AMELIORATING EFFECT OF AQUEOUS GARLIC EXTRACT SUPPLEMENTATION ON CADMIUM INDUCED TOXICITY IN ALBINO MICE.

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Abstract

Cadmium is a highly toxic heavy metal, since it causes deleterious effects in organisms at low levels of exposure. The present study has been undertaken to evaluate the protective efficacy of garlic extract on cadmium induced toxicity in brain and kidney of albino mice. Albino mice were divided into three groups. Group I animals were kept as control. Group II animals were administered a single dose of cadmium chloride (6 mg/kg bw) orally. Group III animals were given a single dose of CdCl₂ followed by a chronic dose of garlic extract (100 mg/kg bw) orally. Autopsies were done at the intervals of 15 and 45 days post treatment. A significant elevation in LPO levels with decreased activity levels of SOD, CAT and GST were observed during Cd intoxication at 15 and 45 days post treatment. With garlic supplementation, a significant reversal in the oxidative stress enzymes was observed and also restored the biochemical changes in brain and kidney tissue. It was concluded that garlic prevented the Cd induced toxicity and this might be due to the antioxidant activity of its constituents.

Introduction:

Heavy metal intoxication, especially by lead, cadmium, arsenic and mercury constitutes serious threats to human health (Wenneberg, 1997; Hu, 2000). Cadmium (Cd) is one of the most toxic substances in the environment with a wide range of organ toxicity and long elimination half life (20-30 years) (Jarup et al., 1998). Both natural and anthropogenic sources of this heavy metal, including industrial emissions and the application of fertilizer and sewage sludge to farm land, may lead to the contamination of soils and the increased Cd uptake by crops and vegetables grown for human consumption (Jarup and Akesson, 2009). Some important sources of Cd exposure for humans can be the emissions from industries of petroleum mining, batteries, metal plating, pigments, plastics, toys and alloy, cigarette smoking and through dietary consumption (De Souza et al., 2010).

Cd is reported to generate reactive oxygen species (ROS) causing oxidative damage in various tissues (Liu et al., 2008). It has been shown that exposure to Cd via different routes causes increased lipid peroxidation (LPO) in membranes of erythrocytes and tissues, such as kidney, liver, brain, and testes where thiobarbituric acid reactive substances (TBARS) and hydroperoxides are used as indicators of oxidative damage (Jahangir et al., 2005; Eybl et al., 2006; Swarup et al., 2007). Previous studies on Cd toxicity have reported behavioral impairments in both animal models and humans after exposure to Cd (Goncalves et al., 2010).
Cadmium can also have neurotoxic effects. In several experimental models, it has been reported that Cd induces oxidative damage in brain mitochondria (Abib et al., 2011; Chen et al., 2011). Oxidative stress that results from the state of imbalance between the concentrations of ROS and the antioxidant defense mechanisms, may be connected to various pathological abnormalities (Valko et al., 2006; Donne et al., 2003; Cooke et al., 2003) e.g. neurodegenerative diseases, diabetes, cancer (Sena and Chandel, 2012). It has been reported that Cd exposure produced long-term impairments of neurobehavioral status such as alterations in attention and memory as well as in the psychomotor and visuomotor functioning and speed in workers (Viaene et al., 2000; Haider et al., 2015).

Cadmium has a very strong ability to accumulate in kidneys and this can be dangerous for them. Chronic exposure to cadmium compounds can damage the renal proximal tubular epithelial cells as a result of dysfunction proximal tubular manifested by low-molecular-weight proteinuria, glucosuria, aminoaciduria, and phosphaturia (Brzoska et al., 2003; Piscator, 1986; Thijssen et al., 2007).

A positive correlation has been established between dietary supplementation with certain vegetables and plants and the reduction of toxic effects of various toxicants and environmental agents, including heavy metals (Sharma et al., 2010).

