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Protective and therapeutic effects of garlic and tomato on cadmium-induced neuropathology in mice

P. Vijaya[1*], Harsimran Kaur[2], Nancy Garg[3] and Suman Sharma[2]

Abstract

Background: Heavy metals are natural components of the earth's crust and are considered as constant environmental pollutants since they cannot be degraded or destroyed easily. Cadmium (Cd) is present primarily in the ores of zinc, copper, or lead; the extraction and processing of which releases large quantities of cadmium into the atmosphere, hydrosphere, and soil thereby contaminating the human environment. The present study aimed to investigate the possible protective and therapeutic effects of garlic and tomato extract on cadmium-induced AChE activity, biochemical parameters along with the pathological changes in the brain tissue of mice.

Methods: Male Swiss albino mice ($n = 40$) were divided into several experimental (protective and therapeutic) groups and were given single dose of cadmium (6 mg/kg bw) with supplementation of garlic (100 mg/kg bw) and tomato (50 mg/kg bw) extract for 15 and 30 days under protective and therapeutic study.

Results: Cadmium-treated mice showed a significant decline in AChE ($p < 0.01$) level and total proteins ($p < 0.0001$) but a nonsignificant decrease in glycogen, cholesterol in the brain tissue activity as compared to control group. The histological study also showed degeneration in the form of vacuolation, congestion, hyperemia, lymphocytic infiltration, and edema in the brain (cortex and hippocampus) of Cd-treated mice. But the antioxidant-treated groups showed significant increment in AChE level and other biochemical parameters in the protective study. But there were significant variations in the therapeutic study, and this was also confirmed by the histological analysis of the brain. Both tomato and garlic administration showed more attenuation in the brain AChE activity, and it may be due to the strong antioxidant potential of their constituents.

Conclusion: We can conclude that cadmium exposure should be avoided as it causes neuropathological effects. But in daily life, it may not be possible, so we should regularly intake natural antioxidants which may neutralize the effects of heavy metals to some extent.

Keywords: Cadmium (Cd), Garlic extract (GE), Tomato extract (TE), Acetylcholinesterase (AChE), histopathology

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Background

Heavy metals are natural components of the earth's crust and are considered as constant environmental pollutants since they cannot be degraded or destroyed easily (Pinot et al., 2000). These metals are emitted into the atmosphere with composition of fine particles or in gaseous form and are transferred by atmospheric fluxes to the unwanted distances where they enter ecosystem of remote regions (Rusconi & Polesello, 2010). Cadmium (Cd) is a toxic metal which was classified as a human carcinogen by the North Carolina National Toxicology Program (NTP, 2000). Cd deposits are found with zinc, copper, or lead, and the smelting processes of these metals releases Cd as a by-product into the atmosphere, water bodies, and soil thereby contaminating the human environment (Pachana et al., 2010).

Cd generates free radicals such as superoxide radicals, hydroxyl radicals, and nitric oxide which cause tissue damage (Galan et al., 2001). Many organs including the brain are affected by free radical accumulation in animals (Kim et al., 2014). The nervous system is highly receptive to free radical damage as it is the site for many unsaturated fatty acids and iron (Bauer & Bauer, 1999). Many epidemiological studies also showed that Cd vulnerably effect renal, prostate, liver, hematopoietic system, urinary bladder, pancreatic, testis and even causes stomach ulcers (Joseph, Muchnok, Klishis, et al., 2001).

Cadmium affects the behavior of both adult and neonatal animals by altering their neurotransmitter levels of the brain (Ozonas, Bstombo, & Santos-Ruiz, 1974). Cd exposure to the brain blocked the synaptic transmission at peripheral cholinergic synapses in vitro (Cooper, Chandhasy, Hastings, & Petering, 1978) and resulted in decreased spontaneous neural firing into the brain stem or cerebral cortex (Rozear, Degroof, & Somjen, 1971). The neurotoxic effects of Cd have also been reported in neonatal mouse brain (Webster & Valois, 1981) besides in young rat brain (Wong & Klaassen, 1982).

The oxidative reaction is one of the important mechanisms of cadmium-induced free radical damage, but this effect can be restricted by the supplementation of some antioxidants (Renugadevi & Prabu, 2010). All the healthy foods such as vegetables, fruits, grain cereals, eggs, meat, legumes, and nuts are the sources of photochemicals that behave as antioxidants and anti-inflammatory agents through different protective mechanisms (Slavin & Lloyd, 2012).

Garlic (*Allium sativum*) is one of the studied plants, with a long history of therapeutic use and its health benefits have been extensively reported (Asdaq & Inamdar, 2010; Sharma, Sharma, & Kansal, 2010). Fresh garlic is reportedly famous for its anti-toxic, antimutagenic, and anticarcinogenic effects due to the presence of sulfhydryl compounds and sulfur-rich compounds, such as diallyl sulfide (DAS), diallyl disulfide (DADS), ajoene, allicin, allyl mercaptans, and allyl methylsulfides (Borek, 2001). Duke (1999) reviewed that various studies conducted on animals have shown improvement in brain functions after eating garlic, and this is possible due to the antioxidants found in garlic which neutralize and destroy the free radicals that have been aggregated in the body. So, garlic extract with its antioxidant ability may improve the cognitive impairment and show some beneficial activities against neurodegenerative disorders such as Alzheimer disease (Jeong et al., 2013). It was further studied that sulfur compound S-allyl cysteine, found in garlic, prevents the degeneration of the brain's frontal lobes (Borek, 2006).

