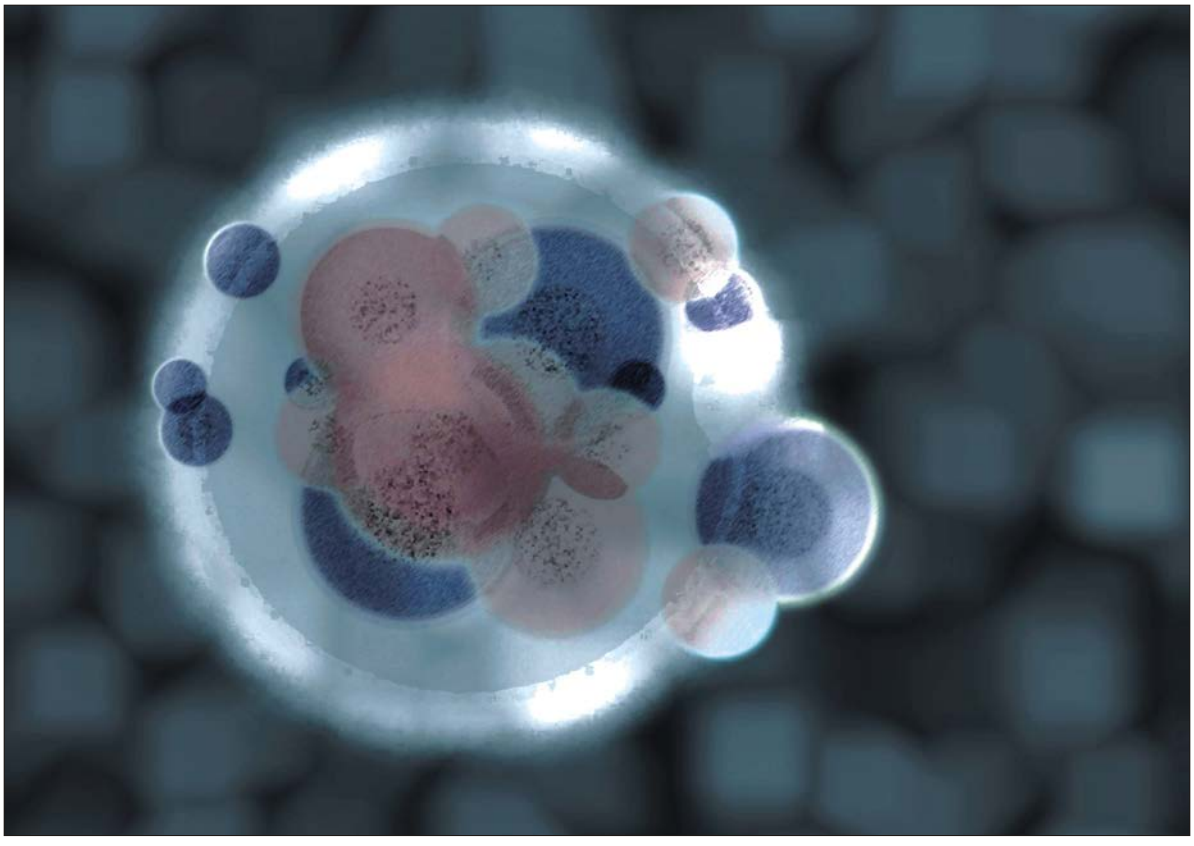

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Research Article
In vivo Amelioration of Oxidative Stress by *Artemisia absinthium* L. Administration on Mercuric Chloride Toxicity in Brain Regions

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Abstract

Background and Objective: *Artemisia absinthium* L. has long been used as traditional herbal medicine for the treatment of gastric pain, cardiac stimulation to improve memory and to restore declining mental function. Aim of the present study was designed to investigate the effects of *Artemisia absinthium* L. on cerebral oxidative stress induced by mercury. Aqueous extract (AEAA) (500 mg L^{-1} b.wt. day^{-1}) was administered orally to experimental rat and brain injury was induced by administration of $HgCl_2$ (5 mg kg^{-1} b.wt., i.p.). **Methodology:** The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione Reductase (GR), catalase (CAT) and thioredoxin reductase (TrxR) were measured in different brain regions: Cerebral cortex (CX), cerebellum (CE), hippocumpus (HI) and striatum (ST). Lipid peroxidation (LPO) and Protein Oxidation (PO) levels were determined by evaluated the levels of malondialdehyde (MDA) and Protein Carbonyl (PC). **Results:** Lipid peroxidation was significantly higher in all brain regions vs. control after $HgCl_2$ treatment. In CX and HI, it is observed a significant depletion of activities, respectively in glutathione peroxydase (11 and 81%), glutathione reductase (32 and 15%), superoxide dismutase (98 and 77%) and catalase (18 and 44%). Further, thioredoxine system was also significantly impaired by mercury as compared with control group. **Conclusion:** Obtained results demonstrated that treatment with AEAA reduce significantly ($p<0.001$) MDA level by 26.99, 31.81 and 80.70% in CE, CX and ST, respectively and increase catalase activity in CE. Furthermore, AEAA significantly ($p<0.001$) restored activities of defense antioxidant enzymes SOD and GPx, GR and TrxR towards normal levels in brain regions.

