International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 85-95
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Neuroprotective and Ameliorative Effect of Caffeic Acid On Antioxidant and Acetylcholine Status of Mercury Intoxicated Rats

Manogaran Manju and Ganesan Jagadeesan*

Department of Zoology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

Received: 10 Jan 2019 / Accepted: 10 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: jaga_zoo@yahoo.co.in

Abstract

Mercury (Hg), a well-known environmental and industrial hazard, exerts a number of toxic effects in humans and animals. And also it acts as a neurotoxin. In the present experimental study, the antioxidant and acetylcholine property of mercury intoxicated brain tissue of rat was investigated. And withdrawal effects of mercury toxicity in the brain tissue with the help of caffeic acid treatments were also investigated. Caffeic acid at a concentration of 5 mg/kg body weight, accelerate the oxidation of neurotoxicity induced by HgCl2 (1.29mg/kg Body weight of the animal). The aim of this study was to investigate the protective potential of Caffeic acid against Hgcl2 induced brain damage. The antioxidant activities of caffeic acid concentration were mainly due to the scavenging of lipid peroxide in this system. The oxidant mechanism for caffeic acid is most likely due to the strong reducing power and weak metal chelating ability. During the Hgcl2 treatment, the mercury toxicants mainly induced the toxicity in the brain tissues or rats to exhibit elevated level of lipid peroxidation (LOP). At the same time the level of enzymic antioxidants Superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPx), AchE activity and non-enzymic antioxidants reduced glutathion (GSH) were significantly decreased in brian tissues. But during the recovery period (Caffeic acid treatment on mercury intoxicated animal), are enhances the not only the GSH levels, and also promote the AchE activities. It leads to protects cell damage against neurotoxicity induced by mercuric chloride. Histological and histopathogical observation are also supporting these findings by the way of restoration of brain level or nearl normal level. Thus, result of the present study caffeic acid exhibited potent antioxidant, neuroprotective activities on HgCl2 induced neurotoxicity in rats.

Keywords
Mercuric chloride, Caffeic acid, Antioxidant, Neurotoxicity.

*****

INTRODUCTION

Mercury toxicity always promoting continuous production of ROS and Oxidative stress in CNS leads to altering their cellular functions and structure of different organs [1, 2, 3] also altering the bio-enzymological properties in the tissues of both animals and humans [4]. Continuous production of free radicals and oxidative stress is one of the main mechanisms of tissue damage in animals due to the heavy metal toxicity [5,6]. The exposure of mercury and its compounds have been found to be associated with different organ injury and malfunctioning of various metabolic activities [7, 8]. Normally oxidative stress promotes reactive oxygen species (ROS) and overproduction of antioxidants [9]. Production of oxidative stress is considered a hallmark in mercury-induced organ injury [10]. The causative factors of mercury and its compounds on tissue damage mechanisms are not clearly established. Most of research workers confirmed that the mercury and its compounds are promoting the oxidative stress in intoxicated tissues leads to cellular damages, because they are transition metal and it can promote the formation of ROS such as hydrogen peroxides [11]. During normal condition, a cell can maintain the balance between ROS and antioxidant defence mechanism at optimum level, but an exposure of certain chemicals can promote ROS in enormous level and simultaneously inhibit the antioxidant defence mechanisms naturally [12, 13] leading to increased lipid peroxidation, protein degradation, and finally cell death [14]. Mercury and its compounds readily binds to protein molecules which is responsible for promoting detoxification mechanisms against free radical induced cell damages [15]. Most of researcher's have explained that the exposure of heavy metals toxicity stimulates the production of ROS and the induction of oxidative stress in different organs [16]. The production of free radicals causes destruction of proteins and DNA results in structural alterations and metabolic disorders [17, 18]. Naturally, formation of free-radical process causes oxidative deterioration of lipids leads to promote the production of LPO content and it also used as an indicator of oxidative stress. Promoting the imbalance between the production of reactive oxygen species (ROS) and free radicals (FR) in the organs due to the accumulation of LPO content caused the pathogenesis of different type of diseases, damages and defects [19, 20, 21]. The accumulation of LPO content in the organs get deleterious effects which results in the production of the following products such as aldehydes, inter alia, and malondialdehyde (MDA) and leads to cellular membrane damages due to oxidative destruction of polyunsaturated fatty acids [22, 23]. Caffeic acid (CA) is one of the non-flavanoid catecholic compounds (the most common phenolic acids). It is easily available in several species of plants and it also occurs in vegetables and part of fruits, tea, coffee and wine [24] and regularly consumed in human diet [25, 26]. In the past 3 decades the understanding Caffeic acid and its derivatives protective role have been clearly demonstrated by number of experimental works showing that these chemicals have a variety of functions including acting as antioxidant and suppressing lipid peroxidation [27, 28, 29]. CA always called as treating agent because it possesses catechol group and double bond in its side chain. CA would act as a treating agent because of it possess antioxidant capacity [30], free radical scavenging capacity [31] and chelation therapy against the heavy metal ions [32]. In addition, CA also carried out number of pharmacological activities such as inhibition of enzyme activity, antitumor activity and anti-inflammatory effect and inhibition of HIV replication [33]. With this point of view, the present experimental work has been carried out to assess the potential efficacy of CA on mercuric chloride induced oxidative damages in the brain tissue of rats.

MATERIALS AND METHODS

Chemicals

In the present experimental work, Mercuric chloride ($HgCl_2$), Caffeic acid and all other necessary reagents of analytical grade were used and it was purchased from Hi-Media laboratories Ltd, Mumbai, India.

Animals

Healthy male albino rats, *Rattus norvigicus* (180–200 g) were obtained from the Central Animal House, Department of Experimental Medicine, Raja Muthiah Medical College and Hospital Annamalai University and maintained in an air condition room (25±3 ◦C) with a 12-h light/12-h dark cycle. Feed, water and ad libitum were provided to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No: 160/1999/CPCSEA, proposal Number: 1156), Annamalai University, Annamalainagar.

Experimental design

Totally 24 animals were acclimatized in the animal cages for 15 days. The animals were randomized and divided into four groups, each group containing six rats. The toxic dosage of mercuric chloride has been obtained from our previous study as sufficient to

elicit mild or moderate oxidative stress for mercuric chloride.

Group I: Untreated control— the animals were received standard diet and clean water *ad labitum* and observed for 15 days.

