micronuclei, Cr, Zn and Co in a short time. The main reason for water pollution in Thailand is the release of untreated industrial waste that leads to a high level of pollution in surface water and underground water[9,10]. Although high levels of metal levels were reported in enormous studies have been reported[11], these metal contaminants tend to toxic alterations in nature, plants, animals and aquatic world. In order to protect local animals in their natural habitats (water resources) it is important to detect their sensitivity and inherent potentiality for the accumulation of metals into different organs[12-16].

Chromium adverse effects at low level are not well established. Hence, the study intended to examine the alterations of chronic exposure to chromium at low dose on antioxidant defense enzymes in mice.

MATERIAL AND METHODS

Procurement and Maintenance Animals

Mice were procured divided into two groups, consisting of 20 animals in each group. Both were allowed *ad libitum* access to tap water normal pellet feed. Both control and experimental group were maintained collectively in polycarbonate cages 30 x 40 x 40 cm (W x L x H) and temperatures maintained at 27°C to 32°C with 45 ± 5 humidity and Light dark cycle 12–12-hours cycle. The body weights of mice were recorded at the beginning and last day of the experiment. The mice were sacrificed by cervical dislocation on the day of experiment. The animals of experimental group were exposed to chromium for thirty days. Experimental animals were received the chromium at 3 ppm through water; whereas; control group received only water i.e. without chromium. All the procedures were approved by institutional ethical committee (Proc. No 11/PH/2019).

Catalase Assay

Catalase was assessed in liver, kidney and testes of mice by using Chance and Machly (1955) method. Tissues were collected and homogenized with 50 mM phosphate buffer (pH 7.0) and centrifuged at 105,000 x g for 60 minutes. Enzymeactivity was assayed with supernatant. H_2O_2 decomposition was measured by decrease in absorbance at 240 nm, at 10 sec intervals for 1 min by spectrophotometer (Hitachi model, U-2001). The activity levels of catalase were expressed as follows n moles of H_2O_2 metabolized/mg protein/min. (E.C. 1.11.1.6)

Glutathione-S-Transferase (GST) activity

GST activity assayed by the method of 1-chloro-2, 4-dinitro benzene (CDNB) (at 340 nm) substrate as described by Habig *et al*. (1974). Tissues were collected and homogenized with 50 mM Tris hydrochloric acid (pH 7.4), containing 1 mM EDTA and 1 mM DDC (diethy1 dithiocarbonate) and centrifuged at 105,000 x g for 60 minutes. The supernatant was used for the assay.

Made it for final volume of 3.0 ml and consists of 150 mM phosphate buffer (pH 7.5), 5 mM glutathione (GSH), 1 mM CDNB and an accurate amount of enzyme protein. The reaction activated by adding GSH and incubated at 37°C. Then thioether formed by the conjugation of CDNB to GSH was monitored at 340 nm in a spectrophotometer (Hitachi model, U-2001); concentration of thioether was assayed by initial reaction rates (slopes). A molar extinction coefficient 9.6 x 10^{-3} cm^{-1} was used in the calculations. The activity of GST was expressed as µ moles of thioether formed/mg protein/min, where one unit of enzyme activity is defined as oneµ mole of thioether formed/mg protein/min. (E.C. 2.5.11.8)

Superoxide dismutase (SOD) activity

Activity levels of SOD was examined by epinephrine assay as described by Misra and Fridovich (1972)[15]. At alkaline pH, superoxide anion O^{-}_{2}completes the auto oxidation of epinephrine to adenochrome; and SOD decreases the formation of adenochrome. One unit of SOD is taken as the amount of extract that suppress the rate of adenochrome formation by 50%. The tissues were homogenized (10% W/V) in 50 mM ice-cold sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 105,000 x g for 60 min. The clear obtained supernatant used for the enzyme activity. The reaction mixture in a final volume of 2.0 ml consist 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the enzyme extract. Changes were recorded at 480 nm, measured at 10 sec intervals for 1 min in a spectrophotometer. The enzyme activity was expressed as Units/mg protein[15,16]. (E.C. 1.15.1.1)

Statistical Analysis

The data was tabulated and statistically evaluated by ANOVA with the help of SPSS version 24.0 package.

RESULTS AND DISCUSSION

Table 1: Levels of Catalase (µ moles of H_2O_2/mg protein / minute) in brain, liver, kidney and testis tissues of control and chromium exposed mice

Tissues	Control	Sodium selenite	Anova
Brain	3.110 ± 0.110	4.291 ± 0.186	p < 0.0001
Liver	8.002 ± 0.92	13.973 ± 0.822	p < 0.0001
Kidney	5.930 ± 0.054	7.881 ± 0.515	p < 0.0001
Testis	0.781 ± 0.0231	1.875 ± 0.162	p < 0.0001

Values are mean ± S.D. of 12 samples. Values in the parentheses are percent increase over control. Values are significantly different from control at p < 0.0001

Table 2: Levels of superoxide dismutase (Units/ mg protein) in brain, liver, kidney and testis tissues of control and chromium exposed mice

Tissues	Control	Sodium selenite	Anova
Brain	28.113 ± 2.701	44.991 ± 1.513	p < 0.0001
Liver	24.011 ± 1.814	34.891 ± 1.376	p < 0.0001
Kidney	15.011 ± 0.099	30.921 ± 0.0989	p < 0.0001
Testis	12.836 ± 0.612	25.851 ± 1.918	t = 27.100 p < 0.0001