Key words: Mercuric chloride, antioxidant enzymes, artemisia absinthium, brain region, rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mercuric chloride was one of the first mercurial compounds to be used as antiseptics and disinfectants. Several mercury compounds have been used as preservatives in drugs and cosmetics. However, mercury was abandoned to avoid its toxic effects after advent of modern antibiotics. Mercury has been used in the manufacture of electrical equipment's, scientific instruments, explosives and chemicals and in the electrolytic production of chlorine and alkali[1]. Exposure to mercury may occur primarily by ingestion, inhalation and through the food chain[2]. Mercurial compounds including organic and inorganic forms exhibit a variety of toxicological effects, including neurotoxicity, nephrotoxicity and gastrointestinal toxicity with ulcération and hemorrhage[3,4].

Some compounds have been reported to protect against Hg toxicity in different experimental models. Vitamin E and K[5,6], thiol compounds[7], natural products[8-10], chelating agents[11], Ca^{2+} channel blockers and glutamatergic antagonists[12]. Although the aforementioned protective effects have been observed under experimental conditions, unfortunately, the clinical practice with humans Hg-exposed has shown the absence of an effective treatment that completely abolishes the toxic effects. In such cases, supportive care is given when necessary to maintain vital functions and the administration of chelator agents is performed in an attempt to assist the body's ability to eliminate Hg from the tissues. However, these drugs have limited use because of incomplete efficacies in removing Hg from tissues and significant adverse side effects[13].

Recently, the clinical importance of herbal drugs has received considerable attention. As many synthetic antioxidants have been shown to have one or the other side-effects, there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury[14]. Numerous plant products have been shown to have an antioxidant activity and the antioxidant vitamins, flavonoids and polyphenolic compounds of plant origin have been extensively investigated as scavengers of free radicals and inhibitors of lipid peroxidation[15].

Excessive production of radical species plays an important role in neuronal pathology resulting from excitotoxic insults and therefore, one plausible neuroprotective mechanism of bioflavonoid is partly relevant to their metal chelating and antioxidant properties. Bioflavonoids are claimed to exert antimutagenic, neurotrophic and xenobiotic ameliorating and membrane molecular stabilizing effects[16].

Artemisia absinthium L. (AEAA) (family: Asteraceae) commonly known as wormwood is an aromatic, perennial small shrub. *Artemisia absinthium* is used as an antiseptic, antispasmodic and febrifuge cardiac stimulant for the restoration of declining mental function, to improve memory and inflammation of the liver[17-19]. Traditional Chinese medicine practitioners use the plant for treating acute bacillary dysentery, cancers and neurodegenerative diseases[20-22]. It has been reported that wormwood enhance the cognitive ability as evidenced by its nicotinic and muscarinic receptor activity in homogenates of human cerebral cortical membranes[18] and his capacity to scavenging the free radical[23,24]. Moreover, it has been reported that ethanolic extract enhanced neurite outgrowth induced by nerve growth factor and PC12D cells[25] and protect against focal ischemia and reperfusion-induced cerebral injury[26].

The present study was undertaken to investigate, whether mercuric chloride induces Reactive Oxygen Species (ROS) and alters antioxidant enzyme activities in different regions of the rat brain and tested the prevented effect of the treatment with AEAA on the oxidative damage and antioxidantes enzymes altered by mercury chloride and to evaluate the action mechanism of $HgCl_2$ on the cytosolic thioredoxin reductase. Additionally, the effects of AEAA on the interaction between mercurials and the thioredoxin system were also addressed.

MATERIALS AND METHODS

Animals and experimental protocol: In the experiment, a total of 32 femelle Wistars rats were used. The rats were housed four per cage and had free access to food and water. They were exposed to a 14-10 h light-dark cycle and the room temperature was controlled at 23±2°C. Animals were first exposed to $HgCl_2$ at the age of 5 weeks, when they weighed 170±30 g. Experiments were performed during 10 weeks.

In a sterile physiological solution and 0.5 mL of suspension containing 50 mg $HgCl_2$ was injected intraperitoneally to each rat in the exposed group:

- **Control group:** Rats received saline, intraperitoneal (i.p.) during 10 weeks
- **AEAA group:** Rats were treated with plant aqueous extract (AEAA) orally at the dose of 500 mg L^{-1} for 10 consecutive weeks
- **AEAA+$HgCl_2$ group:** Rats received a sterile physiological solution containing 5 mg $HgCl_2$ kg^{-1} b.wt., i.p. and were treated with plant aqueous extract (AEAA) orally at the dose of 500 mg L^{-1} for 10 consecutive weeks

- **$HgCl_2$ group:** Rats exposed to 5 mg $HgCl_2$ kg^{-1} (i.p.) for 10 weeks

Animals were sacrificed by cervical decapitation under pentobarbital sodium anesthesia (60 mg kg^{-1}). The brain was removed, washed with normal saline and all the extraneous materials were removed before weighing. The brain was kept at ice-cooled conditions all the time. The brain was dissected using the method of Glowinski and Iversen[27] into different regions; cerebral cortex (CX), cerebellum (CE), striatum (ST) and hippocampus (HI). All these brain regions were frozen on dry ice and stored at -80°C until further use for analysis. Due to the small amount of tissue, tissue of 3 L mates was pooled.

