World Journal of Pharmaceutical and Life Sciences

Impact Factor: 7.409 www.wjpls.org Coden USA: WJPLA7

NEUROTOXICOLOGICAL ALTERATIONS INDUCED BY CADMIUM: AMELIORATIVE AND THERAPEUTIC APPROACH OF ALLIUM SATIVUM AND LYCOPERSICON ESCULENTUM

Puttaganti Vijaya[1*], Suman Sharma[2]

[1*]Department of Zoology, Bangalore University, Bangalore, Karnataka-560056, India.
[2]Department of Zoology & Environmental Sciences, Punjabi University, Patiala, Punjab-147002, India.

***Corresponding Author: Puttaganti Vijaya**

Department of Zoology, Bangalore University, Bangalore, Karnataka-560056, India.

DOI: https://doi.org/10.5281/zenodo.18803502

How to cite this Article: Puttaganti Vijaya[1*], Suman Sharma[2]. (2026). Neurotoxicological Alterations Induced By Cadmium: Ameliorative and Therapeutic Approach of Allium Sativum and Lycopersicon Esculentum. World Journal of Pharmaceutical and Life Science, 12(3), 57–69.

Article Received on 05/01/2026 **Article Revised on 15/01/2026** **Article Published on 10/02/2026**

ABSTRACT

Cadmium (Cd) is a heavy metal and during its extraction process, it gets accumulated into the atmosphere, hydrosphere and even soil, thereby contaminating human environment. The present study was aimed to investigate the cadmium induced damage in some biochemical parameters and the histomorphological changes in adult mice brain along with the protection afforded by the *Allium sativum* and *Lycopersicon esculentum*. Albino mice were divided into several experimental groups and were given single dose of cadmium followed by natural antioxidants i.e. garlic and tomato extract for 45 and 90 days under protective and therapeutic study. At the end of the experiment, the levels of lipid peroxidation were increased while and SOD, CAT, and GST activities along with AChE levels were decreased in cadmium treatments. There was significant decline in the glycogen, cholesterol and total proteins in brain of cadmium treated group as compared to control value. Cadmium induced many pathological changes in the form of vacuolation, hyperemia, necrosis along with pyknotic cells and binucleated cell formation in both cerebral cortex and hippocampal regions of brain. Thus, the result indicated that cadmium induced oxidative stress may be the cause of ultrastructural disturbances in the brain tissue. But, the antioxidant potential of both garlic and tomato along with their constituents afforded significant protection in biochemical and histopathological changes against cadmium toxicity.

KEYWORDS: Cadmium (Cd); *Allium sativum* Extract (AE); *Lycopersicon esculentum* Extract (LE), Antioxidants, Acetylcholinestrase (AChE), Brain damage.

INTRODUCTION

Environmental pollution is caused by the heavy metals that are discharged by various industries. Cadmium doesn't have any physiological role but is highly toxic as it gets accumulated in the living system and causes damage to the soft tissues i.e. liver and kidneys. It enters the human body by different routes and has extremely long half-life with its low rate of excretion.[1] Cd exposure results in renal and hepatic dysfunction, pulmonary edema, testicular damage, osteomalacia, damage to the adrenals and hemopoietic system in humans.[2]

Cd causes many neurodegenerative diseases, Alzheimer's, Parkinson's diseases and other age related disorders by altering many metabolic processes and leads to several pathological conditions.[3] Cd prompted neurotoxicity and neurogenesis that resulted in neuronal differentiation and axonogenesis.[4] Cadmium reacts on mitochondria by instigating oxidative stress and enhancing ROS generation which may further activate apoptosis by mutating mtDNA along with gene expression, reducing ATP synthesis, and finally changing the inner mitochondrial permeability that can results in the development of various human disorders.[5] Cadmium challenge was observed in cortical and trigeminal neurons, in the anterior pituitary cells, in the

glioma and neuroblastoma cells.[6] Cd can enter Cadmium can enter into the brain parenchyma and neurons during the critical point of development.[7] causing neurological alteration.[8] The hippocampus and cortex part of brain are more susceptible to oxidative stress and this may affect the learning and memory status of an individual. Cd targets cardiovascular, cerebrovascular and immune systems; the liver and the reproductive systems that contributes to various diseases like disruption of blood pressure, calcium regulation by resulting in oxidative and DNA damage.[9]

The main mechanism associated with Cd induced damage is the production of oxidative stress along with the formation of reactive oxygen species. The intake of some natural antioxidants can prove helpful in maintaining the antioxidant levels and reversing the Cd-induced injury in the tissues. Many foods such as vegetables, fruits, grain cereals, eggs, meat, legumes and nuts.[10] have different types of antioxidants with various beneficial effects.

Garlic (*Allium sativum*) has been reported as one of the important studied plant with much of the health benefits. It is composed of rich organosulfur compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS), ajoene, allicin, allyl mercaptans and allyl methylsulfides.[11] It contains several enzymes, 17 amino acids, minerals such as selenium and holds at least 33 organosulfur compounds that are responsible both for garlic's pungent odour and its many medicinal properties.[12]

Tomato (*Lycopersicon esculentum*) has immense antioxidants such as carotenoids which may prove helpful in combating the toxic effects of Cd. It was reported that this plant synthesizes metal chelating proteins, peptides, phytochelatins (PC) and other heavy metal binding complexes analogous to metallothioneins.[13] which further help to prevent cellular damage by capturing the heavy metals.[14] Lycopene is a major carotenoid present in tomatoes and is a highly potent antioxidant that provides protection against integral tissue damage caused by oxidative stress.[15] It is naturally found in many plant foods, being especially abundant in tomatoes.[16]

The aim of our present work was to assess the impact of heavy metal cadmium on the various biochemical along with pathological aspects of brain and further to study the protective approach of garlic and tomato against this neurotoxicity induced by Cd in Swiss albino mice.

MATERIALS AND METHODS

Animals: Swiss albino mice weighing 20-25g were used for the experiment. All the animals were housed in hygiene and translucent polypropylene-cage and randomized into different groups. All the animals were kept under 12/12h natural light-dark cycle with controlled temperature and humidity. The overall research procedure was revised and approved by Institutional Animal Ethical Committee (Reg No. 107/99/CPCSEA/2014-33).

Chemicals: Cadmium chloride ($CdCl_2$) was purchased from S.D FINE CHEM LIMITED, Mumbai. It was mixed in distilled water and administered to mice through gavage. Garlic and Tomato was obtained from the local market. Fresh *Allium sativum* extract (AE) was prepared by the method of Iwalokun *et al*.[17] and *Lycopersicon esculentum* extract (LE) was prepared by the method of Salawu *et al*.[18] and administered orally to mice.

Experimental Design: The mice were divided into 8 groups (5 animals each) as follows (Fig.1)
Group I - Control animals, **Group II** - Animals were administered an acute dose of Cd (6 mg/kg bw) orally and were kept for 45 days. **Group III** - Animals were treated with an acute dose of Cd orally followed by aqueous garlic extract (AE) of 100 mg/kg bw for 45 days. **Group IV -** Animals were given a single dose of Cd orally and treated with aqueous tomato extract (LE) of 50 mg/kg bw for 45 days. **Group V** - Animals were administered with an acute dose of Cd orally and combined with an oral supplementation of aqueous garlic (AE) and tomato extracts (LE) for 45 days. **Group VI**- Animals were subjected to Cd at a dose of 6 mg/kg bw orally for 1st day and were left on normal diet for 45 days followed by oral administration of aqueous garlic extract (AE) for next 45 days. **Group VII** - Animals were given an acute dose of Cd (6 mg/kg bw) and were left for 45 days followed by aqueous tomato extract (LE) for next 45 days. **Group VIII** - Animals were given Cd at a dose of 6 mg/kg bw and were kept for 45 days followed by AE + LE for next 45 days.

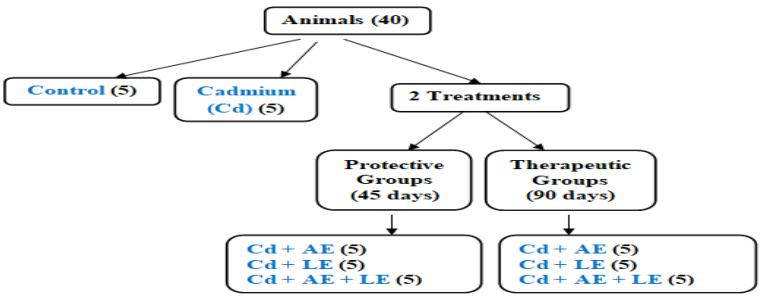

Fig. 1: Division of animals between different groups.