Tomato (*Lycopersicon esculentum*) served as a food additive for fortification and stabilization (Lavelli, Hippeli, Dornisch, Peri, & Elstner, 2001) and is a rich source of antioxidants (Sandhu et al., 2000). Its exposure with heavy metals leads to synthesize metal chelating proteins, peptides, phytochelatins (PC), and other heavy metal-binding complexes analogous to metallothioneins (Tito et al., 2011). Further, these proteins capture the heavy metals and help in preventing cellular damage (Nwokocha et al., 2012). Tomato fruits are a rich source of anti-inflammatory and antioxidant nutrients that includes carotenoids such as lycopene, β-carotene, phytoene, phytofluene, n-carotene (Cohen, 2002), vitamin C, vitamin E, naringenin (a flavonoid), and cholinergic acid (phenolic acid) (Chew & Park, 2004).

Since, the brain is one of the important tissues, so the present investigation was undertaken to study the protective and therapeutic efficacy of garlic and tomato against Cd-induced adverse effects on brain, special emphases on biochemical and histopathological aspects.

Materials and methods

Experimental animals

Swiss albino mice weighing 20–25 g 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 the Institutional Animal Ethical Committee (Reg No. 107/99/CPCSEA/2014-33).

Chemicals

Cadmium chloride ($CdCl_2$) was bought from S.D Fine Chem Limited, Mumbai. It was dissolved in double-glass distilled water and administered orally to mice. Garlic and tomato were obtained from the local market. Fresh garlic extract was prepared by the method of Iwalokun,

Ogunledun, Ogbolu, Bamiro, and Jimi-Omojola (2004), and tomato extract was prepared by the method of Salawu et al. (2009) and administered orally to mice.

Experimental design

The mice were divided into 8 groups (5 animals each) as follows: Group I—control animals treated with only saline water. Group II—animals were administered an acute dose of Cd (6 mg/kg bw) orally as this was the LD_{50} observed for the study, for single acute dose of Cd in the form of $CdCl_2$ in mice. Group III—animals were given an acute dose of Cd orally followed by garlic extract (GE) of 100 mg/kg bw for 15 days. Group IV—animals were given a single dose of Cd orally and treated with tomato extract (TE) of 50 mg/kg bw for 15 days. Group V—animals were administered with an acute dose of Cd orally and combined with an oral supplementation of garlic (GE) and tomato extracts (TE) for 15 days. Group VI—animals were subjected to Cd at a dose of 6 mg/kg bw orally for the 1st day and were left on normal diet for 15 days followed by oral administration of garlic extract (GE) for the next 15 days. Group VII—animals were given an acute dose of Cd (6 mg/kg bw) and were left for 15 days followed by tomato extract (TE) for the next 15 days. **Group VIII**—animals were given Cd at a dose of 6 mg/kg bw and were kept for 15 days followed by GE + TE for the next 15 days. This study, including all the groups, was performed in 3 replicas to confirm the results (Fig. 1).

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 studies.

Preparation of tissue homogenates

The brain was separated and was weighted and homogenized in a tissue homogenizer in 3 ml of phosphate buffer. The crude tissue homogenate was centrifuged at 10,000 rpm for 15 min in cold centrifuge, and the resultant supernatant was used for estimation of biochemical analysis.

Biochemical analysis

Brain homogenates were used for the estimations of AChE activity, glycogen content, cholesterol content, and total proteins in different treatment groups.

Measurement of tissue AChE

AChE activity in brain tissue was estimated using the method of Ellman, Courtney, Andres, and Featherstone (1961). The reaction mixture was composed of 2.8 ml of phosphate buffer, 0.1 ml of Ellman's reagent, and 0.5 ml of tissue homogenate. This reaction was initiated by the addition of 0.1 ml of acetylcholine iodide and the absorbance was measured at 412 nm. Values were expressed in millimoles per gram wet tissue using an extinction coefficient of $13.6 \times 10^3 \, M^{-1} \, cm^{-1}$.

Measurement of tissue glycogen content

The amount of glycogen that was in tissue extract was assessed by the method of Montgomery (1957). The sample was mixed with distilled water followed by phenol. The mixture was shaken well, and concentrated H_2SO_4 was added and made stable at room temperature for 15 min. The absorbance was recorded at 490 nm.