Group II: Mercury treatment—the animals were received 1.29mg of mercuric chloride/kg body weight orally every day up to 15 days.

Group III: Mercuric chloride followed Caffeic acid treatment— the animals were received Mercuric chloride followed by Caffeic acid (1.29 mg $HgCl_2$/kg body weight of animal for 15 days followed by Caffeic acid -5.mg/kg body wt. of animal 15 days administrated) treatment group.

Group IV: Caffeic acid alone treatment—the animals were received Caffeic acid (5mg/kg body weight of animal for 15 days administrated) alone treated.

At the end of the experimental duration, rats were sacrificed by cervical dislocation. The whole brain tissues were isolated immediately from the animals and kept in an ice-cold saline and then used for estimation of oxidant and antioxidant properties studies

Estimation of lipid peroxidation (TBARS)

The concentration of LPO/TBARS in the selected tissues was determined by adopting the method of Nichens and Samuelson [34]. The known weight of selected organs (Brain) was isolated from the animal in a cold room. The isolated organs were immediately homogenized in Tris - HCL buffer (pH 7.5) for 5 minutes. From the homogenized solution, 1 ml of the organ (Brain) homogenate was taken in a clean dry test tube and then 2.0 ml of TBA-TCA-HCL reagent was added to this content. The contents were mixed thoroughly with lateral shaking of the test tubes. The mixture content was kept in an incubation chamber (boiling water bath (60°C) for 15 minutes and then the organ mixture content was cooling with the help of running tap water. After cooling, the mixture was read the absorbance of the chromospheres at 535 nm against the reagent blank in an UV visible spectrophotometer (Spectronic -20, Bausch and Lamb). 1, 1', 3, 3' tetra methoxy propane was used to construct the standard graph. The values were expressed as n moles of MDA released / 100mg.

Estimation reduced glutathione (GSH) activity

The level of GSH in Brain tissue was determined by the method of Beutler and Kelley [35]. The isolated organs (Brain) were homogenized in 5 ml of PBS buffer with the help of morter and pistle. After completing the homogenization, the contents were centrifuged at 2500 rpm for 5 minutes. After completing the centrifugation, 2 ml of clear supernatant was taken in a clean dry test tube and then mixed with 1.8ml of EDTA solution. To this content, 3.0ml of precipitating reagent was added and mixed thoroughly and then kept in the room temperature for 5minutes before centrifugation. The mixed content was centrifuged at 3000 rpm for 5minutes. After centrifugation 2.0ml of the supernatant was taken in a clean test tube and then adding the following reagents. 4.0ml of 0.3M Na_2HPO_4 solutions and 1.0ml of DTNB reagent were added to the supernatant the color developed was read at 412nm in an UV spectrophotometer. A set of standard solutions containing 20-100µg of GSH was treated similarly. The values were expressed as µg/100mg protein for tissues.

Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase in the Brain tissue was assayed by adopting the method of Kakkar [36]. The known weight of selected organs (Brain) were isolated from the animal immediately and then homogenized in 2 ml of 0.25M sucrose solution and then centrifuged at 10,000 rpm for 30 minutes in cold condition. After completing the centrifugation, the supernatant was taken in a test tube and it was dialyzed with Tris-HCL buffer (0.0025M, PH 7.4) and it was used for enzyme assay. In a clean test tube 1.2 ml of dialyzed supernatant was taken and 1.2 ml of $Na_4P_2O_7$ buffer, 0.1 ml of PMS, and 0.3ml of NBT reagents were added. The mixture contents were kept in a water bath at 30ºC for 90 seconds for the purpose of incubation. After completing the incubation, the contents were made up to 3.0 ml by adding glass distilled water and then 0.2 ml of NADH reagents was added for initiating the reaction. The reaction was stopped by the addition of 1.0 ml of glacial acetic acid and then added with 4.0 ml of n-butanol. The reaction mixture was stirred vigorously and shaken well. The mixture was allowed to stand for 10 minutes, and then centrifuged for 15 minutes at 3000 rpm. After centrifugation the butanol layer was separated and the colour intensity of the chromogen was measured at 560 nm in UV spectrophotometer against butanol as blank and the system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme reaction which generates 50% inhibition of NBT reduction in one minute under the assay condition and is expressed as specific activity in unit's / mg protein. The specific activity of the enzyme was expressed as unit/min/mg of protein for tissues.

Estimation of catalase (CAT) activity

The activity of catalase in the Brain tissue was determined by the method of Sinha [37]. The isolated known weight selected organs (Brain) were homogenized in 2.0 ml of PBS solution. After

completing the homogenization, the contents were centrifuged for 10 minutes at 2000 rpm. After centrifugation, 0.1ml organ sample (tissue homogenate) was taken in a clean dry test tube and the 0.9 ml of PBS and 0.4 ml of H_2O_2 were added in contents and kept in room temperature for 30 to 60 seconds and then 2ml of dichromate acetic acid mixture was added in the contents and then kept in an incubation chamber at 37°C for 10minutes. After completing the incubation period, the contents were allowed to cool in the room temperature. The colour developed was read at 620 nm in an UV spectrophotometer. H_2O_2 was used to construct the standard graph. The values are expressed as µ moles H_2O_2 consumed /min/mg protein.

Estimation of glutathione peroxidase (GPx) activity

The activity of GPX in the Brain tissue was measured by the method of Rotruck [38]. The known weight of selected organs (Brain) were homogenized in 2.0 ml of PBS and then centrifuged at 2,500 rpm for 5 minutes. After completing the centrifugation, 0.2 ml of clear supernatant was taken in a clean test tube and then the following enzyme mixture was added. The enzyme mixture contained 0.2 ml of 0.4 mM of EDTA and 0.1 ml 10 mM of sodium azide. The reaction mixture was thoroughly mixed well and kept for two minutes at 37°C in an incubator. After completing the incubation period, 0.2 ml of reduced glutathione and 0.1 ml of H_2O_2 were again added to the above mixture and then again incubated at 37°C exactly for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. The colour was developed and then read at 412 nm in an UV spectrophotometer. Reduced glutathione was used to construct the standard graph. The values are expressed as µ moles of GSH utilized / min / mg protein tissues.