Sample Collection: The mice were sacrificed 24 h after treatment termination. The brain was removed, freed of adipose tissue, washed with cold saline water, blotted dry so as to remove blood and was preceded for biochemical, antioxidant and histological studies (Fig.2).

Preparation of tissue homogenates: Brain was separated, weighted and homogenized in a tissue homogenizer in 3 ml of phosphate buffer. The tissue homogenate was centrifuged at 12,000 rpm for 20 min in cold centrifuge, and the supernatant was collected for estimation of various biochemical studies.

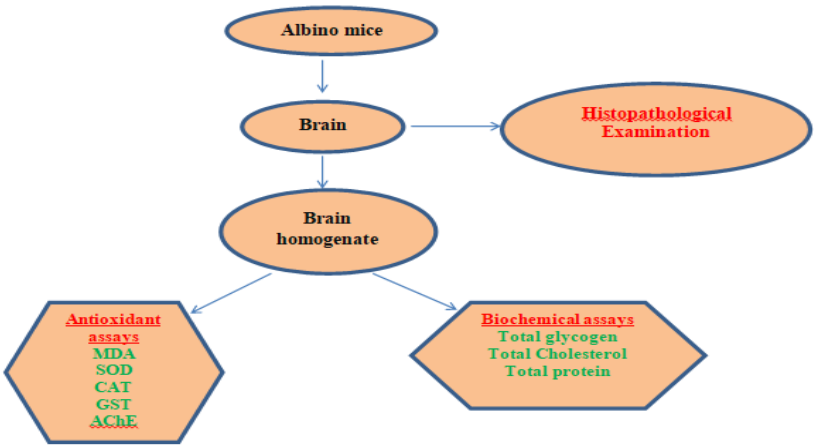

Fig. 2: Graphical representation of methodology.

Biochemical analysis

Brain homogenates were used for the estimations of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT) and Acetylcholinesterase (AChE) activity, glycogen content, cholesterol content and total proteins in different treatment groups.

Measurement of Malondialdehyde (MDA): Lipid peroxidation (LPO) of tissue was measured using the thiobarbituric acid reactive substances (TBARS) test according to Wilbur *et al*.[19] based on the concentration of malondialdehyde (MDA) which is the most important end-product of lipid peroxidation.

Measurement of Superoxide Dismutase (SOD): Superoxide dismutase was measured by an indirect method which involves the scavenging of superoxide radicals by SOD. It was measured by the most sensitive method of Das *et al*.[20] which involves the generation of superoxide radical by photoreduction of riboflavin.

Measurement of Catalase (CAT): Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide to H_2O and O^2. The amount of catalase in the tissue extracts was estimated by the method of Aebi.[21]

Measurement of Glutathione-S-Transferase (GST): GST is a family of enzymes that play an important role in detoxification of xenobiotics. The activity of glutathione-S-transferase in the brain tissue extracts was assessed by the method of Habig *et al*.[22]

Measurement of Acetylcholinesterase (AChE): AChE activity in brain tissue was estimated using the method of Ellman *et al*.[23] The substrate used in the assay system is acetylthiocholine iodine, the ester of thiocholine and acetic acid.

Measurement of Glycogen content: The amount of glycogen was in tissue extract was assessed by the method of Montgomery.[24]

Measurement of Cholesterol content: Cholesterol is a steroid which is a group of lipids and this cholesterol content in the tissues was estimated by the method of Zlatkis *et al*.[25]

Measurement of Total Proteins: The quantitative estimation of total protein in brain tissue was done by the method of Lowery *et al*.[26] It is one of the most common colorimetric assays for the estimation of proteins.

Histopathological study: Brain tissues of treated and control mice were cleaned, washed and cut into pieces and were fixed with Bouin's fixative for 24 hours. Then the tissues were washed in 70% alcohol followed by dehydration in ascending grades of ethanol, cleaned in xylene and embedded in paraffin wax (60^o C melting point) then sectioned at 5-6 µm. These sectioned are stretched on slides and are stained with hematoxylin and eosin staining technique[27] for histopathological studies. These slides were observed under light microscopy.

Statistical analysis: The results were expressed as mean ± standard error (SEM) from all the experiments performed using a standard number of animals (n=5). Statistical significance was assessed by using Student's t-test on GraphPad Prism software. Inter-group comparisons were made by using Two-way analysis of variance (ANOVA) followed by a Tukey's post hoc

multiple range test. The significance level was set as $p \leq 0.01$ and non-significant levels were set as $p \geq 0.05$.

RESULTS

Lipid peroxidation: Cd was found to accelerate the lipid peroxidation in brain tissue as indicated by MDA content. There was statistically significant ($p<0.0001$) increase in the MDA content of Cd treated group in comparison to control value at 45 days. But the treatment with garlic and tomato i.e. in Cd + AE, Cd + LE and Cd + AE + LE groups showed a statistically significant ($p<0.0001$) decline in the MDA content. The reduction was more in Cd + AE + LE treated group as compared to Cd + AE and Cd + LE groups at 45 days which specified decreased peroxidation in the brain tissue with the supplementation of antioxidants i.e. garlic and tomato (Fig. 3). At 90 days also, there was amelioration in the peroxidation of lipids in brain by significantly ($p<0.0001$) reducing the MDA level in these groups. The MDA level was more reduced in Cd + AE + LE group than in other groups i.e. Cd + AE group and Cd + LE groups respectively (Fig. 4). Both protective and therapeutic groups showed affordable protection against Cd induced MDA activity.

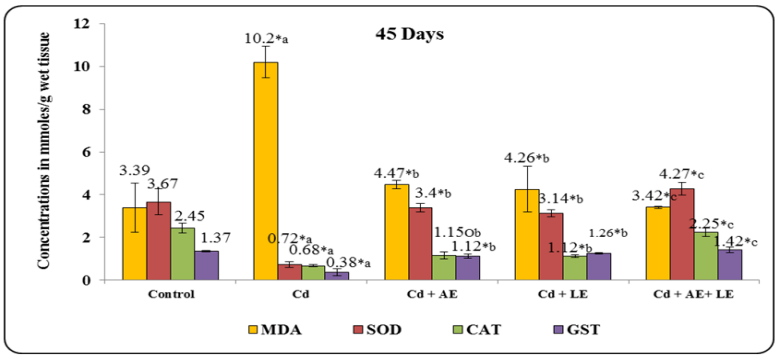

Fig. 3 Lipid peroxidation and antioxidant status in all the treated groups at 45 days.

The values were expressed as mmoles per gram wet tissue. Bars represent means ± SEM for all the experiments. *Significant variations at $p<0.0001$; ^{O} $p<0.01$; a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at $p < 0.05$ in the Tukey's Honestly Significant Difference.

Superoxide Dismutase (SOD): There was significant difference in SOD activity in all the treated groups. Cd caused statistically significant ($p<0.0001$) decline in the SOD level as compared to control group at 45 days. But the antioxidant treated groups showed significant ($p<0.0001$) increase in SOD activity. The values of SOD was enhanced in Cd + AE + LE group than the other groups i.e. Cd + AE and Cd + LE groups at 45 days. Both garlic and tomato prevented the oxidative damage caused by enzyme SOD (Fig. 3). In the therapeutic approach also, there was significant ($p<0.01$) enhancement in the SOD activity. All the groups i.e. Cd + AE, Cd + LE and Cd + AE + LE showed protection in combating the Cd induced damage in brain (Fig. 4). The protective groups showed more prevention than the therapeutic groups.

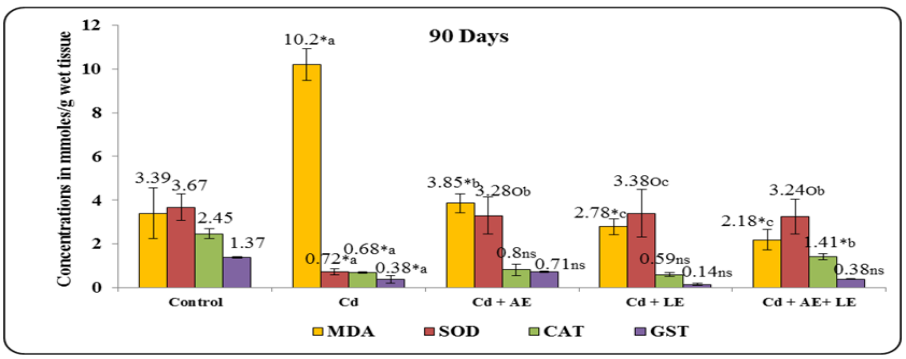

Fig. 4 Lipid peroxidation and antioxidant status in all the treated groups at 90 days.

