PROTECTIVE ROLE OF MAGNESIUM MALATE AND VITAMINE E AGAINST ALUMINIUM CHLORIDE TOXICITY ON ANTIOXIDANT ENZYMEACTIVITY IN ALBINO RATS.

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Abstract

The study was aimed to assess the possible protective effect of vitamin E (vit. E) and magnesium malate against aluminium chloride ($AlCl_3$) toxicity on antioxidant enzymes xanthine oxidase (XOD), super oxide dismutase (SOD) and catalase (CAT)activities in liver tissues of albino rats. Rats were divided in to five groups, group I received normal saline, group II administered orally with $AlCl_3$ (7.5 mg kg bw), group III was received magnesium malate(5.0 mg/ kg bw), group IV was received with $AlCl_3$ and vitamin E(10 mg kg bw) and group V was administered with $AlCl_3$, Magnesium malate and vit E. The antioxidant enzyme XOD activity levels were increased in $AlCl_3$ treated animals and gradual decrease was observed in magnesium malate and vit. E treated animals, SOD activity was decreased in AlCl3 treated animals where increase was observed in magnesium malate and vitamin E (Vit-E) treated animals, the catalase (CAT) activity was decreased in AlCl3 treated animals where increase was observed in magnesium malate and vitamin E (vit-E) treated animals. The results indicated that the toxic effect of $AlCl_3$ could be mediated through modifying antioxidant enzyme activity in rat liver which may lead to impaired function. The combined treatment with magnesium malate and vit E was effective in the restoring the studied parameters near to the normal.

Key Words

AlCl3, magnesium malate, vitamin E, antioxidant enzymes, Ach content, liver, albino rats

Introduction

Aluminum (Al) is one of the most widely used metals and also one of the most frequently found compounds in the earth's crust. Aluminium is present in many manufactured foods, medicines and also added to drinking water for purification purposes thus increasing the human exposure to this toxic metal. The wide distribution of this element ensures the potential for causing human exposure and harm [1]. Aluminum (Al) enters the body with food, air, water, and drugs, it can accumulate in all body tissues and organs [2]. The major route of exposure to aluminium for the general population is through food followed by in drinking water a minor source of exposure. Additional exposures may arise from the use of aluminium compounds in pharmaceuticals and consumer products.

Aluminium chloride (AlCl3) is used in paint manufacturing, in antiperspirants, in petroleum refining and in the production of synthetic rubber. Studies show that regular use of these product can raise the risk of Alzheimer's by as much as three-fold [3]. However, prolonged exposure causes increased mortality in mice [4], and degeneration of astrocytes [5]. The high Al diet may lead to increase the deposition of Al in the Central Nervous system [6]. Al promotes the formation of amyloid-β protein plaques by aggregating tau proteins in Alzheimer's disease [7, 8]. Al increases oxidative burden in the brain and alters post synaptic density and cognitive impairment in the animals [9]. Aluminum toxicity may also cause birth defects in newborns [10].

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the biological system. It requires rapid detoxification of

intermediate reactants or the repair of the damage and it alters the essential processes possibly becoming the origin of tissue damage in the organism [11]. The antioxidant enzymes that catalyze reactions to neutralize free radicals and reactive oxygen species. These enzymes help to protect against free radical-induced cell damage. Superoxide dismutases (SODs) are a class of enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide. These enzymes are present in almost all aerobic cells and in extracellular fluids, Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor [12].

Magnesium supplements may bind with aluminum and will carry it out of the body and act as Chelating agents. Magnesium deficiency increases in oxidative stress in vivo and cardiac susceptibility to ischemia/reperfusion (I/R) injury [13], and increases the susceptibility to toxicoses from various other metals such as aluminum [14]. Vitamins in the body act as potent antioxidants. Vitamin like A, C, E and beta Carotene is a naturally occurring antioxidant nutrient that plays an important role in animal health by inactivating harmful free radicals that are produced during normal metabolic process or induced by external intoxicants such as Al. Vitamin E is the major lipid soluble, chain breaking antioxidant in biological systems. The antioxidant properties of vitamin E have been extensively studied, as the use of this compound as a cell protector and as a potential disease preventing agent [15]. Vitamin E can break the chain of free radical propagation along such structures, thereby preserving cellular integrity and function [16]. Vitamin E has the ability to protect neuronal tissue in several neurodegenerative disorders including Alzheimer'sdisease [17].

Some of the workers observed protective role of magnisium malate and vitamin E against Aluminium toxicity individually. But there was no data available with combined protective role of magnesium malate and vitamin E. Hence, the present study was aimed to study the combined effect of magnisium malate and vitamin E against Aluminium chloride toxicity.