Histology and histopathological study

For the qualitative analysis of Brain tissue histoarchitexture, the Brain tissue sample was fixed in 10% buffered formaldehyde for 48h and dehydrated by passing successfully in different concentrations of ethyl alcohol and cleaned in xylene and embedded in paraffin. Sections of tissue (5-6µm think) was prepared by using a rotary microtome followed by rehydration and then stained with heamatoxylin and eosin dye (H & E), which was mounted in DPX medium for microscopic observations

Statistical analysis

Values are given as mean ± S.D. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of T-test and the group means was compared by Duncan's multiple range test (DMRT) (Duncan) [39] Values were considered statistically significant when $p < 0.05$ and the values sharing a common superscript did not differ significantly

RESULTS

Effect of Caffeic acid on LOP, GSH and GPx in the brain tissue of $HgCl_2$-Neurotoxicity in control and different treated groups

(Values expressed as µg/gm wet weight of tissue)

Groups	LOP	GSH	GPx
Control	0.942±0.01	45.26±2.04	12.037±0.24
$HgCl_2$	2.151±0.01	28.58±6.58	6.743±0.162
$HgCl_2$+Caffeic acid	1.803±0.02	38.42±9.38	10.083±0.38
Caffeic acid	1.246±0.02	48.74±8.61	11.513±0.54

Each value is mean ± SD for 6 rats in each group. The data for various biochemical parameters were analyzed using analysis of 't' test and the group means was compared by Duncan's multiple range test. Values were considered statistically significant at P < 0.05.

Effect of Caffeic acid on SOD and CAT in the brain tissue of $HgCl_2$-Neurotoxicity in control and different treated groups

(Values expressed as µg/gm wet weight of tissue)

Groups	SOD	CAT
Control	4.322±0.25	38.28±2.86

$HgCl_2$	2.332±0.132	21.42±2.14
$HgCl_2$+Caffeic acid	3.681±0.123	32.26±1.26
Caffeic acid	4.827±0.013	40.54±2.17

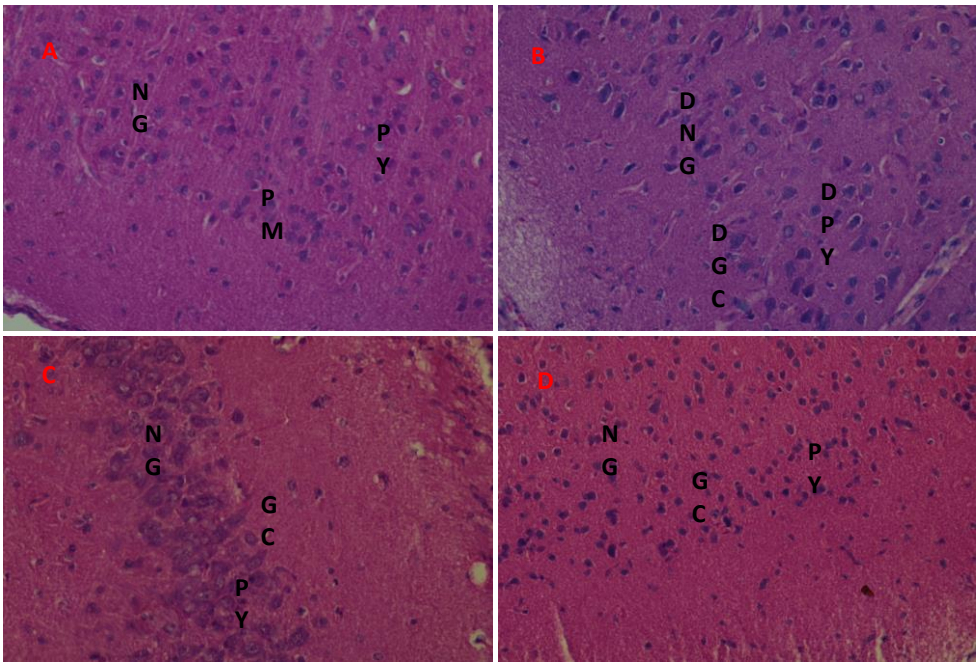
Each value is mean ± SD for 6 rats in each group. The data for various biochemical parameters were analyzed using analysis of 't' test and the group means was compared by Duncan's multiple range test. Values were considered statistically significant at P < 0.05.

Effect of Caffeic acid on Acetyl cholinesterase (AChE) in the brain tissue of $HgCl_2$-Neurotoxicity in control and different treated groups

Groups	AChE
Control	8.437±0.03
$HgCl_2$	4.342±0.15
$HgCl_2$+Caffeic acid	7.031±0.32
Caffeic acid	8.231±0.01

Each value is mean ± SD for 6 rats in each group. The data for various biochemical parameters were analyzed using analysis of 't' test and the group means was compared by Duncan's multiple range test. Values were considered statistically significant at P < 0.05.

Histological and histopathological observation in the brain tissue



A. Control B. Mercury Chloride Treated C. Mercury Chloride + Caffeic acid D. Caffeic acid Alone

Normal untreated **brain** tissue shows the complete histo-architecture of cerebral cortex region, nuclei of neuroglial cell, small blood vessels and Particularly pyramidal cells. (A)

In the present experimental works, the mercury intoxicated brain tissue causes deterioration of neuroglial cells. Neuroglial cells swollen and underlying blood spaces, shape and size of the neuroglial cells uniform all regions, increase in number of vacuolated spaces in the matrix disintegration or decrease of polymorphic cell nuclei (B)

During the recovery period, Caffeic acid treatment on mercury intoxicated rat brain tissue shows that the damages of the numerous neuroglial cells regenerated in the cortex region. Shape and size of

cells increased and inner layer of the cerebral cortex shows larger polymorphic neuronal and sharply demarked smaller neuroglial nuclei. The polymorphic nucleus is surrounded by clear space. (C)
Caffeic acid alone shows a remarkable size of neuroglial cells and to be thick in condition. The complete normal untreated histo-architecture of the brain is maintained (D)

Level of LPO contents in the brains tissue of rats

Table 1 shows the levels of LPO content in the brain tissue of rat when treated with HgCl2-and followed by Caffeic acid, Caffeic acid treated alone rats. Our results indicate that the administrations of Caffeic acid on mercury intoxicated animals promote the healthy conditions. During the mercuric chloride treatment, the level of LPO content was significantly increased. During the recovery period, administration of Caffeic acid on mercury intoxicated brain tissue shows decreased level of LPO content. Treatment with Caffeic acid significantly decreased the levels of LPO.