Values are mean ± S.D. of 12 samples. Values in the parentheses are percent increase from control. Values are significantly different from control at p < 0.0001

Table 3: Levels of GST (µ moles of thioether formed /mg. protein/h) in brain, liver, kidney and testis tissues of control and experimental mice

Tissues	Control	Sodium selenite	Anova
Brain	0.631 ± 0.051	0.901 ± 0.0010	p < 0.0001
Liver	6.109 ± 0.100	10.812 ± 0.190	p < 0.0001
Kidney	0.801 ± 0.008	1.2356 ± 0.0013	p < 0.0001
Testis	2.612 ± 0.0122	6.311 ± 0.00118	p < 0.0001

Values are mean ± S.D. of 12 samples. Values in the parentheses are percent increase over control. Values are significantly different from control at p<0.0001

The activity levels of catalase, superoxide dismutase and GST activity in brain liver and kidney of mice exposed to chromium were presented in Tables 1, 2 and 3 respectively. Exposure to chromium depleted significantly in catalase, superoxide dismutase and GST activity ($p < 0.0001$) in brain, liver and kidney when compared with their respective control group.

Glutathione-S-Transferases (GSTs) present in wide variety of organs and are multifunctional, detoxifying, drug metabolizing enzymes especially involved in the intracellular-transport in steroid hormone metabolism. GSTs are expressed as tissue specific. They have the capacity to detoxify electrophilic xenobiotics, cytotoxic drugs carcinogens by catalyzing their conjugation with reduced glutathione (GSH). GSTs catalyses the reaction between the SH group of GSH and are potential alkylating agents, neutralizing their electrophilic sites and rendering them more water soluble[1,3].

Antioxidant protection system antioxidant enzymes and biological antioxidants[7,8]. Antioxidant enzymes break down superior oxygen and hydrogen peroxide, catalase (CAT) to hydrogen peroxide atomic oxygen and water, glutathione peroxidases, which reduces lipid peroxides to related alcohols and reduces hydrogen peroxide to water, glutathione-es-transfer (GST) alcohol Hydrogen peroxide. Biologic antioxidants are water-soluble antioxidants such as glutathione, ascorbic acid and uric acid and vitamin E (mainly, α-tocopherol), ubiquinone's and fat-soluble antioxidants such as carotenoids.

Antioxidants can be classified into two primary classes through their primary purification activity, including preventive antioxidants and chain breaking antioxidants. Prevention antioxidants prevent the interaction of oxidative actions that destroy cellular molecules. Chain breaking antioxidants disrupt the promotional phase of free radical chain reaction.

Catalase (CAT), a tetrameric haemoprotein, and detoxifies hydrogen peroxide (H_2O_2) into H_2O and O_2. Catalase, a vital antioxidant, consists of iron as the prosthetic group, suppresses the toxic hydrogen peroxide into water and protects the cell from possible oxidative damage. Catalase present mainly in peroxisomes and activity levels alters under pathological and physiological conditions. Catalase acts on H_2O_2, generatesvia metabolism of endogenous substances or metabolism of exogenous compounds. Since it eradicates reactive hydrogen peroxide from the cells, plays a vital role in detoxification mechanisms and able to protect cells from toxic damage[12-14].

Therefore, the present study is aimed to evaluate the oxidative stress indicators such as selected antioxidative enzyme activity levels were examined in brain, testes, liver and kidney tissues of Cr-exposed mice. GST activity may be able to disrupt the superoxide ion radicals made from Xanthine oxidation reaction to detain serious damage to cellular organization in higher levels. In addition, xanthine oxidase activity is currently under investigation and it increases superoxide anion radical generation and SOD operations. Superoxide ion in the proton-free medium contains half an hour, but it converts pure hydrogen peroxide and oxygen to the aqueous medium. In the liver and brain tissues of chick embryos treated with sodium selenite poisoning levels increased levels of catalase activity. Increased cholesterol levels in the chicks developed with the major treatment of the brain and the heart tissue. Elevation of catalyst reported in rats operated with aluminum acetate. Enzyme's increased catalytic potential is identified in motivated urinary rats [14,15].

Lipid peroxidation stimulates the primary cellular depletion process through oxidative stress and occurs instantly in the tissues with highly oxidized polyunsaturated fatty acids. MDA is one of lipid peroxidation products, which is very stable for lipid peroxidation, and is considered a good indicator of lipid peroxidation rate in tissue. During lipid peroxidation, multi-phosphorous fatty acids in the layer have been degraded in a wide variety of aldehydes, including MDA's entire biological samples used for the size of lipid peroxidation. Reddy *et al* reported in 1981 as metals such as Mercury, lead and cadmium also lipid peroxidation are free radicals and cells antibody through the perturbation of the protective system[15-18]. Many studies have been reported on antioxidant enzyme systems that protect cells against ROS which are produced in various cell compartments[17,18]. The catalases are considered one of the most important free radical scavenging enzymes and the body's secondary protection against oxygen metabolites produced by the transformational massive metals.

CONCLUSION

Increased activity levels of CAT, GST and SOD formation were observed experimental animals. The increase in antioxidant enzymes in Cr-treated animals indicated that one of the Cr-induced toxic effects as generated free radicals and in turn damages cell membrane. The results of the present study also indicate the involvement of detoxification mechanism by elevation of SOD, CAT and GST activities to protect the cells from Cr-induced oxidative toxicity.

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