Tissue preparation: Cortex, cerebellum, striatum and hippocampus were excised from the fresh brains then weighted. Each excised region tissues was homogenized in 9 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing KCl (140 mM) with a potter. Homogenates were centrifuged at 900×g for 10 min at 4°C to discard nuclei and cell debris. The supernatant containing the mixed and preserved organelles was centrifuged at 9500×g for 15 min at 4°C. Supernatant containing the fraction cytosolic was collected, aliquoted and stored at -80°C until analysis.

Protein assay: Total protein concentrations were determined using the method described by Bradford *et al.*[28] analytical grade bovine serum albumin was used to establish a standard curve. Chemicals and reagents were purchased from Sigma Chemical Co. (Saint Louis, MO, USA).

Oxidative damage

Evaluation of lipid peroxidation: Lipid peroxidation was indirectly determined by measuring the rate of malondialdehyde (MDA) in the prefrontal cortex, cerebellum and hippocampus extracts according to the method of Ohkawa *et al.*[29] using thiobarbituric acid (TBA) reagent. Malondialdehyde (MDA) produced during lipid peroxidation reacts with thiobarbituric acid (TBA) and generates a pink colored complex.

Breafly 100 µL of sodium dodecyl sulphate 8.1% (w/v) was added to 100 µL of tissues supernatants; 750 µL of 20% (v/v) acetic acid (pH 3.5), 750 µL of 0.8% thiobarbituric acid (TBA) and 300 µL of H_2O. After incubation, for 2 h in a water bath at 95°C the tubes were immediately cooled under running water tap. Each tube was vigorously vortexed and centrifuged at 800×g for 5 min, 2500 µL after the addition of n-butanol-pyridine (15:1 v/v). The upper layer was recuperated and the absorbance was recorded at 530 nm by a visible spectrophotometer (DU 720 UV, Beckman Coulter, Palo Alto, CA). The optical density of the complex formed with the TBA-MDA is proportional to the concentration of MDA and to the lipid peroxide. The concentration of MDA (µmoles/mg proteins) was calculated using the molar extinction coefficient of MDA 1.56×10^5 M^{-1} cm^{-1}.

Protein carbonyle assay: Protein carbonyl essay was performed as described by Mercier *et al.*[30]. Briefly, 800 µL of 10 mM of 2,4-dinitrophenylhydrazine (DNPH), dissolved in 2.5 M HCl was added to 200 µL of the supernatant of prefrontal cortex, cerebellum and hippocampus extracts then incubated for 1 h of at room temperature in the dark. Samples were vortexed every 15 min. After adding 1 mL of 20% TCA, tubes were then left on ice for 10 min and centrifuged for 5 min at 4,000 rpm to collect the protein precipitates. A second wash was performed using 1 mL of 10% TCA and protein pellets were mechanically broken. The DNPH was removed from the pellets by a third wash using 1 mL of ethanol-ethyl acetate (1:1 v/v). The pellets were dissolved in 500 µL of 6 M guanidine hydrochloride and were left for 10 min at 37°C. After being vortexed the absorbance of the samples was recorded at 370 nm using a visible spectrophotometer (DU 720 UV, Beckman Coulter, Palo Alto, CA). Protein carbonyl concentration was determined owing to the molar extinction coefficient of 22.0 mM^{-1} cm^{-1}.

Determination of superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) activities (total SOD: Mn SOD and Cu/ZnSOD) were determined using the pyrogallol assay following the procedure described by Marklund and Marklund[31], based on the competition between pyrogallol oxidation by superoxide radicals and superoxide dismutation by SOD. Briefly, 100 µL of 20 mM pyrogallol solution (in Hcl 0.01 M) was added to 2.8 µL of tris buffer (containing 50 mM of tris buffer and 1 mM of ethylene diamine tetraacetic) and 100 µL of tissues supernatants and then mixed. Measurement was taken at 420 nm after 1 min 30 sec and 3 min 30 sec using a visible spectrophotometer (DU 720 UV, Beckman Coulter, Palo Alto, CA). The percentage of inhibition of pyrogallol autoxidation was calculated and 1 U of enzymatic activity was defined as the quantity of enzyme necessary to achieve a 50% inhibition of autoxidation at 25°C mg^{-1} of protein.

Determination of catalase activity: Catalase activity was measured in prefrontal cortex, cerebellum and hippocampus extracts according to the method of Clairborne[32]. Briefly, 20 µL of the extracts was added to a quartz cuvette containing 780 µL phosphate buffer and 200 µL of 0.5 M H_2O_2. Catalase

activity was spectrophotometrically determined (DU 720 UV/visible spectrophotometer, Beckman Coulter, Palo Alto, CA) determined by kinetic measurement at 240 nm at 25°C by using molar extinction coeficient (ε) 0.04 mM^{-1} cm^{-1}. The results were expressed as micromoles of H_2O_2 per minute per milligram proteins.