The values were expressed as mmoles per gram wet tissue. Bars represent means ± SEM for all the experiments. *Significant variations at $p<0.0001$; ^{O} $p<0.01$; ^{ns} Non Significant variations at $p>0.05$; a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at $p < 0.05$ in the Tukey's Honestly Significant Difference.

Catalase (CAT): Catalase is a crucial enzyme that was statistically ($p<0.0001$) decreased in Cd treated group as compared to nontreated group. There was significant ($p<0.0001$) elevation in Cd + AE ($p<0.01$), Cd + LE and Cd + AE + LE groups as compared to Cd treated group at 45 days. Both garlic and tomato, due to their antioxidant properties, were able to prevent damage

within the brain tissue (Fig. 3). There has been non-significant variations in Cd + AE, Cd + LE groups but a drastic ($p<0.01$) elevation was observed in Cd + AE + LE group in CAT level at 90 days as compared to Cd group (Fig. 4). More amelioration was observed in protective groups compared to therapeutic study.

Glutathione-S-Transferase (GST): Cd treated group showed statistically significant ($p<0.0001$) decline in GST activity as compared to control value. There was significant ($p<0.0001$) elevation in GST content in all the antioxidant treated groups i.e. Cd + AE (1.12 ± 0.10 mmoles), Cd + LE (1.26 ± 0.04 mmoles) and Cd + AE + LE (1.42 ± 0.12 mmoles) in comparison to toxic group. Both the antioxidants were able to prevent the tissue damage in brain from the oxidative stress caused by Cd (Fig. 3). There were non-significant variations in GST activity in all the therapeutic groups i.e. Cd + AE, Cd + LE and Cd + AE + LE groups at 90 days as compared to Cd group (Fig. 4). The protection was more in the protective groups in comparison to therapeutic groups.

Acetylcholinestrase (AChE): There was statistically significant ($p<0.0001$) reduction in AChE activity in Cd treated group in comparison to control group at 45 days. A significant ($p<0.0001$) elevation was observed in AChE level in all the antioxidant treated groups (Cd + AE, Cd + LE and Cd + AE + LE) at 45 days. Both garlic and tomato showed protection and caused increment in AChE level in brain (Fig. 5). A non-significant increase in AChE activity was observed in Cd + AE, Cd + LE ($p<0.0001$) and in Cd + AE + LE groups of therapeutic study in comparison to Cd group at 90 days. There was more afforded protection in the proactive groups than in case of therapeutic groups (Fig.5).

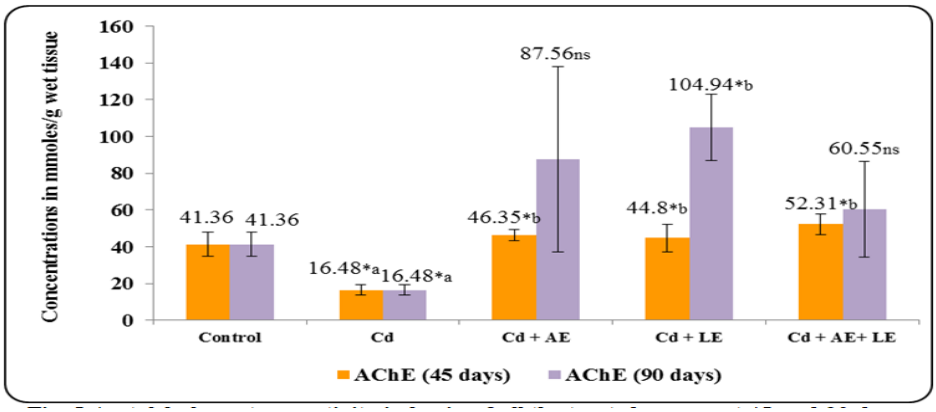

Fig. 5 Acetylcholesnestrase activity in brain of all the treated groups at 45 and 90 days.

The values were expressed as mmoles per gram wet tissue. Bars represent means ± SEM for all the experiments. *Significant variations at $p<0.0001$; ^{ns} Non Significant variations at $p>0.05$; a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at $p < 0.05$ in the Tukey's Honestly Significant Difference.

Total Glycogen: The concentration of glycogen was significantly ($p<0.0001$) decreased in Cd treated group in comparison to control group. But the protective groups showed significant ($p<0.0001$) elevation in the total glycogen in brain at 45 days (Fig. 6). Even the therapeutic groups i.e. Cd + AE ($p<0.01$), Cd + LE and in Cd + AE + LE ($p<0.0001$) groups also showed increment in the glycogen content at 90 days as compared to Cd group. Both garlic and tomato afforded protection against AChE activity in brain. More significant restoration was observed in the protective study in comparison to therapeutic study (Fig. 7).

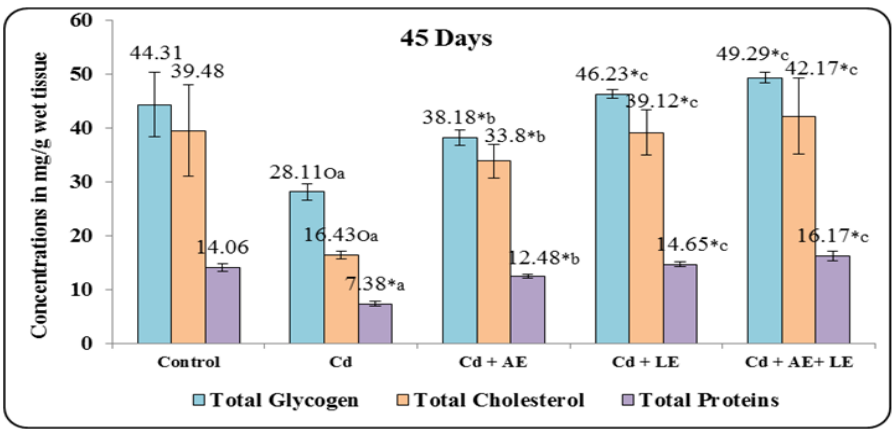

Fig. 6 Glycogen, Cholesterol and Total Proteins in brain of all the treated groups at 45 days.

The values were expressed as milligram per gram wet tissue. Bars represent means ± SEM for all the experiments. *Significant variations at p<0.0001; ^{O} p<0.01; a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at p < 0.05 in the Tukey's Honestly Significant Difference.

Total cholesterol: There was significant (p<0.01) decrement in cholesterol level in Cd treated group in comparison to control value. In the antioxidant treated groups, a significant (p<0.0001) increment was observed in Cd + AE, Cd + LE (p<0.0001) and Cd + AE + LE groups of protective study in comparison to Cd group at 45 days (Fig. 6). A significant (p<0.0001) increase in cholesterol content was observed in the all the therapeutic groups i.e. Cd + AE, Cd + LE and Cd + AE + LE (p<0.01) groups at 90 days. The antioxidants were able to combat cholesterol content in brain of the protective study than the therapeutic study (Fig. 7).

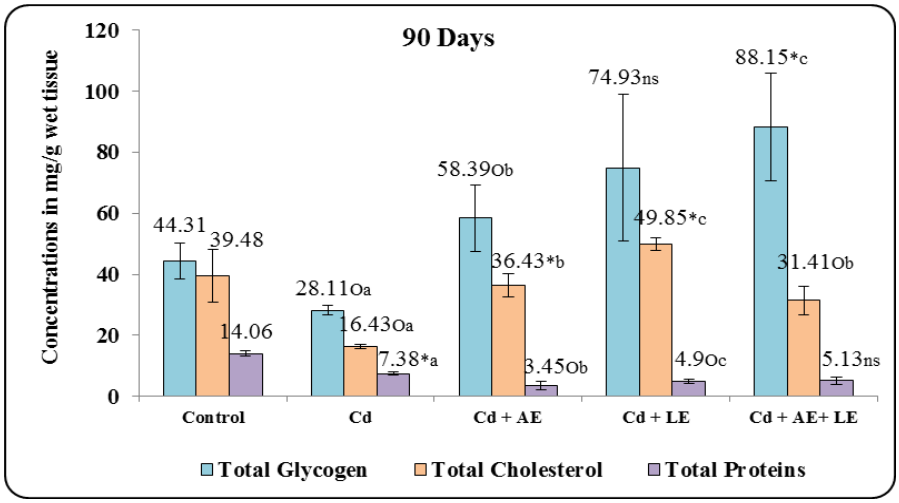

Fig. 7 Glycogen, Cholesterol and Total Proteins in brain of all the treated groups at 90 days.