Fig. 1 Division of animals between different groups
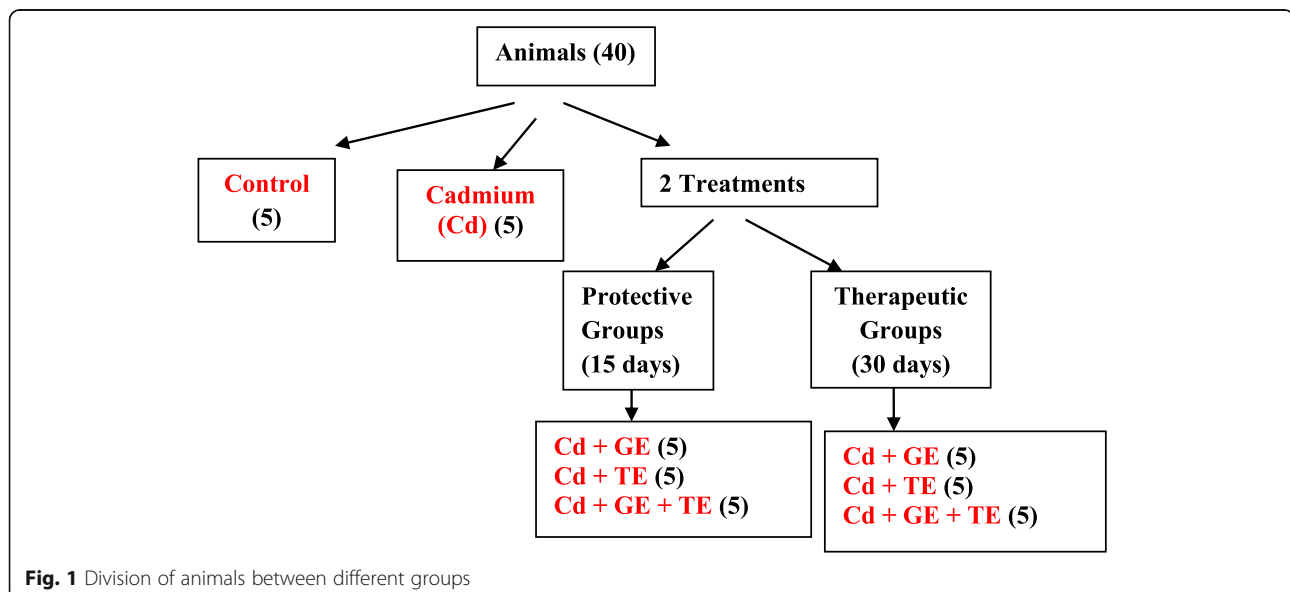
The values were expressed in milligrams per gram of wet tissue, and they were read on a graph sheet against blank.

Measurement of tissue cholesterol content

The cholesterol content in the tissues was estimated by the method of Zlatkis, Zak, and Boyle (1953). The test sample was mixed with ferric chloride-acetic acid reagent and was incubated for 2 min. The sample was centrifuged at 2000 rpm and the supernatant was mixed with 3 ml of concentrated H_2SO_4 followed by incubation for 10 min at room temperature. The optical density was measured at 560 nm, and the values of samples were read against blank. The values were expressed in milligrams per gram of wet tissue.

Measurement of total proteins in tissue

The quantitative estimation of total protein in brain tissue was done by the method of Lowry, Rosebrough, Farr, and Randall (1951). The final mixture consisted of sample and distilled water followed by reagent C (2% Na_2CO_3 and 0.5% $CuSO_4.5H_2O$ solution). This solution was incubated at room temperature and followed by the addition of reagent D (folin-phenol reagent). The intensity of the color was determined at 520 nm. The values were expressed in milligram per gram of wet tissue, and these values were read on graph against corresponding values of standard solution.

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 h. After 24 h of fixative, the tissues were washed in 70% alcohol and then dehydrated in ascending grades of ethyl alcohol (80%, 90%, 95%, and 100%), cleaned in xylene and embedded in paraffin wax (60 °C melting point) then sectioned at 5–6 μm. These sections are stretched on slides and are stained with hematoxylin and eosin staining technique (Drury & Wallington,

1980) for histopathological studies. These slides were observed under light microscopy.

Statistical analysis

The data was analyzed by using Student's t test on the Graph pad online software. All the given values were expressed as mean ± standard error (SEM). Two-way analysis of variance (ANOVA) test was used to compare the differences between the different groups and treatment days followed by Tukey's post hoc multiple range test. $p \geq 0.05$ was considered non-significant and $p \leq 0.01$ was considered as significant values.

Results

Biochemical studies

AChE

A significant decrease was observed in AChE ($p < 0.01$) activity in the brain of Cd-treated group in comparison to control group. The protective groups showed nonsignificant increase with garlic and tomato extract individually but both together showed more significant ($p < 0.0001$) increase in comparison to Cd-treated group at 15 days (Table 1). In the therapeutic study, Cd + GE group and Cd + GE + TE showed significant ($p < 0.0001$) increment, but Cd + TE showed nonsignificant increase in comparison to Cd group at 30 days post treatment (Table 2).

Glycogen content

Cd group showed a nonsignificant decline in glycogen content of brain in comparison to control mice. At 15 days, the antioxidant groups (Cd + GE and Cd + TE) showed nonsignificant elevation but Cd + GE + TE group showed significant ($p < 0.01$) increase in comparison to Cd-treated group (Table 1). The therapeutic groups showed nonsignificant increment in all the antioxidant-treated groups at 30 days as compared to control values.

Table 1 Protective effects of garlic and tomato against cadmium (Cd)-induced biochemical parameters in mice at 15 days

Groups	Autopsy days	Parameters			
		AChE (mmol/g wet tissue)	Glycogen (mg/g)	Cholesterol (mg/g)	Total proteins (mg/g)
Control	15	41.36 ± 6.63	44.31 ± 5.96	39.48 ± 8.54	14.06 ± 0.78
Cd	15	24.05 ± 2.97[o]a	33.90 ± 5.18[ns]	19.90 ± 1.84[ns]	8.82 ± 0.67*a
Cd + GE	15	56.47 ± 2.74[ns]b	35.96 ± 0.54[ns]b	35.68 ± 2.74*b	9.98 ± 0.20*b
Cd + TE	15	58.02 ± 5.76[ns]b	46.63 ± 2.50[ns]c	42.73 ± 3.18*c	11.23 ± 0.40*b
Cd + GE + TE	15	69.20 ± 5.20*c	47.02 ± 1.80[o]c	42.27 ± 2.56*c	15.82 ± 0.46*c