Level of non-enzymatic antioxidants in the brain tissue of rats

Table 1 shows the levels of reduced glutathione (GSH) content in the brain tissue of rat when treated with HgCl2-and followed by Caffeic acid treated alone rats. At sub-lethal dose of mercuric chloride treatment, the level of GSH content in the brain tissue was significantly decreased. During the recovery period, administration of Caffeic acid on mercury intoxicated brain tissue shows that the decreased level of GSH content was increased. This result suggests that the animal get recovery from the toxicity effect of mercury with the help of Caffeic acid administration. Treatment with Caffeic acid alone on control rat also shows significantly elevated levels of GSH content in the brain tissue.

Level of enzymatic antioxidants in the brain tissue of rats

Table 1and 2 show the levels of enzymatic antioxidants, the activities of SOD, CAT and GPx in the tissues are given in respectively. At sub-lethal dose of mercuric chloride treated brain tissue shows the lowered the levels of SOD, CAT and GPx activities. During the recovery period, administration of Caffeic acid on mercury intoxicated brain tissue shows that the decreased level of SOD, CAT and GPx activities. This result also suggests that the animal get recovery from the toxicity effect of mercury with the help of Caffeic acid administration. Treatment with Caffeic acid alone on control rat also shows significantly increased in levels of SOD, CAT and GPx activities in the brain tissue.

Level of AChE activity in the brain tissue of rats

Table 3 shows the levels of AChE activities in the brain tissue of control and treated rats. In the present experiments, the level of AChE activity was significantly decreased in the brain tissue of rat when treated with HgCl2. During the recovery period, the administration of Caffeic acid on HgCl2 intoxicated rats brain tissue shows the remarkable increase in the levels of AChE activity.

DISCUSSION

Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules, including free radicals and it include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, causing the cellular damage. ROS are generated by a number of pathways [40]. Continuous synthesis of ROS promotes major alterations in sub cellular structural molecules such as proteins and DNA, lipid per oxidation (LPO) of polyunsaturated fatty acids and changes of cellular antioxidant system. In tissue level the measurement of Malondi-aldehyde (MDA) is a major indicator of LPO, because it is the end product of LPO [41, 42]. In general, under the normal physiological condition of animal's low level of lipid peroxides occurred in all the tissues [43, 44]. The formation of lipid per oxidation mainly affect the protein molecules which is present in the phosphor lipid bi layers of the plasma membrane to cause number of deleterious effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage [45]. Different type of ROS species (•OH, O2-, RO, ROO, and NO) play a vital role in promotion of chemical-induced cellular damage in tissues and induce oxidative stress to cause neurodegenerative disorders [46]. The brain cell damages are mediated by oxygen derived free radicals by altering the membranes through peroxidation of polyunsaturated fatty acids [47, 48]. The formation of oxidative stress is a serious imbalance between the synthesis of free radicals and anti-oxidant properties [49]. Present experimental study also confirms that an enhanced level of LPO content and simultaneously decreased level of anti-oxidant profiles also noticed in brain tissues of rat when treated with mercuric chloride. Naturally, both types of enzymatic and non-enzymatic anti-oxidants are having high potential to scavenge reactive oxygen species (ROS) [50, 51, 52, 53] had also observed an increased formation of ROS with the administration

of mercuric chloride in animals which could lead to promote the lipid peroxidation and causes oxidative stress. Formation of lipid peroxidation (free-radical system) has been suggested to be closely related with mercury-induced tissue damage [54, 55]. Most of the experimental works suggested that an administration of heavy metals might cause oxidative stress and stimulate ROS [56, 57]. The formation of ROS might react with cellular biomolecules result in promotion of LPO content and leads to membrane damage [58]. The promotion of LPO content induces alteration in the structure and function of cellular membrane which could lead to cell injury in the target organs. An enhanced level of LPO content suggested an increase in the level of lipid peroxidation promoted the brain tissue injury and failure of the antioxidant defense mechanism to suppress the formation of excess free radicals [59]. It is well established fact that the brain tissues are more sensitive to heavy metal toxicity because it is made up of high concentration of unsaturated lipids and high rate of oxidative metabolism leads to oxidative damage occurred [60]. This is also supported by our findings of the formation of increased level of MDA in the brain tissue of mercury-treated animals [61, 62]. The exact mechanism of mercury intoxication has been linked to the formation of ROS and oxidative stress and simultaneously depletion of glutathione and thiols, all of which cause to promote neurotoxicity in animals [63]. GSH play a vital role to neutralize ROS through the glutathione-dependent antioxidant enzymes and it act as co-factor [64]. Promotion of antioxidant enzymes is one of the important factor of defense mechanism against oxidative stress in animals [65, 66]. During the stress condition, the first line of defense mechanism consisting of the following antioxidant enzymes SOD, CAT, and GPx, should be promoted against oxidative stress which converts superoxide free radicals into hydrogen peroxide and then into water and molecular oxygen [67]. Glutathione peroxidase (GPx) is one of important hydro peroxidase enzyme that is responsible for preventing and protecting the organs from the cellular damages caused by the formation of free radicals like hydrogen and lipid peroxides. It also detoxifies the peroxides in cells through the conversion of H2O2 to water in the presence of glutathione as hydrogen donor [68,69]. Normally, the most of the neuro-toxicants exert their toxic effects by the way of inhibiting the AChE activity leads to convulsions [70, 71]. The disturbance and fluctuations in the activity of different enzymes can explain some neurotoxicological characteristics of mercury and its compounds. Most of the experimental work reported that AChE was significantly decreased in the brain [72,73,74,75] and spinal cord [76] of rats when treated with mercury chloride. Coluccia et al., (2007) [77] have also been revealed the behavioral and spatial learning defects in animals due to mercury treatment leads to cognitive impairment in rats. Cognition functions and motor activities of neurons are controlled by normal level of acetylcholine and acetyl cholinesterase (AChE) activity. The decreasing level of AChE leads to an accumulation of acetylcholine causing over stimulation of the receptors of neurons. This mechanism can promote undesirable effects in animals. The brain can produce the signal and pass through the nerve junctions to promote the muscular movements. Normally neurotransmitter (Ach) is liberated from one nerve to another nerve to promote the stimulation of body movements. During this process Ach is split or hydrolyze by AChE. This is an important enzyme which is found in various junctions of CNS and also in nerve endings in various glands and nodes throughout the body. Mercury and its compounds are easily binds to the active site of AChE and decrease its activity. During this time neurotransmitter (Ach) is not hydrolyzed and it can readily accumulate in cholinergic sites of nerve endings in the intoxicated brain [78, 79] leading to impairment of many physiological factions [74,80]. Storage of AChE and Ach are always playing a vital role in a delicate state of balance, interacting to the complex nervous system which controls most of the body functions [81] like learning and memory processes as well as locomotors control and cerebral blood flow [82,83,84,85,86]. Interestingly, AChE will respond to the development of oxidative stress, pathogenesis and progression of CNS disorders in animals [87]. Actually, the use of AChE inhibition as biomarker to assess the toxic effects of different xenobiotics both *in vivo* and *in vitro* [88]. Caffeic acid is phenolic compound which is found in most of the plant parts like fruits, vegetables, tea and wine [24]. Most of the experimental works confirmed the ameliorative potential of Caffeic acid and it has been reported to elicit various bioactive properties including neuroprotective potentials in intoxicated animals [89, 90, 91, 92]. Pereira et al [93] have been proven that the Caffeic acid exerts a protective effect against the oxidative damage in the brain caused by hydrogen peroxide. Caffeic acid prevents brain damage as well as behavioral and biochemical changes caused by heavy metal stress [94]. Further, Caffeic acid has also been demonstrated to exert neuroprotective effect against amyloid-beta (Ab)-