Materials and Methods

The Lethal dose of aluminium chloride was determined by "Probit method" of Finney [18]. It is determined as 7.5mg/kg of bw.

Animal Model & Experimental Design

Healthy adult albino rats of same age group (100±10 days) and weight (200 ±10 g) were obtained from the Indian Institute of Sciences (IISc) Bangalore. Animals were caged in groups and given food and water *ad libitum*. The animal room was maintained at 21–24ºC and 40–60% relative humidity with 12-h light–dark cycles, the light cycle coinciding with the day light hours. After 2weeks of acclimation, the groups were assigned at random to one of the following treatments.Albino Rats were divided in to five groups with six animals in each group, the processes of protocols using the experimental animals were in accordance to the Guide for the Care and Use of Laboratory Animals (8th edition, 2011),the treatments were by oral gavage and the first group of rats was treated as controls received 0.9% normal saline, Second group administered orally with AlCl3 (7.5mg/kg of bw) at 24-hr intervals, third group was received magnesium malate (5.0mg/kg bw) at 24-hr intervals, forth group was administered with aluminiumchloride (7.5mg/kg of wt) and vitamin E(10mg/kg bw) at 24-hr intervals and fifth group was administered with AlCl3 (7.5mg/kg of wt), magnesium malate (5.0mg/kg bw) and vit E (10mg/kg bw)at 24-hr intervals for one week. The control and experimental animals

were sacrificed by cervical dislocation the liver tissues were isolated and processed for oxidative stress studies.

Antioxidant enzymes:

Estimation of Xanthine oxidase (XOD: CE. 1.17.3.2)

Xanthine oxidase activities were estimated by the dye reduction method of Srikanthan and Krishnamoorthy [19]. The assay mixture contained 100 mM sodium phosphate buffer (PH 7.4), 50 µ M of INT and the enzyme source. The reaction was initiated by the addition of enzyme source and incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazon formed overnight was extracted in toluene and read at 495nm against toluene blank.

The activity was expressed as µm of formazon formed/mg protein/hour.

Estimation of Superoxide Dismutase (SOD) Activity (Ec=1.15.1.1)

Superoxide dismutase activity was determined according to the method of Beachamp and Fridovich [20]. Liver tissues were homogenized in ice cold 50mM phosphate buffer (PH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 10 minutes at 0 °C in cold centrifuge. The supernatant was separated and used for enzyme assay. The reaction mixture contained 1.7 ml of phosphate buffer (PH 7.8), 150 ml EDTA (10 mM), 600 ml methionine (130 mM), 300 ml nitro blue tetrazolium (750mM) and the enzyme source. The reaction was initiated by the addition of riboflavin and the samples were placed under 15 watts fluorescence bulb for 30 minutes and the absorbance was taken at 560 nm against reagent blank kept in a dark place. A system, devoid of any superoxide radical scavenger was used as a positive control to compare the results.

The activity of the enzyme was expressed as units/mg protein.

Estimation of Catalase Activity (EC=1.11.1.6)

Catalase activity was measured by a slightly modified version of Aebi [21], at room temperature.

The liver tissue was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 minutes at 0°C in cold centrifuge. The resulting supernatant was used as enzyme source.10 µl of 100% ethyl alcohol was added to 100 µl tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer, 50 µl of tissue extract and 250µl of 0.066 M H_2O_2 (in phosphate buffer) was added and decreases in optical density was measured at 240nm for 60 seconds in a UV spectro-photometer. The molar extinction coefficient of 43.6 $M.cm^{-1}$ was used to determine catalase activity.

One unit of activity is equal to the µm of H_2O_2 degraded / mg protein / min.

Statistical Analysis of the data

Mean, ±SD, Percent changes, Two-way ANOVA by Steel and Torrle, [22] and students "t" test for comparisons were performed using SPSS package. All the values of "t" below 5% levels ($P<0.001$) were considered significant [23].

Results and Discussion

The results of XOD, SOD and Catalase (CAT) activities of liver tissues of control and experimental rats were mentioned in tables 1.

Table:1. Changes in SOD (Units of superoxide anion reduced/mg protein/min), XOD (µm of formazon formed/mg protein/hour) and Catalase (µm of H_2O_2 degraded / mg protein / min)(CAT) enzyme activity levels in rat liver tissue exposed to oral administration of $AlCl_3$, Magnesium malate and Vitamin E.