Determination glutathione peroxidase activity: Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler[33]. The reaction mixture consisted of 300 µL of tissues supernatants, 300 µL of phosphate buffer (0.1 M, pH 7.4), 200 µL of GSH (2 mM), 100 µL of sodium azide (10 mM) and 100 µL of H_2O_2 (1 mM). After incubation at 37°C for 10 min, reaction was stopped by adding 500 µl 5% of TCA, centrifuged at 1500×g for 5 min and then the supernatant was collected. About 700 µL of DTNB (0.4 mg mL^{-1}) and 0.2 µL of phosphate buffer (0.1 M, pH 7.4) were added to 100 µL of the collected supernatant. The optical density was recorded at 420 against blank (i.e., without homogenate). The enzyme activity was calculated as nM of glutathione oxidized/min/mg protein by using molar extinction coefficient 6.22×10^3 M^{-1} cm^{-1}.

Determination glutathione reductase activity: Glutathione Reductase (GR) activity was determined according to Carlberg and Mannervik[34], the oxidation of NADPH was followed for 3 min at 340 nm and the activity of GR was calculated using a molar extinction coefficient of 6.3 mM^{-1} cm^{-1}. Non-enzymatic NADPH oxidation was subtracted from the overall rate. The GR activity was expressed as nmol NADPH oxidized per minute on the basis of total protein content.

Determination of TrxR activity: The TrxR activity was determined according to Cayman's thioredoxin reductase colorimetric assay kit provides a convenient method for detecting mammalian TrxR activity in tissue homogenates and cell lysates. It is based on the reduction of DTNB (5,5'-dithio-bis (2-dinitrobenzoic acid), Ellman's reagent) with NADPH to 5-thio-2-nitrobenzoic acid (TNB), which produces a yellow product that is measured at 405-414 nm. The kit includes all reagents needed to assay mammalian TrxR activity. Measurement of TrxR activity by DTNB reduction in the absence and in the presence of aurothiomalate, a specific TrxR inhibitor included in the kit, allows for correction of non-thioredoxin reductase-independent DTNB reduction (i.e., presence of glutathione). The difference between the two results is the DTNB reduction due to TrxR activity.

Statistical analysis: Results for each measured parameter were expressed as Mean±SD. Quantitative differences were assessed by ANOVA procedure ($p<0.05$) followed by Dunnett-'s (2-sided) comparisons test.

RESULTS

In cerebellum (CE), cerebral cortex (CX), striatum (ST) and hippocampus MDA concentration (Fig. 1) was significantly higher in mercury-treated group compared to control by 68.57, 64.20, 62.40 and 53.73%, respectively ($p<0.05$).

The AEAA administration attenuates the effect of $HgCl_2$ by a significant reduction of MDA in the rats brain regions to -27, -32, -28 and -81% in cerebellum (CE), cerebral cortex (CX), hippocampus (HI) and striatum (ST) ($p<0.05$) compared to $HgCl_2$+AEAA group, respectively. In striatum, the MDA level was significantly reduced by -43% in $HgCl_2$ group vs. control group but remains significantly higher at the all other structures ($p<0.05$). Moreover, no significant alteration was observed in brain regions in any treated groups compared to control group ($p<0.05$) (Fig. 2). Moreover, no significant alteration was observed in brain regions in any treated groups compared to control group ($p<0.05$) (Fig. 2).

In this study, the evaluation of superoxide dismutase activity indicate that no significant variation was observed in the cerebellum and cerebral cortex in $HgCl_2$ groups vs. control (Table 1), while mercury exposure induce a significant reduction by -22% in each other structure, striatum and hippocampus. Treatment with AEAA extract showed a significant amelioration in superoxide dismutase activity in hippocampus and cerebral cortex compared to control by +42 and +20%, respectively.

The rats exposed to mercury induce a significant decreased catalase activity in the cerebral cortex and hippocampus by -81 and -56% ($p<0.05$), respectively in cerebellum, the catalase activity is one and half times higher vs. control ($p<0.05$). The administration of the wormwood extract provides a significant improvement compared to the $HgCl_2$ group, however, no difference is demonstrated in the activity of catalase in the striatum (Table 1).

In $HgCl_2$-treated animals, no changes were observed in the activity of GPx in the cerebellum and cerebral cortex compared to control group. However, GPx activity indicate a significant reduced in hippocampus and striatum by -81 and -32% in $HgCl_2$ groups compared to control ($p<0.05$), the treated group by AEAA remain lower compared to control in this two cerebral structures (Table 1).