induced neurotoxicity in vitro [95]. Know et al [96] have also suggested that Caffeic acid exerts a neuroprotective effect against the oxidative injury in the brain caused by hydrogen peroxide and it also ameliorates cerebral ischemia [97]. Lee et al [98] also established the ameliorative potential of Caffeic acid against brain damage as well as behavioral and biochemical changes caused by aluminum-induced toxicity. Certain type of chemicals alone affects the nervous system of animals to cause the neurotoxicity and leads to promote the degeneration of neuroglial cells [99]. Brain damage, depression and blurred vision are the symptoms of neurotoxicity [100]. Neurotoxicity promoted by neurotoxins such as: aluminum, mercury, copper, arsenic, lead and manganese are altering the normal functions of the nervous system due to the brain cell damage [101, 102]. Caffeic acid is used to treat many type of diseases and it is established in most of the experimental work to proven that it has many very good effects on human health. But the exact mechanisms involved in these ameliorative potentials have not yet been clearly demonstrated. In the present experimental study evaluated in vivo effect of the Caffeic acid on the level of AChE activity in the brain of mercury intoxicated rat. The present result suggested that the decreased level of AChE activity was increased to reach near normal level in the brain tissue of mercury intoxicated rat when treated with Caffeic acid. Margarat and Jagadeesan [80] also observed elevated level of AChE activity in the brain tissue of mercury-intoxicated mice when treated with penicillamine (antidote). They suggested that the administration of penicillamine can promote the increased level of AchE is mainly due to the decrease in their heavy metal toxicity. Normally an antidote not only protects the animal form the adverse effect of heavy metal toxicity but also promote the cellular growth. The above findings are supporting our experimental results.

CONCLUSION

In conclusion, mercuric chloride proved to have a neurotoxic effect and caused neuroglial cell damage and administration of Caffeic acid can reduces the resulting damage probably due to its ability to neutralize or scavenge the free radicals that are generated by mercuric chloride. This study elucidated the protective role of Caffeic acid against mercuric chloride toxicity.

REFERENCES

1. Rao M.V., Purohit A.R., Neuroprotection by Melatonin on Mercury Induced Toxicity in the Rat Brain. Pharmacol Pharm, 2: 375-385, 2011.
2. Xu F., Farkas S., Kortbeek S., Zhang F.X., Chen L., Zamponi G.W., Syed N.I., Mercury-induced toxicity of rat cortical neurons is mediated through N-Methyl-D Aspartate receptors. Mol. Brain, 5: 30. Downloaded on 12/12/2014 from: http://www.molecularbrain.com/content/5 /1/30, 2012.
3. Vanithasri V and Jagadeesan G., Comparative Beneficial Effect of N-Acetylcysteine and Gallic Acid on Antioxidant and Acetyl Choline Status of Mercury Intoxicated Rats. Int. J. Phytopharmacol, 4(5): 293-300, 2013b.
4. Lund B.O., Miller D.M., Woods J.S., Studies on Hg (11) induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol, 45: 2017-24, 1993.
5. Ekambaram M., Ramalingam K.A., Balasubramanian A., Effect of solanum triloba-tum linn on mercury-induced hepatotoxicity in Swiss albino mice. J Drug DelivTher, 2(6):68–70, 2012.
6. Malini P., Kanchana G., Rajadurai M., Antibiabetic efficacy of ellagic acid in strep-tozotoc in induced diabetes mellitus in albino wistar rats. Asian J Pharm Clin Res, 4(3):124–8, 2011.
7. Jagadeesan G and Sankarsamipillai S., J. Env.Biol, 28(4):753-756, 2007.
8. Sugunavarman T., Jagadeesan G and Sankarsamipillai S., J. Ecobiotech, 2/1:59-65, 2010.
9. Liu B.Y., Jiang H.J., Lu J.J., Baiyun R.Q., Li S.Y., Lv Y.Y., Li D., Wu H., Zhang Z.G., Grape seed procyanidin extract ameliorates lead-induced liver injury via miRNA153 and AKT/GSK-3β/Fyn-mediated Nrf2 activation in rats. J. Nutr. Biochem, 52, 115–123, 2017a.
10. Sener G., Sehirli O.A., Tozan A., Velioglu-ovuç and Gedik N., Ginkgo biloba extract protects against mercury (II)-induced oxidative tissue damage in rats. Food Chemical Toxicol, 45: 543-550, 2007.
11. Kumar S., Dhankhar N., Kar V., Shrivastava M., Shrivastava S., Myocardial injury provoked by cyclophosphamide, protective aspect of hesperidin in rats. Int JRes Pharm Biomed Sci, 2(3):1288–96, 2011.
12. Nordberg J., & Arner E.S.J., Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Rad. Biol. Medic, 31, 1287-1312, 2001.
13. Padma V.V., Sowmya P., Felix T. A., Baskaran R., & Poornima P., Protective effect of gallic acid against lindane induced toxicity in experimental rats. Food Chem. Toxicol, 49: 991-998, 2011.
14. Sorg O., Schilter B., Honegger P., Monnet-Tschudi F., Increased vulnerability of neurones and glial cells to low concentrations of methylmercury in a prooxidant situation. Acta Neuropathol (Berl.), 96: 621-7, 1998.