The values were expressed as milligram per gram wet tissue. Bars represent means ± SEM for all the experiments. *Significant variations at p<0.0001; ^{O} p<0.01; ^{ns} Non Significant variations at p>0.05; a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at p < 0.05 in the Tukey's Honestly Significant Difference.

Total Proteins: The total proteins were significantly (p<0.0001) decreased in the Cd treated group as compared to control value. All the protective groups i.e. Cd + AE, Cd + LE and Cd + AE + LE groups showed significant (p<0.0001) enhancement in the total proteins at 45 days in comparison to Cd group (Fig. 6). There was significant (p<0.01) decrement in total proteins of Cd + AE and Cd + LE group but non-significant decrease was observed in Cd + AE + LE group at 90 days. The prevention was more in the protective study than the therapeutic study. Both garlic and tomato showed protection against Cd induced deterioration in total proteins (Fig. 7).

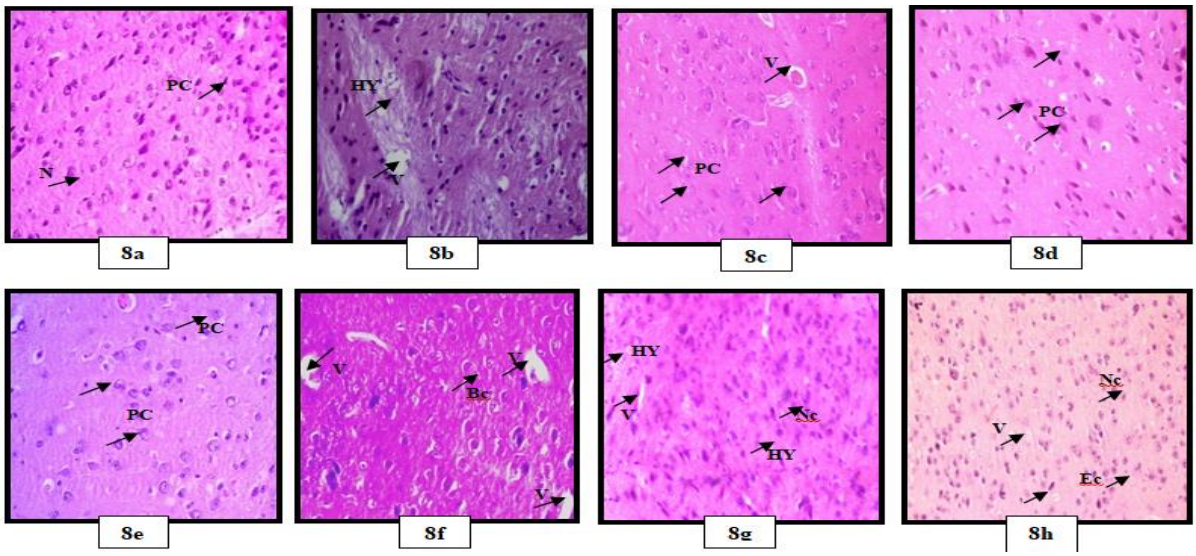

Fig. 8: Photomicrograph of cerebral cortex of mice.

8a: Control group showing normal architecture of cells of cerebral cortex (Cc) with pale open face nucleus and neuroglia (N). **8b:** Cd group showing much vacuolation (V), pericelluar halos, pyknotic cells (Pc) and binucleated cells (Bc) **Protective Groups 8c:** Cd + AE group showed more or less normal pyramidal cells (PC) with normal neuroglial cells (N). **8d:** Cd + LE treated group, the cortex appeared to be normal with pyramidal cells (PC) with a dark stained nucleus. **8e:** Cd + AE + LE group showing normal cortex structure with a little degeneration in the cells. **Therapeutic Groups 8f:** Cd + AE treated group, the cells showed normal structure with mild vacuolation (V) in the neurophil. **8g:** Cd + LE group showed some shrinkage of cells, necrosis (Nc) and hyperaemia (HY) in the cytoplasm. **8h:** Cd + AE + LE group, the cortex showed many normal cells with slight hyperaemia (HY) and necrotic cells (Nc). Shrinkage was also seen in the cells with pericellular halos. X400, H & E stain.

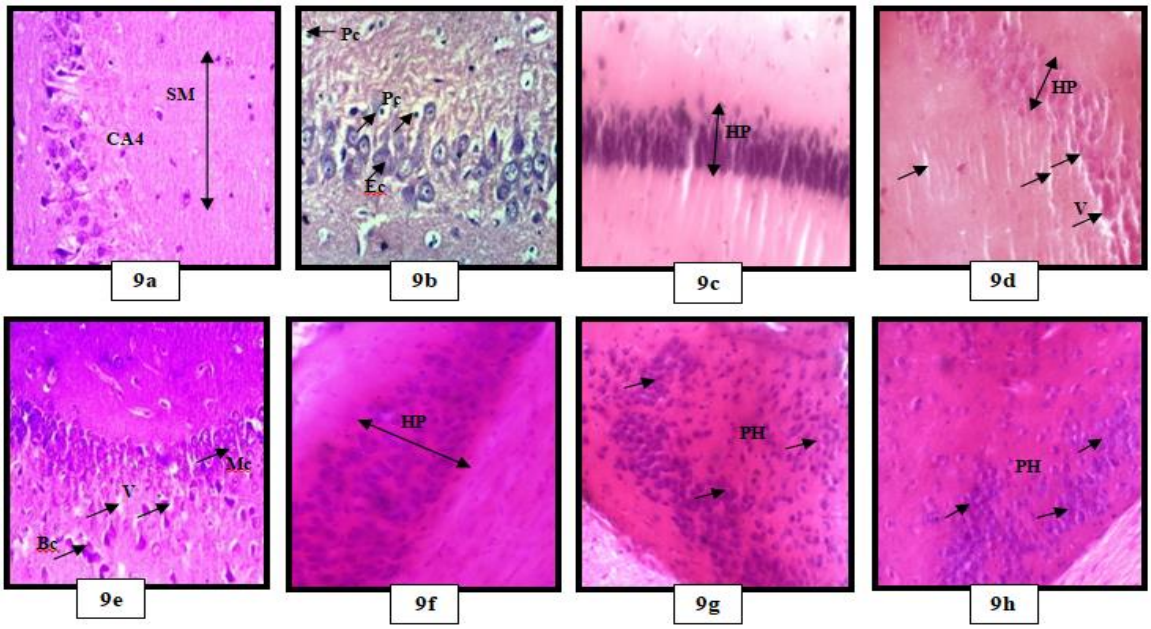

Fig. 9: Photomicrograph of hippocampus of mice. **9a.** Control group showing normal structure of hippocampus (Hp) with CA4 region having a layer of Stratum Moleculare (SM). **9b:** Cd group showing degeneration in the nerve cells and glial cells with enucleated cells (Ec) and pyknotic nuclei. **Protective Groups 9c:** Cd + AE group showing thick layers of cells with slight shrinkage in the structure of neurons was visible in the hippocampal proper (HP). **9d:** Cd + LE showing little vacuolation (V) and deshaped structure of neurons. **9e:** Cd + AE + LE showing normal compact structure with some vacuolation (V) and binucleated cells (Bc) **Therapeutic groups 9f:** Cd + AE showing normal structure of neurons with pyramidal (PC) and glial cells. **9g:** Cd + LE showing differential pattern of distribution of neurons with pericellular halos. **9h:** Cd + AE + LE showing heterogeneous morphology. Different layers are visible with cellular halos (PH). X400, H & E stain.