Aqueous extract of garlic is one of the widely used herbal medicines which is used as an additive in foods (Ghalehkandi et al., 2013). Garlic is one of the condiments necessary in everyday life from the past until now. It contains active compounds that are responsible for its effect on almost every part of the human body. Garlic is considered an excellent tonic for the humans. It has antioxidant, anti-thrombotic, hypo-cholesterolemic and anti-hypertensive properties (Petrovska and Cekovska, 2010). The bulb of the plant has been used as a carminative, anti-septic, expectorant, anti-helmintic and diuretic (Mikail, 2010).

The present work was aimed to evaluate the protective role of garlic extract on cadmium induced biochemical changes in brain and kidney of albino mice.

**Materials and Methods:**

**Animals:**
Swiss albino mice weighing 20-25g were procured from CRI, Kasauli. They were kept and acclimatized to the laboratory conditions for 15 days under optimal conditions of light and temperature. They had *ad libitum* access to tap water. The animals were handled with humane care in accordance with the guidelines of the ‘Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)’, India and all experimentation procedures were approved by Institutional Animal Ethical Committee (Reg No. 107/99/CPCSEA/2014-33).

**Chemicals:**
Cadmium chloride (CdCl₂) was bought from S.D FINE CHEM LIMITED, Mumbai. It was dissolved in double glass distilled water and administered orally to mice. Garlic was obtained from the local market. Fresh garlic extract was prepared by the method of Iwalokun et al. (2004) and administered orally to mice.

**Experimental Design:**
The mice were divided into three groups of five mice each. **Group I** – Control animals were given distilled water. **Group II** – Animals were administered a single dose of 6 mg/kg bw of cadmium orally. **Group III** – Animals were given an acute dose of 6 mg/kg bw of cadmium followed by a daily dose of 100 mg/kg bw of garlic extract orally. Autopsies were done on 15 and 45 days post treatment.

Brain and kidneys were excised, freed of adipose tissue, blotted dry so as to remove blood and were weighed separately.

**Biochemical Analysis:**
Brain and kidney homogenates were prepared with the help of tissue homogenizer in 3 ml of phosphate buffer and used for estimation of antioxidant enzymes. Lipid peroxidation was measured as malondialdehyde a thiobarbutaric acid reacting substance, using the method of Wilbur et al. (1949). SOD activity was determined by the method of Das et al. (2000). The catalytic activity (CAT) was estimated from the rate of decomposition of H₂O₂ by the method of Aebi (1983) and GST activity was determined by the method of Habig et al. (1974).
Statistical Analysis:
The data was analyzed by using Student’s t-test.

Results and Discussions:
The Cd is known to be one of the most dangerous occupational and environmental toxins. It can be found in water, atmospheric air and even in food. Products of vegetable origin are the main carrier of Cd compounds in food (Sullivan and Krieger, 2001). Cd administration does not produce any discernible signs and symptoms of sickness in mice. Also, there was observed no mortality during the entire period of experiment.

Oxidative damage induced by long-term cadmium intoxication has been demonstrated by the increased lipid peroxidation and/or inhibition of antioxidant enzymes that are required to prevent such oxidative damage (Kelley et al., 1999).

Cadmium induces oxidative damage due to its ability to indirect production of ROS such as hydrogen peroxide, superoxide radicals, hydroxyl radicals and lipid peroxides (Percival, 1998). MDA content in brain as well as kidney showed significant (p<0.01) increase at 15 and 45 days post treatment in Cd treated group as compared to control (Fig.1 and 2). Excessive production of free radicals or ROS is mainly responsible for peroxidation of cell membrane lipids and other unsaturated lipids (especially LDL) which are the chief mechanisms of cell damage leading to necrosis or apoptosis and the terminal product of lipid peroxidation is MDA. The determination of MDA levels is usually the most practical and reliable method for detecting and screening for oxidative stress (Chlubek et al., 2003). Such increment in lipid peroxidation has been attributed to alterations in the antioxidant defense system that normally protects the body against free radical toxicity (Patra et al., 2011).