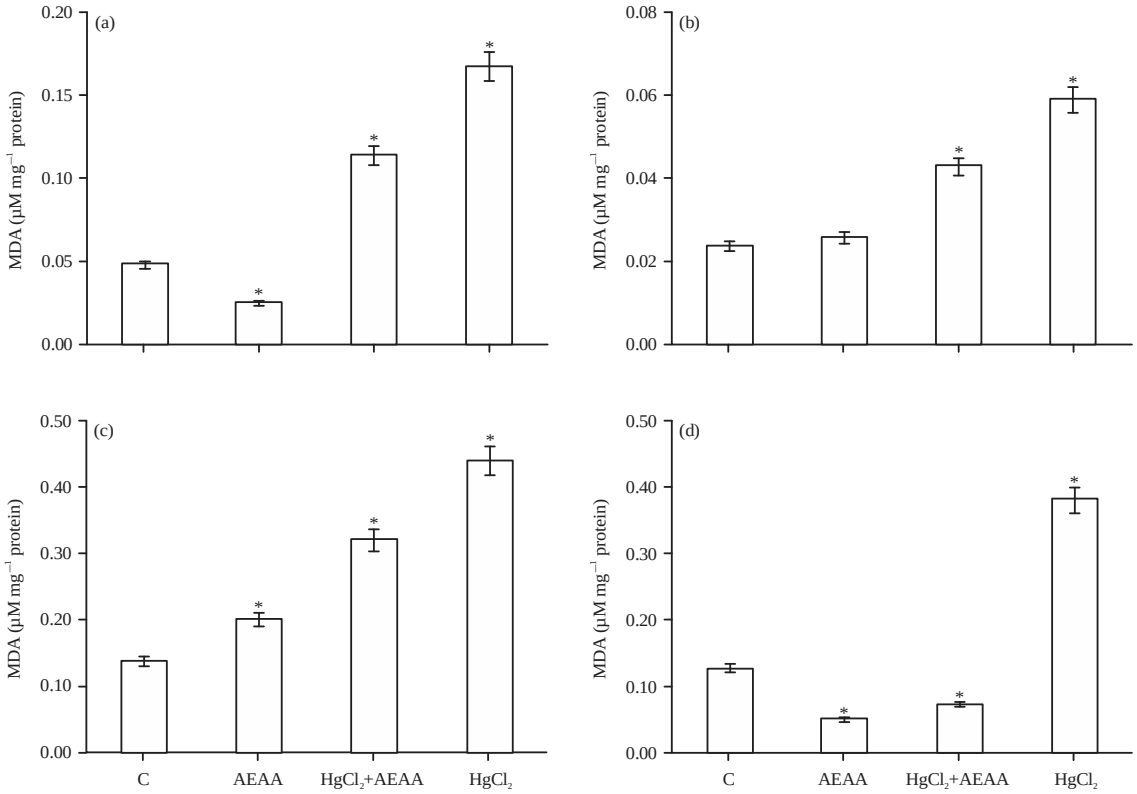

Fig. 1(a-d): Effect of aqueous extract of *Artemisia absinthium* (AAEA) on mercuric chloride ($HgCl_2$) on lipid peroxidation (LPO) level in all rats brain regions, (a) Cerebral cortex, (b) Hippocampus, (c) Cerebellum and (d) Striatum. Values are Mean±SE (n = 6), *p<0.05, AEAA group, $HgCl_2$+AEAA group and $HgCl_2$ group were compared vs. control. Data were expressed as Mean±SD (n = 6)

Table 1: Brain glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activities in different brain region and in all treated or no treated groups

	GPx	GR	SOD	Catalase
	(U mg $^{-1}$ proteins)			
Cerebral cortex				
Control	0.573±0.003	2.437±0.074	0.030±0.003	0.548±0.184
AEAA	0.534±0.040	2.080±0.056*	0.029±0.003	0.297±0.086
$HgCl_2$+AEAA	0.573±0.005	2.070±0.040*	0.036±0.012	0.404±0.003
$HgCl_2$	0.507±0.002*	1.643±0.246*	0.029±0.004	0.103±0.032*
Hippocampus				
Control	0.136±0.006	2.353±0.031	0.032±0.002	0.559±0.050
AEAA	0.131±0.020	2.493±0.075	0.031±0.003	0.478±0.051
$HgCl_2$+AEAA	0.035±0.013*	2.067±0.047*	0.045±0.016	0.450±0.031*
$HgCl_2$	0.025±0.006*	1.983±0.083*	0.025±0.001*	0.246±0.008*
Cerebellum				
Control	0.135±0.002	2.710±0.187	0.023±0.005	0.391±0.044
AEAA	0.138±0.010	1.820±0.030*	0.025±0.003	0.427±0.058
$HgCl_2$+AEAA	0.130±0.007	2.057±0.116*	0.024±0.006	0.468±0.011
$HgCl_2$	0.126±0.002*	1.860±0.030*	0.024±0.006	0.652±0.064*
Striatum				
Control	0.127±0.006	2.347±0.100	0.024±0.001	0.440±0.018
AEAA	0.117±0.003	2.363±0.064	0.023±0.001	0.479±0.063
$HgCl_2$+AEAA	0.115±0.005	3.670±0.176*	0.022±0.002	0.463±0.012
$HgCl_2$	0.076±0.008*	3.213±0.096*	0.018±0.002	0.497±0.052

*p<0.05, AEAA group, $HgCl_2$+AEAA group and $HgCl_2$ group were compared vs. control