Histopathological Studies

Normal Brain: The Cerebral cortex (Cc) was composed of six different layers of cells. These were characterized by the neurons associated with particular functions and unique connections that show normal variations in the arrangements of cells in cerebral hemispheres (CH). Underneath the outermost molecular layer, four layers of pyramidal cells were observed along with the multiform sixth layer that was composed of fusiform cells. The cells in these layers are the commonly neurons especially pyramidal and granule cells in addition to neuroglial cells. The neurophil was a mat of neuronal and glial cell processes (Fig. 8a). The hippocampus (Hp) section single layer of very densely packed neurons which curls into a tight S shape. It further has two parts: hippocampal proper (HP) and dentate gyrus. The three major Cornu Ammonis (CA) regions i.e. CA1, CA2 and CA3 forms the hippocampal proper. The dentate gyrus (DG) consists of granular layer that is characterized by the small cells. The axons of the granule layer pass through the polymorphic layer and the dendrites of the granule layer enter the molecular layer. The polymorphic layer consists of several cell types including pyramidal neurons (Fig. 9a).

Effects of Cadmium (Cd): The tissue disclosed many multifocal histological alterations in all the layers of the cerebral cortex as compared to control group. There was the presence of large round cells (astrocytes) with foamy cytoplasm. Astrocytes showed hypertrophy and alterations in the neurofibrils that have clumped and twisted into odd shapes. The neuronal cell body also showed tremendously distended patterns in its structure. The cortex showed the formation of many pyknotic nuclei and binucleated cells along with hyperemia. The granule cells were more affected with ill-defined

boundaries and loss of nuclei. The overall structure showed vacuolated background (Fig. 8b). The hippocampus showed decreased thickness in all the layers of hippocampus with the formation of pyknotic cells and enucleated cells. Hippocampus proper showed distorted structure with much undesirable changes. It is further correlated with many areas of vacuolation, hyperemia and showed much of the edema in the tissue (Fig. 9b).

Effects of Protective treatment: In Cd + AE treated group, the pyramidal and granule cells were appeared to be normal with nucleus and basophilic cytoplasm. The neurophil was apparently normal with many neuroglial cells (Fig. 8c). The different layers of Hp appeared to be thick with increase in number of neurons. There was slight shrinkage in the structure of neuron (Fig. 9c). In Cd + LE treated group, the cortex appeared to be normal with some of the pyramidal cells that contained dark stained nucleus in the neurophil. Very few pyknotic cells with compact cytoplasm were observed (Fig. 8d). The Hp showed normal structure with slight vacuolation and deshaped nature of neurons in the tissue (Fig. 9d). In combined treated group, Cd + AE + LE showed normal cortex structure as that of control group with the pyramidal cells and glial cells along with little degeneration in the neural cells. (Fig. 8e). Even the hippocampus showed compact structure with somewhat thick layer of neurons but slight vacuolation and binucleated cells were seen in the hippocampal proper (Fig. 9e).

Effects of Therapeutic treatment: In Cd + AE treated group, the cortex showed vacuolation with almost normal neuroglial cell, pyramidal and granule cells. Some of the neurons were slightly affected with a dark stained nucleus (Fig. 8f). The Hp tissue showed almost normal pattern of distribution of cells. Neurons and glial cells were more prominent and all the cells were clear with mild degeneration (Fig. 9f). The tomato extract treated group, Cd + LE showed some necrosis, hyperamia in the cytoplasm with slight degeneration in the cells of the cerebral cortex (Fig. 8g). The Hp area also showed slight differential pattern of distribution of neurons. Some of the areas were more predominant with densely packed pyramidal cells than the other areas in the cytoplasm of the hippocampal proper (Fig. 9g). In the combined treated group, Cd + AE + LE, the cortex showed many normal cells with slight vacuolation and necrosis in the cells. Shrinkage was also observed more in the cells with pericellular halos (Fig. 8h). The hippocampal neurons showed diversified morphology. Some areas were predominated with pyramidal cells and some areas with glial cells in the neurophil (Fig. 9h).

DISCUSSION

Cadmium (Cd) is a toxic heavy metal that induced many biochemical and pathological changes in the brain. It further may get accumulated the tissue and disturb the physiological and metabolic activities in the cells. MDA is the most popular and widely used indicator for oxidative stress in various tissues and organs. The acute effect induced by low dose of Cd in the present study was confirmed by statistically significant ($p<0.0001$) increase in the level of MDA in brain tissue extracts. The direct targets of lipid peroxidation are the cell membranes as they are composed of phospholipid bilayers with extrinsic proteins and this may further lead to various detrimental effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage.[28] that influences the fluidity of the cell membrane along with DNA.[29] The intensity of the Cd intoxication not only depends on the route but also on the dose and duration of the exposure to the metal.[30] So, lipid peroxidation constituted a major consequence of Cd- induced oxidative damage and also was correlated with the exposure levels to Cd.[31]

The brain is believed to be more vulnerable to oxidative stress, among all the organs because it has high concentrations of polyunsaturated fatty acids, nondegenerative nature of neurons along with high oxygen consumption, which may further lead to many neurodegenerative diseases.[32] The main event associated with Cd evoked neurotoxicity is its ability to penetrate blood brain barrier (BBB) and accumulate within the brain to induce lipid peroxidation. Therefore, the Cd-elicited lipid peroxidation was mediated by overproduction of superoxide radicals to make a toxic product, MDA.[33]

The analysis of various enzymatic activies in the brain is very crucial in determining the toxic effects of heavy metals.[34] and AChE is an important enzyme that is responsible for hydrolyzing and deactivating acetycholine in the body.[35] In the present study, a statistical significant ($p<0.0001$) decrement in brain AChE activity was observed in Cd group. The attenuated AChE activity within the brain may be one of the symptoms for Cd-elicited complication in the brain as, brain AChE activity is an important regulator and controller of all the behavioral processes.[36] Even many studies also interpreted that the free radical production may be related to the decrease in the AChE level.[37] Cadmium induced peroxidation as well as oxidative stress is also related to the fact that metallic cadmium will pass through the BBB and gets concentrated in the brain to cause tissue injury.[34]

AChE activity was also inactivated due to high Cd concentration because Cd^{2+} is one of the metal inhibitors of the enzyme (where Ca^{2+} is one of the activators) that can induce a conformational change in the protein structure, which further leads to the formation of an "unreactive" enzyme.[38] Regarding calcium (Ca), it should be noted that calmodulin does not differentiate between Ca and Cd, resulting in alteration of cholinergic functioning.[39] Cd induced oxidative stress may also play a significant role in the process of down regulating nAChRs (acetylcholesnestrase recepters) that leads to

marked decline in AChE level so, all the observed changes in AChE activity probably indicated that Cd can affect brain cholinergic or dopaminergic mechanisms.[40]

The most important defense mechanisms that help in neutralizing free radical chain reactions and protect the cell against their toxic effects[41] are mostly connected with the antioxidant effect of SOD, CAT, GPx and GST. With the long term exposure to Cd, the activity of SOD, CAT and GST was reduced as many of the essential metals from their active sites and were replaced by other nonessentials metals by making the enzymes less active.[42] Even Cd may change the protein conformation by interacting with the enzyme and finally changing its functional activity which may further result in a number of deleterious effects due to the aggregation of superoxide radicals.[43] So, the decreased SOD activity in Cd treated group might be due to its inhibition by the immense production of ROS.[44] as noticeable by the LPO levels in the present study.

CAT is an inducible cytosolic enzyme which serves to protect the biological system against reactive oxygen species, rapidly converting hydrogen peroxide to non-toxic oxygen and water.[45] There was significant decline in CAT level in Cd treated group. This decrease was attributed to the possibility of high production of ROS and their increased intracellular accumulation which further exceed the detoxification capacity of antioxidant enzymes, finally resulting in subsequent development of tissue injury.[46] The decrease in CAT level could also result from iron deficiency due to cadmium intoxication as iron acts as a composing element for the interaction between Cd and catalase.[47] and Cd decreases the iron level in blood.

GST is an important enzyme that catalyses the reaction of the thiol group of the GSH with electrophilic reagents by neutralizing their electrophilic sites and providing more water soluble products.[48] The overconsumption of the enzyme GST to escape from the toxicity of peroxides under Cd insult resulted in decrement of GST activity and further this decrease in the GST concentration might be correlated with the effect of Cd on GSH because of its high affinity to this molecule where a sulfhydryl acid, an amino acid and two carboxylic acid groups as well as two peptide linkages represent reactive sites for metals.[44] making the enzymes nonfunctional. Even Shagirtha et al.[49] also recorded a decrease in the activities of enzymatic antioxidants in rats intoxicated with Cd.