15. Elkhadragy M.F., Abdel Moneim A.E., Protective effect of fragaria ananassa methanolic extract on cadmium chloride (CDCL2)-induced hepatotoxicity in rats. Toxicol. Mech. Methods, 27: 335–345, 2017.
16. Thompson J., Bannigan J., Cadmium: Toxic effects on the reproductive system and the embryo. Reprod. Toxicol, 25: 304–315, 2008.
17. Ognjanovic B.I., Markovic S.D., Ethordevic N.Z., Trbojevic I.S., Stajn A.S., Saicic Z.S., Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: Protective role of coenzyme Q 10 and vitamin E. Reprod. Toxicol, 29: 191–197, 2010.
18. Medina M.F., Arrieta M.C., Villafañe M.N., Klyver S.M.R., Odstrcil I.M.A., González M.E., Early signs of toxicity in testes and sperm of rats exposed to low cadmium doses. Toxicol. Ind. Health, 2017.
19. Bartosz G., Druga Twarz Tlenu. Wolne Rodniki W Przyrodzie, Wydawnictwo Naukowe PWN, Warszawa, Poland, 2004.
20. Niki E., "Lipid peroxidation: physiological levels and dual biological effects," Free Radical Biology and Medicine, vol. 47, no. 5, pp. 469–484, 2009.
21. Moreira da Silva F., Marques A., Chaveiro A., Reactive oxygen species: a double-edged sword in reproduction. The Open Vet Sci J, 4: 127-133, 2010.
22. Esterbauer H., Waeg G., Puhl H., Dieber-Rotheneder M., Tatzber F., Inhibition of LDL oxidation by antioxidants. EXS, 62:145, 1992.
23. Cheeseman K.H., Slater T.F., An introduction to free radical biochemistry. Br Med Bull, 49:481, 1993.
24. Clifford M., Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden, J. Sci. Food Agric, 79: 362–372, 1999.
25. Silva T., Oliveira C., Borges F., Caffeic acid derivatives, analogs and applications: a patent review (2009-2013). Expert Opin Ther Pat, 24: 1257-1270, 2014.
26. Jiang R.W., Lau K.M., Hon P.M., Mak T.C., Woo K.S., Fung K.P., Chemistry and biological activities of caffeic acid derivatives from Salvia miltiorrhiza. Curr Med Chem, 12: 237–46, 2005.
27. Parlakpinar H., Sahna E., Acet A., Mizrak B., Polat A., Protective effect of caffeic acid phenethyl ester (CAPE) on myocardial ischemia-reperfusion-induced apoptotic cell death. Toxicology, 209, 1-14, 2005.
28. Kitsati N., Fokas D., Ouzouni M.D., Mantzaris M.D., Barbouti A., Galaris D., (2012). Lipophilic caffeic acid derivatives protect cells against H2O2-Induced DNA damage by chelating intracellular labile iron. J Agric Food Chem, 60: 7873-7879, 2005.
29. Du Q., Hao C., Gou J., Li X., Zou K., He X., Li Z., Protective effects of p-nitro caffeic acid phenethyl ester on acute myocardial ischemia-reperfusion injury in rats. Exp Ther Med, 11: 1433-1440, 2016.
30. Chan J.H., Ho C., Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. J Agric Food Chem, 45: 2374–8, 1997.
31. Gulcin I., Antioxidant activity of caffeic acid (3,4-dihydroxycinnamicacid), Toxicology, 217: 213–220, 2006.
32. Psotova J., Lasovsky J., Vicor J., Metal chelating properties, electrochemical scavenging and cytoprotective activities of six natural phenolics, Biomed. Pap, 147: 147–153, 2003.
33. Pari L., & Prasath A. (2008). Efficacy of caffeic acid in preventing nickel induced oxidative damage in liver of rats. Chemico-Biological Interactions, 173(2): 77–83, 2008.
34. Nichens W.G., Samuelson B., Fomulation of malondialdehyde from phosphor lipid arachidouate during microsomal lipid peroxidation. Eur J Biochem, 6: 126–30, 1968.
35. Beutler E., and Kelley B.M., The effect of disodium nitrate on RBC glutathione. Experim, 29: 97-101, 1963.
36. Kakkar P., Das B., Viswanathan P.N., A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 1984; 21(2): 130-132.
37. Sinha A.K., Colorimetric assay of catalase. Anal Biochem, 47(2): 389-394, 1972.
38. Rotruck J.T., Pope A.L. Ganther H.E., Swanson A.B., Hateman D.G., and Hoekstra W.G., Science. 179: 588-590, 1973.
39. Duncan B.D., Duncan's multiple range tests for correlated and heteroscedastic mean. Biometr, 13: 359-364, 1957.
40. Krishnamurthy P., & Wadhwani, A., Antioxidant Enzymes and Human Health. Antioxidant Enzyme, 2012.
41. Uzun F.G., Kalender Y., Chlorpyrifos induced hepatotoxicity in rats and the protective role of quercetin and catechin. Food Chem. Toxicol. 55, 549–556, 2013
42. Apaydın F.G., Baş H., Kalender S., & Kalender Y. Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats. Environmental Toxicology and Pharmacology, 41: 219–224, 2016.
43. Bashandy S.A., Alhazza I.M., El-Desoky G.E., Al-Othman Z.A., Hepatoprotective and hypolipidemic effects of Spirulina platensis in rats administered mercuric chloride. Afr J Pharm Pharmacol. 5(2):175–82, 2011.
44. Oda S.S., El-Ashmawy I.M., Protective effect of silymarin on mercury-inducedacute nephro-hepatotoxicity in rats. Global Veterinaria, 9(4): 376–83, 2012.
45. Milton prabu S., Shagirtha K., Renugadevi J., Quercetin in combination with vitamins (C and E) improves oxidative stress and renal injury in cadmium intoxicated rats. Eur Rev Med Pharmacol Sci, 14: 903–14, 2010.
46. Nabavi S.F., Nabavi S.M., Habtemariam S., Moghaddam A.H., Sureda A., Jafari M., Hepatoprotective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride

induced oxidative stress. Ind Crops Prod, 44: 50–5, 2013.
47. Sankarsamipillai S., Jagadeesan G., Ramesh S., Arumugam P., Role of Taurine andGlutathione treatment on lipid peroxidation and antioxidant defense in mer-cury induced toxicity in rats. Int J Hum Sci Tech, 1(1): 72–81, 2010.
48. Malini P., Kanchana G., Rajadurai M., Antiperoxidative and antioxidant effect of ellagic acid on normal and streptozotocin induced diabetes in albino wistarrats. Res J Pharm Biol Chem Sci, 2(3): 24–34, 2011.
49. Halliwell B., Free radicals and antioxidants-quovadis? Trends Pharmacol Sci, 32: 125-130, 2011.
50. Apaydın F.G., Kalender S., Demir F., Bas H. Effects of sodium selenite supple-mentation on lead nitrate-induced oxidative stress in lung tissue of diabetic andnon-diabetic rats. *GU. J. Sci.* 2014; 27(2): 847–853.
51. Kalender S., Apaydın F.G., Uzunhisarcikli M., Aslanturk A., Protective role of sodium selenite and vitamin e against rat erythrocytes induced by mercury chloride. GU. J. Sci, 27(4): 1123–1129, 2014.
52. Durak D., Kalender S., Uzun F.G., Demvr F., Kalender Y., Mercury chloride-induced oxidative stress in human erythrocytes and the effect of vitamins C and E in vitro. Afr. J. Biotechnol, 9(4): 488–95, 2010.
53. Haibo Y., Zhaofa X., Wei L., Yu D., Bin X., The protective role of procyanidins and lycopene against mercuric chloride renal damage in rats. Biomed Environ Sci, 24: 550-559, 2011.
54. Tunali-Akbay T., Sener G., Salvarli H., Sehirli O., Yarat A., Protective effects of Ginkgo biloba extract against mercury(II)-induced cardiovascular oxidative damage in rats. Phytother Res, 21: 26-31, 2007.
55. Karaboduk H., Uzunhisarcikli M., & Kalender Y., Protective Effects of Sodium Selenite and Vitamin E on Mercuric Chloride-Induced Cardiotoxicity in Male Rats. Braz. arch. Biol. technol, 58(2): 229–238, 2015.
56. Kalender S., Uzun F.G., Demir F., Uzunhisarcikli M., Aslanturk A., Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol, 55: 456-462, 2013.
57. Kalender S., Apaydin F.G., Bas H., Kalender Y., Protective effect of sodium selenite on lead nitrate-induced hepatotoxicity in diabetic and non-diabetic rats. Environ. Toxicol. Pharmacol, 40: 568–574, 2015.
58. Amamou F., Nemmiche S., Meziane R., Didi A., Protective effect of olive oiland colocynth oil against cadmium-induced oxidative stress in the liver of wistarrats. Food Chem. Toxicol, 78: 177–184, 2015.
59. Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., Telser J., Free radical and antioxidants in normal physiological functions and human disease. Int J Biochem cell Biol, 39: 44-84, 2007.
60. Goering P.L., Morgan D.L., Ali S.F., Effect of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. J. Appl. Toxicol, 22: 167–172, 2002.
61. Agarwal R., Behari J.R., Effect of selenium pretreatment in chronic mercury intoxication in rats. Bull. Environ. Contam. Toxicol, 79: 306–310, 2007a.
62. Agarwal R., Behari J.R., Role of selenium in mercury intoxication in mice. Ind. Health, 45: 388–395, 2007b.
63. Pacitti D., Wang T., Page M.M., Martin S.A., Sweetman J., Feldmann J., Secombes C.J., Characterization of cytosolic glutathione peroxidase and phospholipid-hydroperoxide glutathione peroxidase genes in rainbow trout (Oncorhynchus mykiss) and their modulation by in vitro selenium exposure. Aquat Toxicol, 130–131: 97–111, 2013.
64. Pacitti D., Wang T., Page M.M., Martin S.A., Sweetman J., Feldmann J., Secombes C.J. Characterization of cytosolic glutathione peroxidase and phospholipid-hydro peroxide glutathione peroxidase genes in rainbow trout (Oncorhynchus mykiss) and their modulation by in vitro selenium exposure. *Aquat Toxicol*. 2013; 130–131: 97–111
65. Velisek J., Stara A., Li Z.H., Silovska S., Turek J., Comparison of the effects of four anaesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. Aquaculture, 310: 369–375, 2011.
66. Jagadeesan G., Mercury poisoning and its antidotes. Biochem. Cell. Arch, 4: 61-83, 2004.
67. Ural M.S., Chlorpyrifos-induced changes in oxidant/antioxidant status and hematological parameters of Cyprinus carpio: ameliorative effect of lycopene. Chemosphere, 90(7): 2059–2064, 2013.
68. Prabhakar R., Vreven T., Morokuma K., Musaev D.G., Elucidation of the mechanism of selenoprotein glutathione peroxidase (GPx) catalyzed hydrogen peroxide reduction by two glutathione molecules: a density functional study. Biochemistry, 44: 11864-11871, 2005
69. Alwaleedi S.A., Alterations in antioxidant defense system in hepatic and renal tissues of rats following aspartame intake. Int J Health Sci Res, 6(3): 267-276, 2016.
70. Harrison R.M., Jones A.M., and Lawrence R.G. Major component composition of PM10 and PM2.5 from roadside and urban background sites. Atmospheric Environment, 38(27): 4531–4538, 2004.
71. Bajgar J., Fusek J., Kassa J., Jun D., Kuca K., Hajek P. An attempt to assess functionally minimal acetylcholinesterase activity necessary for survival of rats intoxicated with nerve agents. *Chem Biol Interact.* 2008; 175: 281–285.
72. El-Demerdash F.M., Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver and blood of rats. J Environ Sci Health, 36:489-499, 2001.
73. Margarat A., and Jagadeesan G., Antidotal recovery in carbohydrate metabolism intoxicated mice. Mus musculus. U.P. Zool. Soc, 19(3): 193-196, 1999.
74. Sankarsami pillai S., and Jagadeesan G., Protective role of Taurine on mercuric chloride induced neurotoxicity in rats. Poll Res, 25(2): 353-356, 2006.