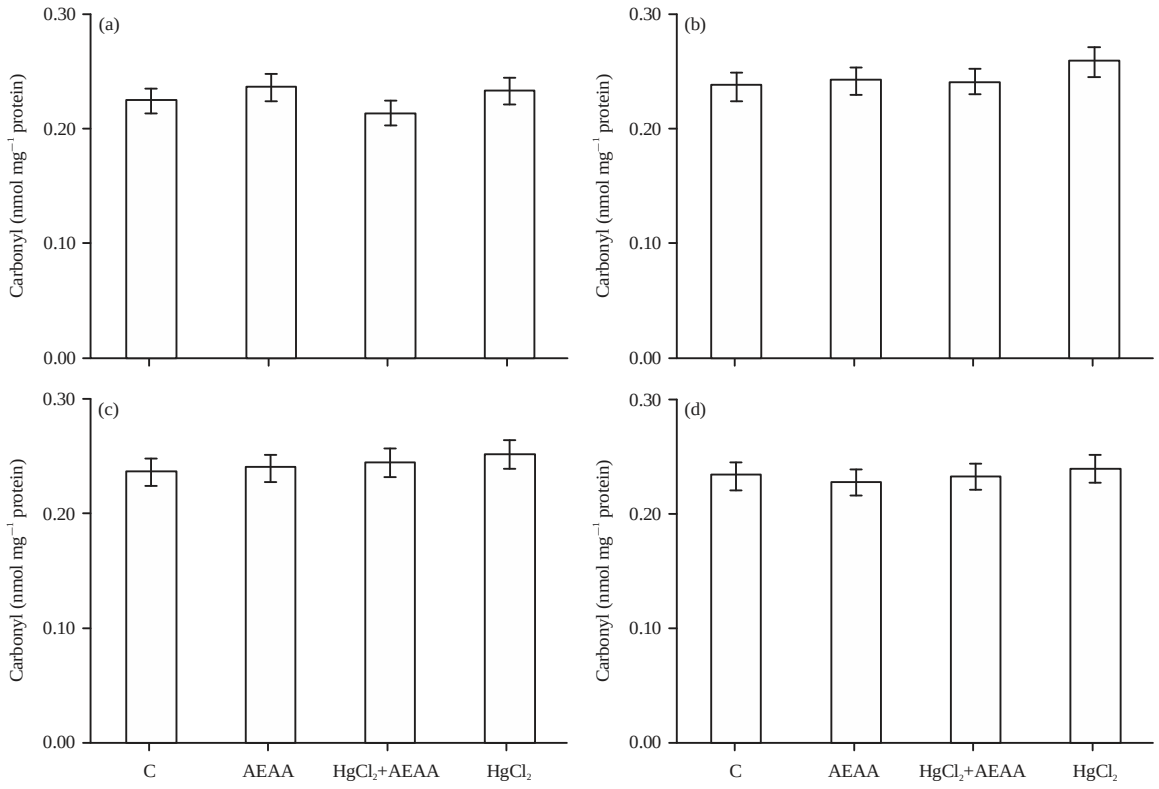

Fig. 2(a-d): Brain protein carbonyl level in all groups treated or no treated by *Artemisia absinthium* extract, (a) Cerebral cortex, (b) Hippocampus, (c) Cerebellum and (d) Striatum. Values are Mean±SE (n = 6), *p<0.05, AEAA group, $HgCl_2$+AEAA group and $HgCl_2$ group were compared vs. control

Table 1, indicate that the GR activity in cerebellum, cerebral cortex and hippocampus was significantly reduced in $HgCl_2$-treated rat compared to control, by respectively -31, -32 and -15%. The AEAA administration with $HgCl_2$ remain significantly reduced in all brain regions, except in striatum where the activity is higher (+37%) vs. control.

It is observed a significant decrease ($p<0.05$) in the TrxR activity in different cerebral areas in the $HgCl_2$ group compared with the control group in the hippocampus: -58%, in the striatum: -67%, in the cerebral cortex: -86% and in the cerebellum: -78%. Treatment with wormwood extract (AEAA) indicate that the TrxR activity remain lower vs. control group in all brain region, in cerebral cortex by -46%, in hippocampus by -23%, in cerebellum by -62% and in striatum by -29% ($p<0.05$) (Fig. 3). However, a marked improvement was noted in TrxR activity in $HgCl_2$+AEAA group compared to $HgCl_2$ group in all brain regions.

DISCUSSION

In the present study, it is used differents brain regions to evaluate the ability of mercuric chloride to generate oxidative stress and cerebral dysfunction; mercuric ion is one of strongest thiol-binding agents[35] is known to increase the intracellular levels of reactive oxygen species and induce oxidative stress resulting in tissue damage[36,37]. The aim of this study was to evaluate the potential capacity of aqueous *Artemisia absinthium* extract to protect brain against the neurotoxic effect of mercuric chloride.

This study results also indicated that lipid peroxidation was increased in all brain area after exposure to $HgCl_2$ (Fig. 1). From the results discussed, it is concluded that mercuric chloride is associated with superoxide radical generation and glutathione depletion[38,39] and induces oxidative stress, mainly by disturbing the antioxidant defense of the body. In these cases, the administration of exogenous antioxidants to counteract the proportionate magnitude of the cell injury plays an important role in the treatment of free radical mediated injury or disease[40,41].