The brain is an important part of biological system whose function is to help and regulate other parts along with various mechanisms of the body but any damage or stress experienced to this part of the body may have a serious effect on the entire organism.[50] Cd causes an adverse effect on cellular defense systems and thiol status, since metallothionein (MT) is cysteine rich molecule and Cd has high affinity for MT, so cadmium is stored as a Cd-MT complex and further it is metabolized in lysosomes to liberate cadmium ions. The Cd toxicity occurs when the MT synthesis cannot keep up with the demand and the non-MT bound Cd overwhelms the defense systems as Cd ions gets bound to the existing MT.[51] The attainable mechanism in Cd-elicited antioxidant enzyme depletion in the present study was also because of the interaction with SH groups in some enzymes to exchange essential metals from their active sites, or alteration in amino acid chain due of the radical mediate reaction so, these toxic changes results in inactivation and loss of protein function.[34]

Glycogen is an imperative source of energy for the general metabolism of the body.[52] A reduced insulin release/activation of gluconeogenic enzymes may also results in decrement of tissue glycogen.[53] and are further linked to the depressed feeding and/or elevated levels of the stress hormones: cortisol and adrenaline.[54] Glycogen can be depleted in response to some physiological processes or nonchemical stresses such as temperature and hypoxia.[55] This decreased glycogen levels in the brain can lead to neurological disruption and this further promotes cortical spreading depression due to disturbed K^+ regulation by astrocytes.[56] along with altered astrocyte morphology.

Cholesterol is an essential part of cell membrane and helps in the maintenance of cellular homeostasis along with the communication between and with cells.[57] There was decrement in the cholesterol level in response to Cd treatment. This decrease in the cholesterol content may be because of Cd-induced production of free radicals that can damage the glial cells leading to depletion in tissue cholesterol.[58] The decreased concentrations of cholesterol might be due to increased ACTH secretion by pituitary leading to decreased cholesterol concentration.[59]

Proteins are the constituents of cell membranes and are manufactured by the ribosomes of the cells. There was decreased in the total protein content in Cd treated group as compared to control value. This decrease may be correlated to with the decreased protein synthesis due to hepatic dysfunction under heavy metal exposure.[60] Even the chronic renal diseases that are associated with heavy metal toxicity can cause excessive loss of proteins.[61] Hypoproteinuria is generally regarded as a nonspecific indicator of toxicity and can be caused due to several factors such as the reduction in food intake, chronic liver function and renal protein loss.[62] Moreover, Cd retards the protein synthesis by binding itself to sulfhydryl group (SH) of many enzymes and this may further cause inhibition of many enzymatic activities.[63]

Cd treatment induced histological changes in all the layers of the cortex which involved different types of cells especially pyramidal and granule cells along with neuroglia. Cd induced changes in the cerebral cortex were hyperaemia, lymphocytic infiltration, vacuolation

in the neurophil, astrocytes with ill-defined boundaries and pericellular halos as compared to control tissue. The hippocampus of Cd treated mice also showed many remarkable and undesirable changes in the form of vacuolation, edema, pyknotic nuclei and the formation of many binucleated cells in comparison to normal structure.

The present study showed that Cd intoxication resulted in degeneration in the pyramidal neurons of the CA3 subfield of the hippocampus and this is implicated due to the possibility of interruption of the nervous impulses transmitted from the granule cell layer of the DG of the hippocampus top the CA3 area.[64] Even, the neurotoxic effects of Cd due to oxidative injury may bring many necrotic changes in the brain as it is high susceptible to Cd toxicity.[65] The astrocytes in the neuroglia may form enlarged processes due to the result of lipid peroxidation theory and also cause swelling of the cells due to an increase in the sodium permeability resulting in sodium accumulation along with an increase in water content inside the cell.[66] Further, Cd-mediated neurotoxicity may be due to Cd evoked free radicals that may cause the oxidation of macromolecules to make carbonyl groups and result in toxicity.[67]

AGE treatment showed significant amelioration in all the biochemical and pathological studies. Garlic increases the antioxidant levels by elevating homeostatic regulation of cellular GSH contents as well as by promoting removal of metabolic intermediates through induction of phase II metabolizing enzymes such as GST.[68] Zaidi et al.[69] concluded that the intra-gastric administration of crude garlic extract significantly elevated the circulating activities of SOD, CAT and GST, therefore found to prevent and normalize oxidative stress generated by immobilization stress. They further suggested that garlic along with its constituents behaved as double-edged swords to up-regulate the antioxidant activities during stress.[69] and this might be possible due to the organosulfur contents in garlic which are potent free radical scavengers.

ATE treatment showed significant changes in the protective groups ($p<0.0001$) but non-significant variations were observed in the therapeutic groups. These changes may be correlated to the protection against per oxidative injury induced by ROS which is associated with the risk of osteoporosis and can be reduced by certain dietary antioxidants.[70] The administration of antioxidant i.e. tomato extract as a dietary supplement showed remarkable protection by restoring the general structure of the tissues. These results indicated that carotenoid (lycopene) is a highly efficient scavenger of singlet-oxygen ($^1O_2$) and other excited species. The energy is transferred from $^1O_2$ to the lycopene molecule during quenching, thereby converting it to the energy-rich triplet state by preventing their damage.[71]

The administration of garlic and tomato (AGE + ATE) singly or in combination showed good amelioration in biochemical parameters in all the protective as well as therapeutic groups as compared to toxic groups. Garlic may participate in the chelation of heavy metals due to the presence of an important constituent i.e. allicin.[72] Even Tomato, has some of the metal chelating proteins and phytochelatins that has been proven effective in counteracting and ameliorating some of the biomarkers of toxicity.[13] The combination of both the antioxidants has played a significant role in attenuating Cd induced biochemical changes in brain of mice.

CONCLUSION

Cd administration resulted in severe toxic effects in both biochemical and histological aspects of brain tissue in mice. But the intake of antioxidants i.e. garlic and tomato reversed the toxic effects of Cd to some extent. But the synergistic action of garlic and tomato proved even more satisfactory and encouraging in reducing the damage caused by Cd. So, it is concluded that regular intake of both the antioxidants may be beneficial in minimizing and counteracting the damage caused by heavy metals in the brain tissue of humans and other animals exposed to environmental pollution.

ACKNOWLEDGEMENTS

The authors like to thank the Department of Zoology & Environmental Sciences, Punjabi University, Patiala, for providing the necessary facilities to pursue this research work. The authors also likes to acknowledge all the people who have directly or indirectly helped in the completion of this work.

Author's contributions

PV have drafted the manuscript along with research and statistical analysis and this work was supervised and finalized by SS.

Declaration of Conflicting Interests

The authors declare that they have no conflict of interest with respect to the research, authorship or publication of this article.

Funding

This authors received no financial support for the research, authorship and for the publication of the article.

Ethics approval

Animals (Albino mice) were treated in accordance with the guidelines of the Institutional Animal Ethical Committee with the approval number 107/99/CPCSEA/2014-33.