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
AChE acetylcholinesterase, GE garlic extract, TE tomato extract
*Significant variations at $p < 0.0001$
[o]$p < 0.01$
[ns]Non significant variations at $p > 0.05$

Table 2 Therapeutic effects of garlic and tomato against cadmium (Cd) induced biochemical parameters in mice at 30 days

Groups	Autopsy days	Parameters			
		AChE (mmol/g wet tissue)	Glycogen (mg/g)	Cholesterol (mg/g)	Total proteins (mg/g)
Control	15	41.36 ± 6.63	44.31 ± 5.96	39.48 ± 8.54	14.06 ± 0.78
Cd	15	$24.05 \pm 2.97^{o}a$	33.90 ± 5.18^{ns}	19.90 ± 1.84^{ns}	$8.82 \pm 0.67^{*}a$
Cd + GE	30	$60.22 \pm 9.14^{*}b$	58.92 ± 22.52^{ns}	28.73 ± 13.59^{ns}	7.80 ± 2.80^{ns}
Cd + TE	30	48.23 ± 13.49^{ns}	43.46 ± 4.19^{ns}	32.18 ± 13.06^{ns}	$5.91 \pm 0.56^{o}b$
Cd + GE + TE	30	$51.08 \pm 7.04^{*}c$	51.93 ± 9.71^{ns}	25.41 ± 13.98^{ns}	6.25 ± 1.56^{ns}

a vs. control; b, c vs. Cd. The mean values having dissimilar superscripts differ at $p < 0.05$ in the Turkey's Honestly significant difference
AChE acetylcholinesterase, *GE* garlic extract, *TE* tomato extract)
*Significant variations at $p < 0.0001$
$^{o}p < 0.01$
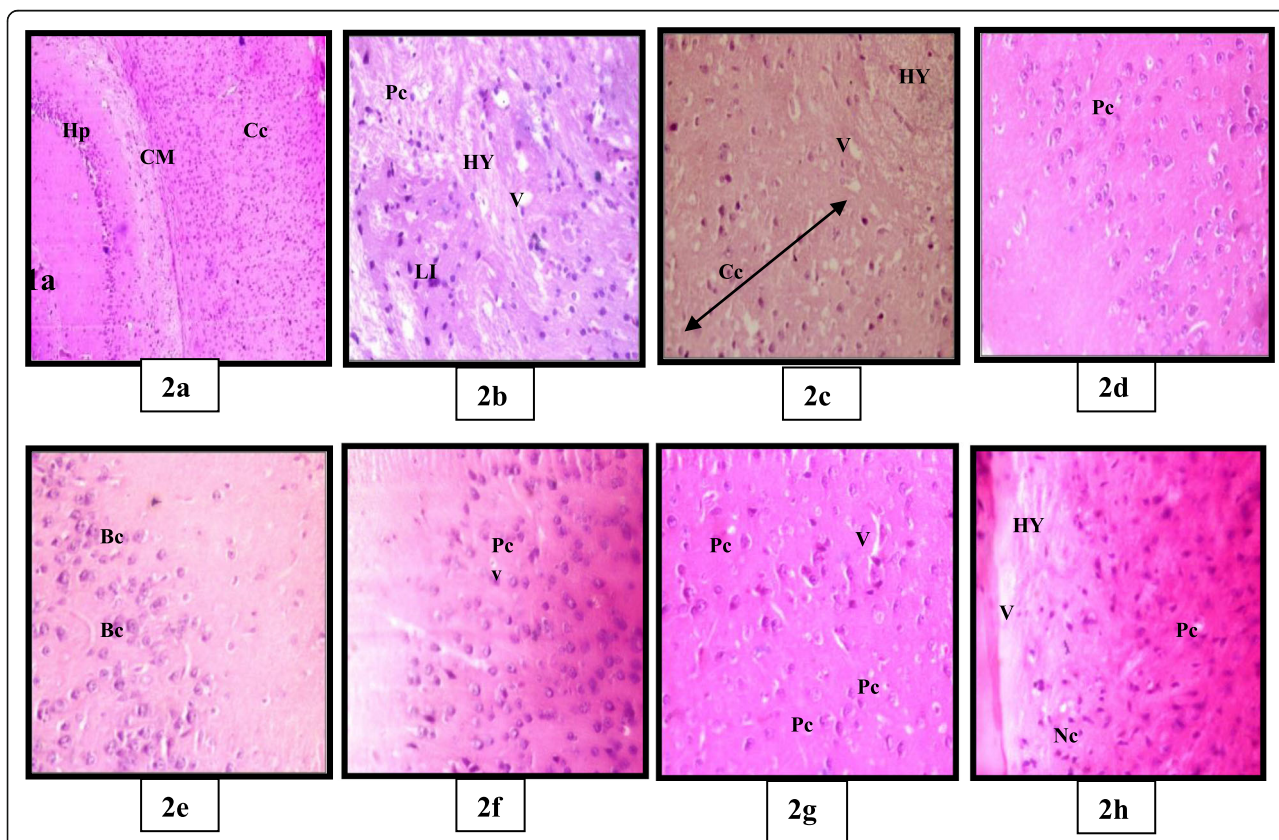^{ns}Non significant variations at $p > 0.05$