Mercuric chloride is also known to increase the production of many endogenous oxidants such as H_2O_2 and O_2, which cause lipid peroxidation in tissues[40]. It is demonstrated that mercury accumulation is associated with high levels of lipid peroxidation in different regions of the

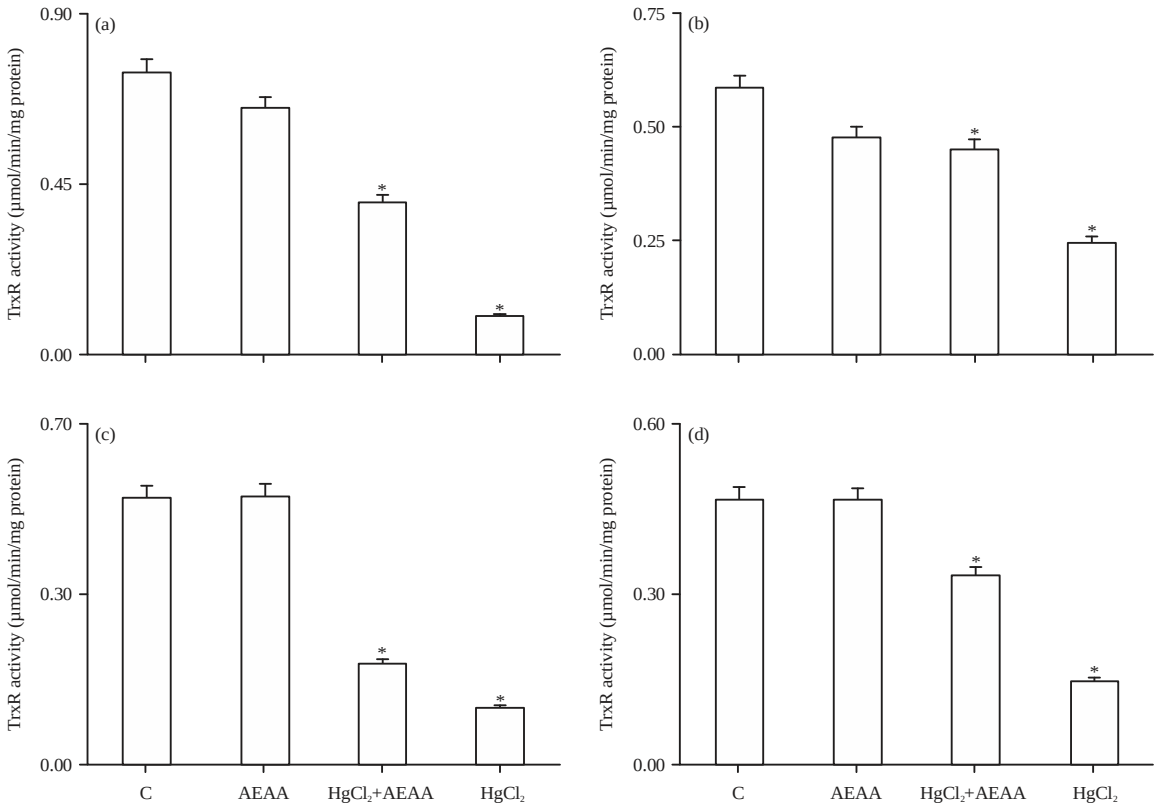

Fig. 3(a-d): Brain thioredoxin reductase activity in all groups treated or no treated by *Artemisia absinthium* extract, (a) Cerebral cortex, (b) Hippocampus, (c) Cerebellum and (d) Striatum. Values are Mean±SE (n = 6), *p<0.05, AEAA group, $HgCl_2$+AEAA group and $HgCl_2$ group were compared vs. control

brain, such as cerebral hemisphere and cerebellum[42]. The vulnerability of neuronal membrane oxidative stress and cellular peroxidation induced by mercury is due to the presence of a relatively high concentration of fatty acids that are readily oxidizable. In addition, production of ROS and alteration of homeostasis *in vivo* may be major factors in the severity of mercuric poisoning.

Findings reported earlier indicate that the wormwood extract administration provided protection against deleterious effect of free radical attack in the brain by reduction the lipid peroxidation and restoration of endogenous antioxidants. The present investigation revealed that treatment with plant extract containing antioxidant components offered effective protection against neuronal damage induced by mercury (Fig. 1).

The TBARS concentration in brain mitochondrial and supernatant fractions was significantly elevated in rat ischemic brain due to focal ischemia and 24 h reperfusion injury. methanol extract of *Artemisia absinthium* pre-treatment markedly decreased the elevated TBARS concentration in brain mitochondrial and supernatant fractions in rat ischemic brain due to focal ischemia and 24 h reperfusion injury as compared to control group[26].

Artemisia absinthium leaves extracts assayed through all the three methods was found to be water extract>methanol extract>ethyl acetate extract. This order is similar to the phenolic contents of the extracts that showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract. The UHPLC analysis of the phenolic compounds profile revealed that salicylic acid was the dominant phenolic compound present in the wormwood leaves extract followed by myricetin, caffeic acid, gallic acid and ferulic acid. The knowledge of the phenolic profile, occurring in the wormwood holds great significance from both dietary and nutritional point of view[43].