REFERENCES

1. Branca JJV, Morucci G, Alessandra Pacini A. Cadmium induced neurotoxicity: still much ado. Neural Regen Res., 2018; 13(11): 1879-1882. doi: 10.4103/1673-5374.239434

2. Tinkov AA, Filippini T, Ajsuvakovae OP, Skalnaya MG, Aasethf J, Bjørklundh G, Gatiatulinai ER, Popova EV, Nemereshinai ON, Huangk PT. Cadmium and atherosclerosis: A review of toxicological mechanisms and a meta-analysis of epidemiologic studies. Environ Res., 2018; 162: 240–260. doi: 10.1016/j.envres.2018.01.008
3. Tamás MJ, Fauvet B, Christen P, Goloubinoff P. Misfolding and aggregation of nascent proteins: A novel mode of toxic cadmium action in vivo. Curr Genet. 2018; 64: 177–181. doi: 10.1007/s00294-017-0748-x
4. Vijaya P, Kaur H, Garg N, Sharma S. Protective and therapeutic effects of garlic and tomato on cadmium-induced neuropathy in mice. J Basic Appl Zool., 2020; 81: 23. doi: 10.1186/s41936-020-00160-4
5. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The Effects of Cadmium toxicity. Inter J Environ Res Public Health. 2020; 17: 3782. doi: 10.3390/ijerph17113782
6. Marini HR, Puzzolo D, Micali A, Adamo EB, Irrera N, Pisani A, Pallio G, Trichilo V, Malta C, Bitto A, Squadrito F, Altavilla D, Minutoli L. Neuroprotective Effects of Polydeoxyribonucleotide in a Murine Model of Cadmium Toxicity. Oxid Med Cell Longev, 2018 Aug 29; 4285694. doi: 10.1155/2018/4285694
7. Nishimura Y, Yamaguchi JY, Kanada A, Horimoto K, Kanemaru K, Satoh M. Increase in intracellular Cd(2+) concentration of rat cerebellar granule neurons incubated with cadmium chloride: Cadmium cytotoxicity under external Ca (2+) – free condition. Toxicol In Vitro, 2006; 20(2): 211-216. doi: 10.1016/j.tiv.2005.06.006
8. Lukawski K, Nieradko B, Sieklucka- Dziuba M. Effects of cadmium on memory processes in mice exposed to transient cerebral oligemia. Neurotoxicol Teratol, 2005; 27(4): 575-84. doi: 10.1016/j.ntt.2005.05.009
9. Yang Y, Hassan MF, Ali W, Zou H, Liu Z, Ma Y. Effects of Cadmium Pollution on Human Health: ANarrative Review. Atmosphere, 2025; 16(2): 225. https://doi.org/10.3390/atmos16020225
10. Megha J, Ganesh, N, Versha S. In vitro evaluation of free radical scavenging activity of Pistia stratiotes. Inter J ChemTech Res., 2010; 2(1): 180-184.
11. Borek C. Antioxidant health effects of aged garlic extract. J Nutr., 2001; 131: 1010-1015. doi: 10.1093/jn/131.3.1010S
12. Papu S, Jaivir S, Sweta S, Singh BR. Medicinal values of Garlic (Allium sativum L.) in Human Life: An Overview. Greener J Agricul Sci., 2014; 4(6): 265-280. DOI: http://dx.doi.org/10.15580/GJAS.2014.6.031914151
13. Tito A, Carola A, Bimonte M, Barbulova A, Arciello S, deLaurentiis F, Monoli I, Hill J, Gibertoni S, Colucci G, Apone F. A tomato stem cell extract, containing antioxidant compounds and metal chelating factors, protects skin cells from heavy metal-induced damages. Inter J Cos Sci. 2011; 33(6): 543-552. doi: 10.1111/j.1468-2494.2011.00668.x
14. Nwokocha CR, Nwokocha M I, Aneto I, Obi J, Udekweleze DC, Olatunde B, Owu DU, Iwuala MO. Comparative analysis on the effect of Lycopersicon esculentum (tomato) in reducing cadmium, mercury and lead accumulation in liver. Food Chem Toxicol. 2012; 50(6): 2070-2073. doi: 10.1016/j.fct.2012.03.079
15. Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr Res., 1999; 19(2): 305-323. https://doi.org/10.1016/S0271-5317(98)00193-6
16. Agarwal A, Shen H, Agarwal S, Rao AV. Lycopene content of tomato products: its stability, bioavailability and in vivo antioxidant properties. J Med Food., 2001; 4(1): 9-15. doi: 10.1089/10966200152053668
17. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. In-vitro antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J Med Food. 2004; 7(3): 327-333. doi: 10.1089/jmf.2004.7.327
18. Salawu EO, Adeleke AA, Oyewo OO, Ashamu EA, Ishola OO, Afoladi AO, Adesanya TO. Prevention of renal toxicity from lead exposure by oral administration of Lycopersicon esculentum. J Toxicol Environ Health Sci., 2009; 1(2): 22-27. https://doi.org/10.5897/JTEHS.9000058
19. Wilbur KM, Bernhein F, Shapiro OW. The thiobarbituric acid (TBA) reagent as a test for the oxidation of unsaturated fatty acid by various agents. Acta Biochimica et Biophysica Sinica., 1949; 24(2): 305-313. PMID: 15405719
20. Das K, Samamta L, Chainy GBN. A modified spectrophotometric assay for superoxide dismutase using nitrite formation by superoxide radicals. Ind J Biochem Biophy, 2000; 37(3): 201-204.
21. Aebi HE. Catalase. In: Methods of enzymatic analysis. Bergmeyer, H.U. (ed.) Verlag Chemiew, Weinheim, 1983; 3: 273-286.
22. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem., 1974; 249(22): 7130-7139. PMID: 4436300
23. Ellman GL, Courtney D, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol., 1961; 7: 88-95. doi: 10.1016/0006-2952(61)90145-9
24. Montgomery R. Determination of glycogen. Arch Biochem Biophy. 1957; 67: 378-381. doi: 10.1016/0003-9861(57)90292-8
25. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clinical Med., 1953; 41(3): 486-492. PMID: 13035283

26. Lowery OH, Roseburg NJ, Farr AL, Raudall RJ. Protein measurement with the folin-phenol reagent. J Biol Chem., 1951; 193(1): 265-275. PMID: 14907713
27. Drury RA, Wallington EA. Carleton's Histological Techniques, 5th ed, Oxford University Press. London, New York Toronto., 1980; 1: 653-661.
28. Ognjanovic BI, Pavlovic SZ, Maletic SD, Zikic RV, Stajn AS, Radojicic RM, Saicic ZS, Petrovic VM. Protective influence of vitamin E on antioxidant defense in the blood of rats treated with cadmium. Physiol Res., 2003; 52: 563-570.
29. Zama D, Meraihi Z, Tebibel S, Benayssa W, Benayache FS. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: the protective role of butanolic extract Paronychie argentea L. Ind J Pharmacol., 2007; 39(3): 145-150. doi:10.4103/0253-7613.33434
30. Djokic J, Ninkov M, Mirkov I, Aleksandrov AP, Zolotarevski L, Kataranovski D, Kataranovski, M. Differential effects of cadmium administration on peripheral blood granulocytes in rats. Environ Toxicol Pharmacol., 2014; 37(1): 210-219. doi: 10.1016/j.etap.2013.11.026
31. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem-Biol. Interact., 2006; 160: 1-40. doi: 10.1016/j.cbi.2005.12.009
32. Halliwell B, Gutteridge JMC. Free radicals in Biology and Medicine. 4th ed. Oxford UK: Clarendon Press, Oxford Science. 2007.
33. El-Tarras AES, Attia HF, Soliman MM, El-Awady MA, Amin AA. Neuroprotective effect of grape seed extract against cadmium toxicity in male albino mice. Int J Immunopathol Pharmacol., 2016; 29(7): 398-407. doi: 10.1177/0394632016651447
34. Alnahdi HS, Sharaf IA. Possible prophylactic effect of omega-3 fatty acids on cadmium induced neurotoxicity in rats' brain. Environ Sci Pollut Res Int., 2019; 26(30): 31254-31262. doi: 10.1007/s11356-019-06259-8
35. Kaoud HA, Mekawy MM. Effect of cadmium pollution on neuromorphology and function of brain in mice offspring. Nat Sci., 2011; 9(4): 28-35.
36. Pari L, Murugavel P. Diallyl tetrasulfide improves cadmium induced alterations of acetylcholinestrase, ATPases and oxidative stress in brain of rat. Toxicol., 2007; 234(1-2): 44-50. doi: 10.1016/j.tox.2007.01.021
37. Pervin M, Abul Hasnat MD, Yoon YM, Kim DH, Jo JE, Lim BO. Antioxidant activity and acetylcholenestrase inhibition of grape skin anthocyanin (GSA). Mol., 2014; 19(7): 9403-9418. doi: 10.3390/molecules19079403
38. Tomlinson GB, Mutus, McLennan I. Activation and inactivation of acetylcholenestrase by metal ions. Can J Biochem., 1981; 59(9): 728-735. doi: 10.1139/o81-101
39. Sutoo D, Akiyama K, Imamiya S. A mechanism of cadmium poisoning: the cross effect of calcium and cadmium in the calmodulin-dependent system. Arch Toxicol, 1990; 64: 161-164. https://doi.org/10.1007/BF01974404
40. Klegeris A, Korkina LG, Greenfield SA. A possible interaction between acetylcholenestrase and dopamine molecules during autoxidation of the amine. Free Radic Biol Med., 1995; 18(2): 223-230. doi: 10.1016/0891-5849(94)00115-z
41. Liczmanski A. Oxygen toxicity. I: damage of living cells. Post Biochem. 1988; 34(4): 273-291.
42. Winiarska-Mieczan A. Protective effect of tea against lead and cadmium-induced oxidative stress-a review. Biometal, 2018; 31(6): 909-926. doi: 10.1007/s10534-018-0153-z
43. Nagaraj M, Sumitha S, Varalakshmi P. Effect of lupeol, a pentacyclic triterpene, on lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure. J Appl Toxicol, 2000; 20(5): 413-417. doi: 10.1002/1099-1263(200009/10)20:5<413::AID-JAT706>3.0.CO;2-Y
44. Jamakala O, Rani UA. Amelioration effect of zinc and iron supplementation on selected oxidative stress enzymes in liver and kidney of cadmium treated male albino rat. Toxicol Inter. 2015; 22(1): 1-9. doi: 10.4103/0971-6580.172289
45. Hussein SA, Abd El-Hamid OM, Fayed AMS. Protective effects of alpha-lipoic acid and melatonin against cadmium-induced oxidative stress in erythrocytes of rats. J Pharmacol Toxicol, 2014; 9(1): 1-24. doi: 10.3923/jpt.2014.1.24
46. De Castro MAC, Neto FFC, Lima LMC, Da Silva FM, De Oliveira RJ, Zanesco A. Production of free radicals and catalase activity during acute exercise training in young men. Biol Sport, 2009; 26(2): 113-118. doi:10.5604/20831862.890157
47. Jurczuk M, Brzoska MM, Moniuszko-Jakoniuk J, Galazyn-Sidorczuk M, Kulikowska-Karpinska E. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. Food Chem Toxicol, 2004; 42(3): 429-438. doi: 10.1016/j.fct.2003.10.005
48. Han XY, Xu ZR, Wang YZ, Huang QC. Effect of cadmium on lipid peroxidation and activities of antioxidant enzymes in growing pigs. Biol Trace Elem Res., 2006; 110(3): 251-263. doi: 10.1385/BTER:110:3:251
49. Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. European Rev Med Pharmacol Sci., 2011; 15(9): 1039-1050. PMID: 22013727
50. Ogunrinola OO, Wusu DA, Fajana OO, Olaitan SN, Smith ZO, Bolaji ARI. Effect of low level cadmium exposure on Superoxide dismutase activity in rat. Trop J Pharma Res., 2016; 15(1): 115-119. doi:10.4314/tjpr.v15i1.16
51. Nishitai G, Matsuoka M. Differential regulation of HSP70 expression by the JNK kinases SEK1 and