Fig. 2 Photomicrograph of cerebral cortex of mice. **a** Control group showing normal architecture of cells of cerebral cortex (Cc) with medulla (CM). × 200. **b** Cd group showing hyperemia (HY) in the cortex with vacuolation (V), lymphocytic infiltration (LI), and with the appearance of pyknotic cells (Pc). Protective Groups **c** Cd + GE group showed improvement in the nerve cells in many areas with little vacuolation (V) in the tissue. **d** Cd + TE-treated group, the cortex appeared to be normal with all the different types of cells having a compact nucleus in the neurophil. **e** Cd + GE + TE group showing normal cortex structure with pyramidal cells but some binucleated cells were also seen. Therapeutic Groups **f** Cd + GE-treated group, the pyramidal and granule cells were slightly affected with a dark-stained nucleus but mild shrinkage in the cells with a pericellular halo was visible. **g** Cd + TE group showed almost normal with pyramidal cells, granular cells, and neuroglial cells in the neurophil of the cortex with less pyknotic cells (Pc) and vacuolation (V). **h** Cd + GE + TE group, the cortex showed many normal cells with slight hyperemia of blood capillary and necrotic cells (Nc). Shrinkage was also seen in the cells with pericellular halos. × 400, H & E stain

Cholesterol content

The cholesterol level in the brain of Cd-treated mice showed nonsignificant decrement in comparison to the control group. All the protective groups, i.e., Cd + GE, Cd + TE, and Cd + GE + TE showed statistically significant ($p < 0.0001$) increase in accordance to control values after 15 days (Table 1). Table 2 showed a nonsignificant elevation in all the antioxidant groups at 30 days post treatment in comparison to toxic (Cd) group.

Total proteins

The amount of proteins in the brain was decreased significantly ($p < 0.0001$) in Cd group as compared to control values. The protective groups showed significant increment in all the treated groups, i.e., Cd + GE ($p < 0.0001$), Cd + TE ($p < 0.01$), and Cd + GE + TE ($p < 0.0001$) at 15 days post treatment (Table 1). At 30 days, the total proteins in brain tissue showed nonsignificant decrease in Cd + GE and Cd + GE + TE group but a

significant ($p < 0.01$) decrease in Cd + TE group in comparison to Cd group (Table 2).

Histological studies

Normal brain

The cerebral cortex (Cc) was characterized by the neurons associated with particular functions and unique connections that showed normal variations in the arrangements of cells in the different parts of the six layers of cerebral hemispheres (CH). The common cells inside these layers are the neurons especially pyramidal and granule cells in addition to neuroglial cells. The neurophil was a mat of neuronal and glial cell processes (Fig. 2a). The hippocampus (Hp) section shows a zone where the cortex narrows into a single layer of very densely packed neurons which curls into a tight S shape. It consists of two parts: hippocampal proper (HP) and dentate gyrus. The hippocampal proper consists of three major cornu ammonis (CA) regions, i.e., CA1, CA2, and CA3. The pyramidal neurons, the most important cells, have
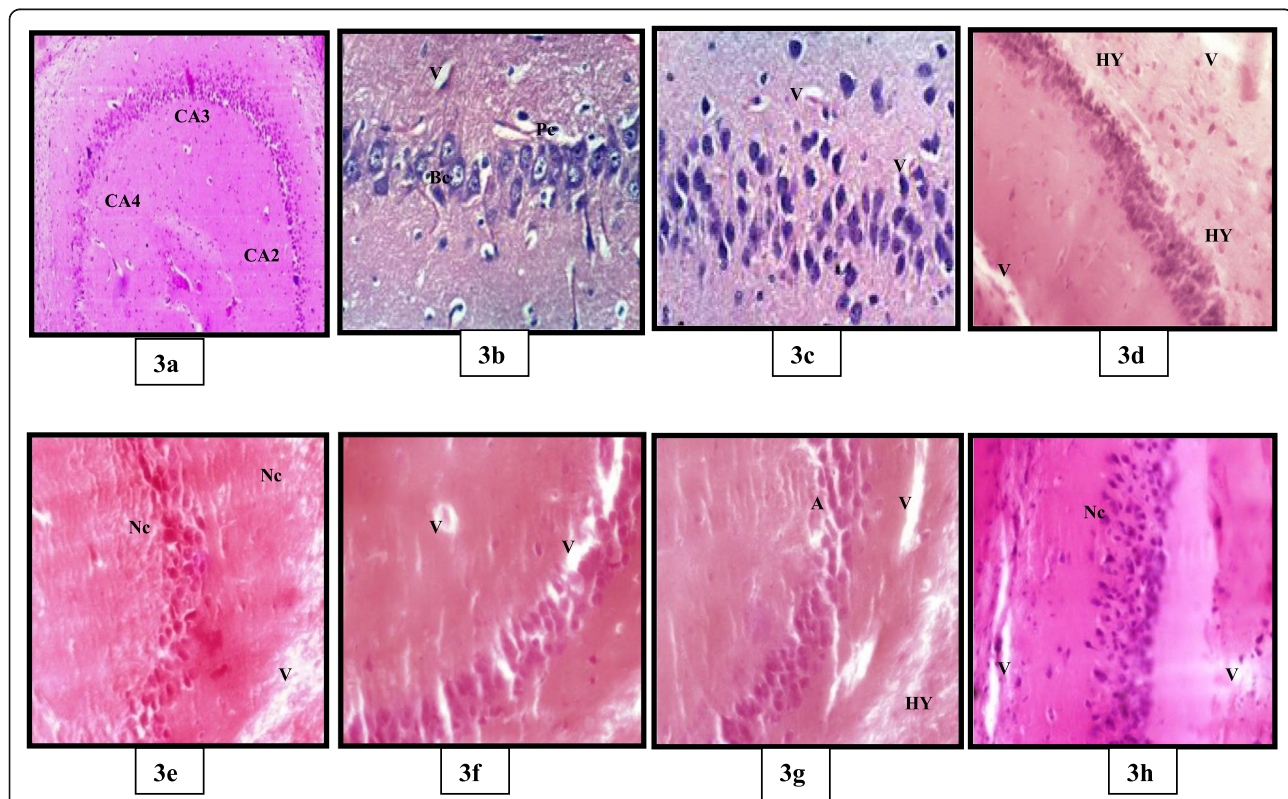