Parameter	**Control**	**Al Cl3**	**Magnesium malate**	**Vitamin E**	**AlCl3+ MM+Vit-E**
SOD	7.573 ±0.024	3.737 ±0.198 (-50.65)	5.189 ±0.142 (-31.48)	5.944 ±0.112 (-21.51)	6.491 ±0.164 (-14.287)
XOD	2.66 ±0.043	4.823 ±0.124 (+81.31)	2.378 ±0.224 (-10.60)	2.836 ±0.132 (6.61)	3.602 ±0.106 (35.41)
CAT	0.649 ±0.124	0.240 ±0.053 (-61.47)	0.411` ±0.065 (-36.67)	0.459 ±0.056 (-29.27)	0.491 ±0.044 (-24.64)

All the values are mean ± SD of six individual observations; SD – Standard Deviation; PC – Percent change over control, Values in parenthesis indicate percent change over control.

It was found that the XOD activity levels were increased in $AlCl_3$ treated animals and gradual decrease was observed in magnesium malate and vit. E treated animals. Combined administration of magnesium malate and vitamin E to the aluminium chloride treated animals showed moderate change to the control group animals.

The SOD activity was decreased in AlCl3 treated animals where increase was observed in Magnesium malate(MM) and Vitamin E (Vit-E) treated animals. The combined treatment of magnesium malate and vitamin E to the aluminium choride treated animals shows moderate increment in SOD activity as controls.

It is found that the CAT activity was decreased in AlCl3 treated animals where increase was observed in Magnesium malate (MM) and Vitamin E (Vit-E) treated animals. The combined treatment of magnesium malate (MM) and vitamin E

(Vit-E) group animals shows moderate increment in CAT activity.

The elevated levels of xathine oxidase in the present investigation indicates the over production of superoxide anions ($\mathbf{O_2^-}$) in the liver tissues of albino rats treated with aluminium chloride. Under aluminium stress significant increased xanthine oxidase activity (Table.1) might be due to conversion of xanthine dehydrogenase to xanthine oxidase. For nitrogen balance of the tissue, xanthine oxidase is produced when the native form of xanthine dehydrogenase is altered either by sulphydryl oxidation or by limited proteolysis [24]. Superoxide dismutase (SOD) and Catalase (CAT) have been detected in a wide variety of mammalian cells. Superoxide dismutase and catalase are generally involved in the detoxification of superoxide anion radical generated by xanthine oxidase. These enzymes have an important role in protecting the cell against the toxic effects of toxic pollutants [25]. The reaction diminishes the destructive oxidative processes in cells. Catalases play an important role in protection of cell from the hydrogen peroxide toxicity [14, 26].

In the present study the superoxide dismutase activity was decreased (table. 1) according to the doses. This result was in agreement with the result of Manna *et al.*, [27]. Hexachlorohexane (HCH) effect on immature chick tissues decreased SOD activity (Manna *et al*., [28]. SOD activity was significantly inhibited in both the brain and liver of albino rat during the development of behavioral tolerance to organophosphate compound phosphomidon [29]. A gradual decrease in catalase activity was observed after Isoproterenol administration in to the tissues of rats [30]. The increased XOD and decreased SOD, Catalase activities were observed in albino mice under fluoride toxicity [31]. In *Boleophthalmuspectinirostris* liver the

heavy metal cadmium (Cd2+) caused an increased XOD activity levels [32].

Low levels of magnesium have been associated with a number of chronic and inflammatory disease such as Alzheimer's disease [33]. Magnesium affects many biochemical mechanisms that are prevented synapse loss and memory decline in a transgenic mouse model of AD [34]. Magnesium depletion has been found in the hippocampus in patients with AD, providing more evidence that magnesium may be a target of treatment [35].

Vitamin E has valuable properties and able to antagonize aluminum toxicity [36]. Vitamin E was able to reduce histological and biochemical changes induced by aluminum chloride [37]. Antioxidants are recognized to decrease oxidative radical-induced responses. Vitamin E (α-Tocopherol is a vital antioxidant which prevents peroxidation of membrane lipids by converting lipid peroxyl radicals into a tocopheroxyl radical [38]. Al exposure is known to produce neurotransmission disruption and cholino toxicity and acetylcholine is usually related to short-term memory [39]. Al causes disturbances in cholinergic neurotransmission [40].

The aluminium toxicity in the production of reactive oxygen species may be due to their Redox– cycling activity, they readily accept an electron to form free radicals and then transfer them to oxygen to generate Superoxide anions and hence H_2O_2 formation through dismutation reaction. Generation of free radicals probably because of the alterations in the normal homeostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained [41].

The result of the present study clearly shows significant alterations in oxidative enzymes in albino rats. Magnisium malate is a chelating agent and Vitamin E is

the most effective fat-soluble antioxidant. This study strongly suggested that Magnesium malate and vitamin E could possibly restore the altered antioxidant power in rats, and it could even be a good alternative to chelating agents or other chemical medicines against Al-induced intoxication.

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