MKK7 in mouse embryonic stem cells treated with cadmium. J Cell Biochem, 2008; 104(5): 1771-1780. doi: 10.1002/jcb.21743
52. Desai KR, Moid N, Patel PB, Highland HN. Protective effect of Allium sativum on deltamethrin induced toxicity in reproductive tissues of male mice. Inter J Pharm Sci Res., 2015; 6(4): 1711-1720. doi:10.13040/IJPSR.0975-8232.6(4).1711-20
53. Demir H, Kanter M, Coskun O, Uz YH, Koc A, Yildiz A. Effect of Black Cumin (Nigella sativa) on heart rate, some hematological values and pancreatic β-cell damage in cadmium-treated rats. Biol Trace Elem Res., 2006; 110(2): 151-162. doi: 10.1385/BTER:110:2:151
54. Heath AG. Water pollution and fish physiology. 2nd Edition, CRC Press, Boca Raton. 1995. https://doi.org/10.1201/9780203718896
55. Yuness HAM. Role of melatonin in reducing hypoxia-induced oxidative stress. M.Sc. Thesis, Assuit University, Egypt. 2005.
56. Kilic K, Karatas H, Donmez-Demir B, Eren-Kocak E, Gursoy-Ozdemir Y, Can A, et al. Inadequate brain glycogen or sleep increases spreading depression susceptibility. Ann. Neurol. 2018; 83(1): 61-73. doi: 10.1002/ana.25122.
57. Liu JP, Tang Y, Zhou S, Toh BH, McLean C, Li H. Cholesterol involvement in the pathogenesis of neurodegenerative diseases. Mol Cell Neurosci. 2010; 43(1): 33-42. doi: 10.1016/j.mcn.2009.07.013
58. Samarghandian S, Azimi-Nezhad M, Shabestari MM, Azad FJ, Farkhondeh T, Bafandeh F. Effect of chronic exposure to cadmium on serum lipid, lipoprotein and oxidative stress indices in male rats. Interdiscip Toxicol. 2015 Sep; 8(3): 151-154. doi: 10.1515/intox-2015-0023
59. Nagler K, March D, Pfriegar FW. Glia derived signals induced synapse formation in neurons of the rat central nervous system. J Physiol. 2001; 533(Pt 3): 665-679. doi: 10.1111/j.1469-7793.2001.00665.x
60. Tchounwou PB, Yedjou C, Patlolla A, Sutton D. Heavy metal toxicity and the environment. Exp Suppl. 2012; 101: 133-164. doi: 10.1007/978-3-7643-8340-4_6
61. Barbier O, Jacquiller G, Tauc M, Cougnon M, Poujeol P. Effect of heavy metals on, and handling by the kidney. Nephron Physiol. 2005; 99(4): 105-110. doi: 10.1159/000083981
62. Jadhav SH, Sarkar SN, Patil RD, Tripathi HC. Effect of Subchronic Exposure via drinking water to a mixture of Eight Water-contaminating metals: A biochemical and histopathological study in male rats. Arch Environ Contamin Toxicol, 2007; 53(4): 667-677. doi: 10.1007/s00244-007-0031-0
63. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis: a review. Toxicol. 2003; 192(2-3): 95-117. doi: 10.1016/s0300-483x(03)00305-6
64. Vivar C, van Praag H. Functional circuits of new neurons in the dentate gyrus. Front Neural Circuit. 2013; 7: 15. doi: 10.3389/fncir.2013.00015
65. El-Sokkary RT, Awadalla E. The protective role of vitamin C against cerebral and pulmonary damage induced by cadmium chloride in male adult albino rat. Open Neuroendocrinol J. 2011; 4: 1-8. doi: 10.2174/1876528901104010001
66. Panickar KS, Norenberg, MD. Astrocytes in cerebral ischemic injury: Morphological and general considerations. Glia. 2005; 50(4): 287-298. doi:10.1002/glia.20181
67. Engstrom KS, Vahter M, Johansson G, Lindh CH, Teichert, F, Singh, R, Kippler, M, Nermell M, Raqib R, Stromberg U, Broberg K. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7, 8-dihydroguanosine in urine. Free Radic Biol Med. 2010; 48(9): 1211-1217. doi: 10.1016/j.freeradbiomed.2010.02.004
68. Park EY, Ki SH, Ko MS, Kim CW, Lee MH, Lee YS, Kim SG. Garlic oil and DDB, comprised in a pharmaceutical composition for the treatment of patients with viral hepatitis, prevents acute liver injuries potentiated by glutathione deficiency in rats. Chemico-Biol Interac. 2005; 155(1-2): 82-96. doi: 10.1016/j.cbi.2005.04.006
69. Zaidi SK, Ansari SA, Md.Ashraf G, Jafri MA, Tabrez S, Banu N. Reno-protective effect of garlic extract against immobilization stress induced changes in rats. Asian Pacific J Trop Biomed. 2015; 5(5): 364-369. doi:10.1016/S2221-1691(15)30370-1
70. Moawad KM. Possible prophylactic effects of vitamin E or lycopene treatments on renal toxicity induced by CCl_4 administration in albino rats. World J Zool., 2007; 2(2): 19-28.
71. Atessahin A, Yilmaz S, Karahan I, Ceribasi AO, Karaoglu A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicol., 2005; 212(2-3): 116-123. doi:10.1016/j.tox.2005.04.016
72. Chowdhury R, Dutta A, Chaudhuri SR, Sharma N, Giri AK, Chaudhuri K. In vitro and in vivo reduction of sodium arsenite induced toxicity by aqueous garlic extract. Food Chem Toxicol. 2008; 46(2): 740-751. doi: 10.1016/j.fct.2007.09.108.