Fig. 3 Photomicrograph of the hippocampus of mice. **a** Control group showing different regions of cornu ammonis (CA) are visible, i.e., CA2, CA3, and CA4 with innermost network of fibres. × 200 **b** Cd group showing vacuolation (V), binucleated cells (Bc), and glial cells containing vesicular nucleus. Protective groups **c** Cd + GE group showing almost normal neurons with a slight distortion in the structure of pyramidal cells. **d** Cd + TE showing normal thick layer of neurons with normal structure but with slight hyperemia (HY) and vacuolation (V) in the tissue. **e** Cd + GE + TE showing slight degeneration in the neurons of the hippocampus. Mild necrosis (Nc) and vacuolation (V) is observed in the tissue. Therapeutic groups **f** Cd + GE showing vacuolation (V) and shrinkage in the neurons, slight hyperemia (HY), and distortion in the shape of the cells. **g** Cd + TE showing slight atrophy (A) in the structure of neurons. Number of neurons is reduced in the hippocampus (Hp). **h** Cd + GE + TE showing normal structure. Some necrosis (Nc) and shrinkage of the neurons in all the layers are visible. × 400, H & E stain

dendrite plexus, which arise from both apical and basal poles of the cells. The dentate gyrus (DG) is composed mainly by its granular layer which is characterized by the small granule cells that are densely packed and are arranged in U-shaped configuration (Fig. 3a).

Effects of cadmium
The tissue revealed severe multifocal histological changes in all the layers of the cortex as compared to the control group. Many vacuoles of variable sizes either of single or multiple appeared between, and inside most of the cells of all layers were seen. Some areas became more cellular especially in outer granular and pyramidal layer while others are less crowded with the cells. The tissue (neurophil and neuroglia) has vacuolated structure with hyperemia and lymphocytic infiltration in many areas (Fig. 2b). The hippocampus showed CA region with much undesirable changes in the structure. It is further associated with an area of vacuolation, pyknotic nuclei, and many numerous binucleated cells (Fig. 3b).

Effects of protective treatment
In Cd + GE-treated group, the cortex showed improvement in the nerve cells in many areas. Most of the pyramidal and granule cells were more or less as that of control with little vacuolation in the tissue (Fig. 2c). The Hp also showed numerous neurons as well as glia with normal morphology and normal pattern of distribution (Fig. 3c). In Cd + TE-treated group, the cortex appeared to be normal with all the different types of cells having a compact nucleus in the neurophil. Mild vacuolation was observed (Fig. 2d). The Hp showed thick layer of neurons with normal structure. Slight hyperemia and vacuolation was seen in the tissue (Fig. 3d). In combined-treated group, Cd + GE + TE showed normal cortex structure with pyramidal cells but some binucleated cells were also seen (Fig. 2e). Even the hippocampus showed little degeneration in the neurons of hippocampal proper with necrosis and vacuolation in the tissue (Fig. 3e).

Effects of therapeutic treatment
In Cd + GE-treated group, the pyramidal and granule cells were slightly affected with a dark-stained nucleus. Mild shrinkage in the cells with pericellular halos was visible (Fig. 2f). The Hp tissue showed vacuolation and shrinkage in between the neurons along with slight hyperemia with a little distortion in the structure of neurons (Fig. 3f). The tomato extract-treated group, Cd + TE, showed almost normal pyramidal cells, granular cells, and neuroglial cells in the neurophil of the cortex (Fig. 2g). The Hp area also showed slight atrophy in the structure of neurons, but less number of neurons were observed in the hippocampal proper (Fig. 3g). In the combined-treated group, Cd + GE + TE, the cortex showed many normal cells with slight hyperemia of blood capillary and apoptotic cells. Shrinkage was also seen in the cells with pericellular halos (Fig. 2h). The hippocampal neurons showed heterogeneous morphology. Some areas were predominated with pyramidal cells and some areas with glial cells in the neurophil (Fig. 3h).