It has been demonstrated that the natural compounds that are rich in antioxidants help to reduce oxidative stress thus alleviating the effect of oxidative agents[44,45]. These antioxidants play significant roles in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity on the metabolic and the neuronale function[46].

Lipid peroxidation is known to be one of the molecular mechanisms for cell injury in acute mercury poisoning and is associated with a decrease in cellular antioxidants such as superoxide dismutase (SOD) and catalase (CAT)[47].

According to Hijova *et al.*[48], catalase, glutathion peroxidase and glutathion reductase, activities were found to be decreased in all brain area, except in catalase activity in striatum. Those enzymes were responsible for balancing the production of H_2O_2 and superoxide radicals, reduction of enzymes activities in the brain regions reflected an increased free radical (ROS) generation after mercury intoxication. However, the SOD activity remains without change in all brain regions. It has been reported that toxic effects of mercury have also been observed in oligodendrocytes, astrocytes, cerebral cortical and cerebellar granular neurons obtained from embryonic and neonatal rat brains[40,49].

Antioxidant enzymes are crucial for the removal of oxygen free radicals produced during oxidative stress[50]. The first line of defense against toxic oxygen species are enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and Glutathione Reductase (GR). Induction of these enzymes attenuates the accumulation of toxic oxygen species thereby protecting against potential cell injury and death, tissue dysfunction and numerous pathologies including aging and cancer[51,52].

This study results, suggested that AEAA could exert its antioxidant and/or radical scavenging activities thus preventing the formation of the carbon free radicals originated from mercury toxicity as well as ROS and peroxidation products. Treatment with wormwood significantly prevented mercury-induced decline in GPx, SOD and catalase activity.

Pre-treatment with methanol extract of *Artemisia absinthium* EAA significantly reversed the alterations in biochemical parameters brought by focal I/R. The GSH, SOD and CAT were significantly elevated in the EAA subjected to MCAO and reperfusion as compared to control group[26].

Although the literature survey reveals that *Artemisia absinthium* possesses antioxidant, anti-inflammatory and cognition enhancement activity. Therefore, the present study indicate that the *Artemisia absinthium* extract protect brain against the effect of oxidatif stress generated by mercury. The mechanisms of neuroprotective activity of AEAA was due to the reactivation of the brain antioxidant enzymes such superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) who are the critical antioxidant enzymes, which playing a role in the cellular defense against the deleterious action of ROS and cellular products of free radical chain reactions and canal so directly detoxify lipid peroxides generated by ROS[53].

Thioredoxin reductase is a head-to-tail homodimeric selenoprotein with a molecular mass of 112 kDa containing an N-terminal redox-active disul de/dithiol and a C-terminal active site containing a Cys residue adjacent to a penultimate selenocysteine (Sec) residue, with the conserved sequence[54] Gly-Cys-Sec-Gly-COOH.

Toxicity of mercury compounds arises from their interaction with nucleophilic groups such as selenols and thiols[55,56]. Selenols (–SeH) have a lower pKa than thiols (5.3 vs. 8.5) and under physiological conditions are fully ionized to selenolates ($–Se^-$) and thus are more reactive and can easily interact with mercury[56]. The selenoenzymes such as glutathione peroxidases (GPx) are good targets for mercury[57] but the involvement of the thioredoxin system-comprising thioredoxin (Trx), the selenoenzyme thioredoxin reductase (TrxR) and NADPH on the molecular mechanism of mercury toxicity was proven, the inhibitory effects of mercurials on the thioredoxin system have been shown both *in vitro*[56,58] and *in vivo*[58-60]. Administration of wormwood extract induced a significant amelioration in TrxR activity in all brain region compared to $HgCl_2$ group, this finding is certainly due to the composition of this plant on flavonoids, alcaloids and tannic components and his ability to possess a potent antioxidant activity, free radical scavenging and a anti-inflammatory activity[23,24,61,62]. The mechanisms underlying the protective role of antioxidants molecules against CNS toxicity by $HgCl_2$ will likely be related to their thiol groups (-SH) binding capacity and to the reduction lipid peroxidation and equilibrate the calcium and sulphydryl homeostasis.

CONCLUSION

The molecular mechanism of mercurial damage in both adult and developing CNS is not fully understood. Also, the susceptibility of neurons to mercuric intoxication has been associated to the absence or limited presence of inherent protective mechanisms such as metallothioneins. Who corroborates with the major role of ROS production in mediating mercurial toxicity in the CNS. Various studies have confirmed that metals activate signalling pathways and the cytotoxic effect of MeHg has been related to activation of mainly redox sensitive transcription factors. In this study, it is demonstrated that mercuric chloride administration produce a significant increased lipid peroxidation level along with decrease in various enzymatic activities in different brain region as compared to mercuric chloride treated groups. Treatment with *Artemesia absinthium* L., show a significant amelioration in activity of antioxidant enzymes, which may be associated with the level of non-enzymatic antioxidants than

the activity of enzymatic antioxidants and protects brain dysfunction induced by $HgCl_2$. It can suggest that this molecular approach can be a strategy to a possibly therapeutic action of *Artemisia absinthium* in the treatment of neurodegeneration produced by $HgCl_2$.

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