Fig 1:- MDA content in brain tissue. Control vs. Cd, Cd vs. Cd+AGE (*p<0.01), (ns p>0.05)

Fig 2:- MDA content in kidney tissue. Control vs. Cd, Cd vs. Cd+AGE (*p<0.01), (ns p>0.05)
It has been shown in many studies that Cd induces oxidative damage by producing ROS (Liu et al., 2008; Chen et al., 2008) and decreasing the biological activities of some antioxidant enzymes, such as SOD and CAT (Ikediobi et al., 2004; Uchida et al., 2004) which play an important role in antioxidant profile, and in scavenging of free radicals. The Cd has also been reported to cause damage to lipids, and to generate LPO (El-Sharaky et al., 2007; Renugadevi and Prabu, 2010).

Fig 3:- SOD activity in brain tissue. Control vs Cd, Cd vs Cd+AGE (*p<0.01), (ns p>0.05)

Fig 4:- SOD activity in kidney tissue. Control vs Cd, Cd vs Cd+AGE (*p<0.01), (ns p>0.05)

SOD activity showed non significant decrease at 15 day and a significant decrease at 45 day post treatment in Cd treated mice as compared to controls (Fig. 3 and 4) Casalino et al. (2002) demonstrated that SOD activity is strongly inhibited by cadmium, probably by interacting with metal moieties of SOD (Cu, Zn or Mn) and thus reducing its activity. Alternatively, cadmium may alter the protein conformation by interacting with the enzyme, thereby altering its functional activity (Nagaraj et al., 2000).This decrease is in accordance with the results of others workers (Hussain et al., 1987; Stajn et al., 1997; Sarkar et al., 1998; Yalin et al., 2006).
A significant (p<0.01) decrease in CAT activity was observed at 15 and 45 days post treatment in brain and kidney of Cd treated mice as compared to normal mice (Fig. 5 and 6). Due to cadmium intoxication, catalase activity gets disturbed. There are many suggested mechanisms behind the inhibition of catalase activity; one of them is attributed to the possibility of high production of ROS and their increased intracellular accumulation which exceed the detoxification capacity of antioxidant enzymes with subsequent development of liver and kidney injury (De Castro et al., 2009). An interaction between cadmium and catalase subunits which contain iron as a composing element was suggested (Wronska-Nofer et al., 1999). The presence of cadmium in the organism is well known to decrease the levels of iron in the blood, so the decreased activity of catalase could be resulted from iron deficiency (Jurczuk et al., 2004).
GST can remove free radicals and its levels can reflect the antioxidant capacity in the body (Wang et al., 2006). Similarly, GST activity also showed significant (p<0.01) decrease at 15 and 45 days post treatment in brain as well as kidney of Cd treated mice as compared to control (Fig. 7 and 8). Reactions of metals with glutathione might lead to either the formation of complexes or the oxidation of glutathione. The decreased GST activity in the test tissues is in agreement with El-Missiry and Shalaby (2000) in Cd-treated rat brain and kidney. Moreover, the decrease in the activity of each of them would induce increased free radicals, thus injuring the corresponding tissues (Jamakala and Rani, 2015).

This indicates that some of the responses of cellular protective mechanisms of tissue against Cd insult may also be different depending on nature, the dose, duration and route of Cd exposure and the stage of life at which Cd were administered (Wang et al., 2006).

Garlic extract showed significant decrease in MDA content and elevated antioxidant enzymes in both brain and kidney of mice as compared to toxic group (Fig. 1-8). Allicin is a major organo-sulfur component of garlic and its antioxidant properties has been confirmed by many investigations (Leelarungrayub et al., 2006). In addition to allicin; other garlic organosulfur compounds, like allyl-trisulfide, also possess antioxidant properties and can neutralize several types of ROS (Chung, 2006). Increased level of total protein in *Allium sativum* extract treated groups suggests the ability of garlic to stimulate the regeneration of tissues. Garlic has been reported to increase protein synthesis in damaged tissues and improve the functional status of the cells (Sharma et al., 2010).
Conclusion:-
The aqueous extracts of garlic exhibited therapeutic and chelating effects against Cd induced toxicity. This investigation fundamentally cleared the protective role played by garlic against toxicity incurred by Cd. The current data reveals that garlic administration possesses conspicuous modulating effects and is capable to overcome the oxidative stress and subsequently rectify the biochemical perturbations induced by the Cd administration through its antioxidant properties.

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