Discussion
Cadmium is a known heavy metal with considerable toxicity in humans and experimental animals after acute or chronic exposure (Bernhoft, 2013). It has the ability to induce severe alterations in various organs and tissues including the nervous system (Manca, Ricard, Trottier, & Chevalier, 1991). Cadmium administration decreased the body weight of mice and brain weight in comparison to control. In the present study, a significant decrease in brain AChE activity was observed in Cd group. AChE is a key enzyme in the brain to detect the neurotoxic effect of certain heavy metals Hao, Pan, Zhang, and Wang (2015, 2015). Numerous studies have suggested that the free radical production could at least partly be associated with the decreased activity of brain AChE (Tsakiris, Angelogianni, Schulpis, & Starridis, 2000). The inhibited AChE activity in the brain is in confirmation with the observations of El-Demerdash, Yousef, and Elagamy (2001). Inactivation of AChE enzymes as a result of the occupation of its active sites by heavy metals has been suggested by Shaw and Panigrahi (1990). Other workers also reported a decrease in the AChE level in the brain after Cd intoxication (Abu-Taweel, 2016; Hao et al., 2015, 2015; Maodaa et al., 2016; Shi et al., 2019).

The impairment in the cholinergic brain synapses and neuromuscular junctions may also alter the AChE levels in the brain (Mushtaq et al., 2018). Cd inhibited the K^+-evoked release of ACh, dopamine, serotonin, GABA, and glutamate from rat brain slices (Harvey, Wedley, Findlay, Sidell, & Pullar, 1996). Moreover, a decreased function of dopaminergic and serotonergic system of certain brain areas of rats has been reported after Cd exposure to 21 days (Carageorgiou et al., 2000). AChE was inhibited by high Cd concentration in vitro, and Cd^{2+} is one of the metal inactivators of this enzyme and is capable of inducing a conformational change in the protein, which leads to the formation of an "unreactive" enzyme (Ma, Zhang, & Jiang, 2017). This decreased AChE may also be associated with the risk that cadmium has the ability to cross the blood brain barrier and get concentrated in the brain which induced peroxidation as well as oxidative stress (Alnahdi & Sharaf, 2019) leading to the production of free radicals (Pervin et al., 2014). Our results are in accordance with previous investigations (Antonio,

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Corredor, & Leret, 2003; Eck & Wilson, 1989; Gupta, Gupta, Murthy, & Chandra, 1993) in rats.

Glycogen is an imperative source of energy to the brain, and blood glucose is requisite for normal human life (Chen & Weinstein, 2016). Glycogen content was found to be reduced nonsignificantly in Cd-treated brain. These variations in the glycogen level may be due to increased breakdown of biomolecules to meet the energy requirement of the animals under stress or their reduced synthesis due to impaired tissue function (Ivanova-Chemishanska, 1982). Bhushan, Saxena, and Saxena (2013) stated that high amount of catecholamine production decreases the glycogen reserves. The decreased feeding and the elevated levels of stress hormones, cortisol and adrenaline, may also result in the loss of glycogen content (Heath, 1995).

The total cholesterol in the brain was decreased non significantly in Cd-treated group. Wassermann, Wassermann, and Aronovaski (1970) suggested that the decrease in cholesterol level may be related to its enhanced utilization in corticosteroidogenesis and/or its de novo synthesis. Involvement of thyroid hormones has been suggested in cholesterol metabolism and an enhanced breakdown in hyperthyroidism is known to result in hypocholesterolemia (Yadav, Jindal, & Goyal, 2005).

Cholesterol is a vital component of all the membranes that surround the human cells, and the brain is the most cholesterol-rich organ (Björkhem, Meaney, & Fogelman, 2004) as it contains 20% of the total body's cholesterol. The decline in cholesterol level may result in synaptic and dendrite degeneration, failed neurotransmission, and decreased synaptic plasticity (Koudinov & Koudinova, 2005).This decrease in the cholesterol content in the present study may be because of inhibition of lecithin cholesterol acyltransferase activity in Cd-induced mice (Newairy, El-Sharaky, Badreldeen, Eweda, & Sheweita, 2007). This decline in cholesterol can also be correlated with the impaired developmental gene hedgehog and organization of cell membranes during the developmental processes of various tissues and cells (Roux et al., 2000). Martin, Dotti, and Ledesma (2010) specified that cholesterol biosynthesis as well as its degradation are active processes in the adult brain, and any variation in these mechanisms will further influence the higher order brain functions.

The total protein content in the present study was significantly decremented ($p < 0.0001$) in the brain of Cd-treated group. These results are in agreement with the observations of other workers (Babaknejad, Moshtaghie, Nayeri, Hani, & Bahrami, 2016; El-Demerdash, Yousef, Kedwany, & Baghdadi, 2004). Christopher (1991) stated that this decrease in total proteins may be due to the deleterious effects of Cd which resulted in increased excretion of high molecular weight proteins. Heavy metals

also resulted in the death of ribosomes by acting on its membranes and finally causing cellular death, so it may decline the synthesis of proteins (Yadav et al., 2005). Cd has direct toxic effect on brain as it enters the brain parenchyma and neurons that may lead to neuronal alterations (Yuan et al., 2018) by decreasing proteins synthesis. Cd critically affects the function of nervous system by promoting neuronal apoptosis (Lopez, Figueroa, Oset-Gasque, & Gonzalez, 2003). Jan et al. (2015) reported that even the protein structure is also greatly affected by the heavy metals as they displace the physiological metals bound to the protein ligands causing disruption in the cell physiology by changing their steric arrangements. Swamy, Ravikumar, and Murali Mohan (1992) suggested that the decrease in total proteins and soluble proteins indicates their metabolic utilization. They also associated the increase in proteases with decrease of soluble and total proteins.