75. Vanithasri Varadharajan and Jagadeesan Ganesan. Restoration of Antioxidant Activity by N-Acetylcysteine and Gallic Acid on Kidney Tissue of Mercuric Chloride Intoxicated Wistar Rats. *Int. J. Biol. Pharmaceut. Res*. 2013a; 4(4): 302-307.
76. Kozik M.B., and Wygtadatska H., Enzymatic activity of hydrolases in the spinal cord of rat after acute and subacute catomel intoxication. Folia Histochem Cytochem 15:79-85, 1977.
77. Coluccia A., Borracci P., Giustino A., Sakamoto M., Carratu M.R., Effects of low dose methylmercury administration during the postnatal brain growth spurt in rats. Neurotoxicology & Teratology, 29: 282–287, 2007.
78. Dorman D.C., Brenheman K.A., and Bolon B., Nervous system, hand book of toxicology pathology, edited by WM Haschek, CG Rousseaux and MA Walling, 2nd Edn. (Academic press, sandiego New York boston) 509, 2002.
79. Farina H., Brandao R., Lara F.S., Soares F., Souza D.O., and Rocha J.B., Mechanism of inhibitory effect of selenium and mercury on the activity of delta-aminolevulinate dehydratase from mouse liver, kidney and brain. Toxicol let, 139, 2003.
80. Margarat A., and Jagadeesan G., Effect of mercuric chloride and influence of mercuric chloride and influence of penicillamine on acetyl cholinesterase (AchE) activity in mice poll. Res 18(3): 293-296, 1999.
81. Bretaud S., Toutant J.P., and Saglio P., Effects of carboduran diuron and nicosulfuron on acetylcholinesterase activity in goldfish Carassius auratus. Ecotoxicol. Environ. Saf, 47: 117-124, 2000.
82. Kiehn O., Locomotor circuits in the mammalian spinal cord. Annu Rev Neurosci, 29: 279–306, 2006.
83. Deiana S., Platt B., Riedel G., The cholinergic system and spatial learning. Behav Brain Res, 221: 389–411, 2011.
84. Hut R.A., Van der Zee E.A., The cholinergic system, circadian rhythmicity, and time memory. Behav Brain Res, 221: 466–80, 2011.
85. Klinkenberg I., Sambeth A., Blokland A., Acetylcholine and attention. Behav Brain Res, 221: 430–42, 2011.
86. Havekes R., Abel T., Van der Zee E.A., The cholinergic system and neostriatal memory functions. Behav Brain Res, 221: 412–23, 2011.
87. Ozkul A., Akyol A., Yenisey C., Arpaci E., Kiylioglu N., Tataroglu C., Oxidative stress in acute ischemic stroke. J Clin Neurosci, 14: 1062–6, 2007.
88. Sanchez-Hernandez J.C., Walker C.H., In vitro and in vivo cholinesterase inhibition in Lacertides by phosphonate- and phosphorothioate- type organophosphates. Pest. Biochem. Physiol, 67: 1–12, 2000.
89. Oboh G., Agunloye O.M., Akinyemi A.J., Ademiluyi A.O., Adefegha S.A., Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain-in vitro. Neurochemical research, 38(2): 413-419, 2013.
90. Liang G., Shi, B., Luo, W., Yang, J., The protective effect of caffeic acid on global cerebral ischemia-reperfusion injury in rats. Behavioral and Brain Functions, 11(1): 18, 2015.
91. Soliman A.M., Fathalla A.M., Moustafa A.A., Dose-dependent neuroprotective effect of caffeine on a rotenone-induced rat model of Parkinsonism: A histological study. Neuroscience letters, 623, 63-70, 2016.
92. Oboh G., Agunloye O.M., Akinyemi A.J., Ademiluyi A. O., Adefegha S. A. Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain-in vitro. *Neurochemical research*. 2013; 38(2): 413-419
93. Pereira, P. Oliveira, P. Ardenghi, L. Rotta, J. Henriques, J. Piccada, Neuropharmacological analysis of caffeic acid in rats, Basic Clin. Pharmacol. Toxicol, 99(2006): 374–378, 2017.
94. Yang J.Q., Zhou Q.X., Liu B.Z., He B.C., Protection of mouse brain from aluminum-induced damage by caffeic acid. CNS Neurosci Ther, 14:10–6, 2008.
95. Vauzour D., Rodriguez-Mateos A., Corona G., Oruna-Concha M.J., Spencer J.P., Polyphenols and human health: prevention of disease and mechanisms of action. Nutrients, 2(11): 1106–31, 2010.
96. Know S., Lee H., Kim K., Hong, S., Kim, H., Jo, T., 2010. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and antioxidative activities in mice. Eur J Pharmacol, 649, 210–217.
97. Zhou Y., Fang G., Ye Y., Chu L., Zhang W., Wang M., Caffeic acid ameliorates early and delayed brain injuries after focal cerebral ischemia in rats. Acta Pharmacol Sin, 27: 1103–10, 2006.
98. Lee E.S., Uhm K.O., Lee Y.M., Han M., Lee M., Park J.M., 2007. CAPE (caffeic acid phenethyl ester) stimulates glucose uptake through AMPK (AMP-activated protein kinase) activation in skeletal muscle cells. BiochemBiophys Res Commun, 361, 854–858, 2007.
99. Troshin V.V., Pathogenesis and classification of chronic encephalopathy due neurotoxic chemicals. Med Tr Prom Ekol, 7: 21–6, 2009.
100. Asada K., Toyota K., Nishimura T., Ikeda J., Hori K., Accumulation and mobility of zinc in soil amended with different levels of pig-manure compost. J Environ Sci Health B, 45: 285–92, 2010.
101. Belyaeva E.A., Sokolova T.V., Emelyanova L.V., Zakharova I.O., Mitochondrial electron transport chain in heavy metal-induced neurotoxicity: effects of cadmium, mercury, and copper. Sci World J, 1360-63, 2012.
102. Pohl H.R., Roney N., Abadin H.G., Metal ions affecting the neurological system. Met Ions Life Sci, 8: 247–62, 2011.