Cd administration induced histological changes in all the layers of the cortex and hippocampus which involved different types of cells especially pyramidal and granule cells along with neuroglia. Cd caused neuropathological and neurochemical alterations in the brain which further results in encephalopathy, peripheral neuropathy, and even hemorrhages (Afifi & Embaby, 2016). Cd also affects the brain parenchyma and other neurons which causes hypernociception, olfactory dysfunction, and mental deficits (Wang et al., 2013). These findings are in accordance with the results of Jadhav, Sarkar, Patil, and Tripathi (2007); Ojo, Oyinloye, Ajiboye, and Onikanni (2014); Allam, Maodaa, Abo-Eleneen, and Ajarem (2016); and Maodaa et al. (2016).

The brain tissue showed the appearance of dark neurons which may be due to the condensation of cytoplasm and neucleoplasm as a result of apoptosis (Ratan, Murphy, & Baraban, 1994) and it was further stated that these neurons are ischemic due to the abnormalities in the capillary wall with the adjacent disorders related to the structural components of blood brain barrier (Sobaniec-Lotowska, 2001). The pyramidal cells in the present work were observed to be irregular due to loss of shape that corresponds with the cytoskeletal disorganization (Kumar, Abbas, & Fausto, 2008).

The vacuolation in the surrounding neurophil might be attributed to the shrinkage of cells and withdrawal of their processes by leaving pericellular spaces (Sobaniec-Lotowska, 2001). The enlarged processes of astrocytes in the neuroglia may be the result of lipid peroxidation theory and an increase in the sodium permeability resulting in sodium accumulation in the cell followed by the swelling of the cell due to an increase in water content inside the cell (Panickar & Norenberg, 2005). The necrotic as well as degenerative changes of the brain may be attributed to the high susceptibility of mice to Cd

toxicity, and it is considered as important indicator for neurotoxic effects of Cd due to oxidative injury (El-Sokkary & Awadalla, 2011). Williams (1995) confirmed that the oxidative stress is one of the mechanisms that contribute to these structural changes and plays an important role in neuro-degeneration. Further, Kumar, Asic, Agarwal, and Seth (1996) also observed the biomembrane changes in different regions of brain due to Cd toxicity.

GE-treated group showed increase in the AChE activity and other biochemical parameters. The effectiveness of garlic compound, i.e., diallyltetrasulfide on the AChE activity and other antioxidant enzymes in the brain of rats has been well studied by Pari and Murugavel (2007) against Cd-intoxicated rats. Even cortex and hippocampus of AGE-treated groups showed much more amelioration in the protective study in comparison to the therapeutic study against Cd-induced toxicity. The structures were reverted to normal but with some amount of degeneration in the form of vacuolation and shrinkage in the neurons. Zeng et al. (2017) reviewed that the functional properties of garlic are due to allicin, S-propargyl-cysteine, and diallyl trisulfide. Panyod et al. (2016) stressed that allicin showed therapeutic activities by reducing oxidative stress, inflammation, vascular dysfunction, and aortic pathology.

TE as a dietary supplementation showed remarkable protective effects in all the biochemical parameters and restored the general structure of the tissue. These results showed that carotenoid (lycopene) is a highly efficient scavenger of singlet-oxygen ($^1O_2$) and other excited species. During $^1O_2$ quenching, energy is transferred from $^1O_2$ to the lycopene molecule, converting it to the energy-rich triplet state and thus preventing their damage (Atessahin, Yilmaz, Karahan, Ceribasi, & Karaoglu, 2005). Rao and Agarwal (1998) observed that the dietary supplementation of lycopene from traditional tomato products increased lycopene concentration in plasma by reducing oxidative damage to lipids and proteins.

AGE + ATE administration singly or in combination showed significant increment in all the parameters in both protective as well as therapeutic groups as compared to toxicant groups. Garlic, due to the presence of allicin, may participate in the chelation of heavy metals (Chowdhury et al., 2008). Tomato extract has been proven effective and useful in counteracting and ameliorating some of the biomarkers, oxidative stress parameters, and tissue injury against acetaminophen-induced acute toxicity (Jamshidzadeh, Baghban, Azarpira, Bardbori, & Niknahad, 2008). This may be due to the presence of certain metal chelating proteins and phytochelatins (Tito et al., 2011). This protective effect may also be correlated with the presence of lycopene which acts as a potent antioxidant and aids in chemoprevention of cancer in various tissues (Palozza, Simone, Catalano, & Mele, 2011).

Conclusions
From the present biochemical and histological data, it can be concluded that cadmium intoxication resulted in severe toxic effects in the brain of albino mice. Garlic and tomato supplementation counteracted this toxicity to some extent. Moreover, the synergistic action of garlic and tomato provided even more satisfactory and encouraging results. Thus, it is suggested and recommended that regular intake of garlic and tomato in diet may play a beneficial role in reducing the toxic effects of the heavy metals in human beings.

Abbreviations
AChE: Acetylcholinesterase; A: Atrophy; Bc: Binucleated cells; Cc: Cerebral cortex; Cd: Cadmium; CH: Cerebral hemispheres; CM: Cerebral medulla; DG: Dentate gyrus; GE: Garlic extract; HP: Hippocampal proper; Hp: Hippocampus; HY: Hyperemia; LI: Lymphocytic infiltration; Nc: Necrotic cells; Pc: Pyknotic cells; TE: Tomato extract; V: Vacuolation

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