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The neuroprotective effects of garlic, ginger, and sodium selenite on mercuric chloride toxicity in mice

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Abstract. Mounting evidence suggests that mercuric chloride ($HgCl_2$) exposure is associated with a broad spectrum of toxicological signs, including neurobehavioral and neurochemical abnormalities. It was owing to the antioxidant, anti-apoptotic, and anti-inflammatory properties of garlic, ginger, and sodium selenite (Na_2SeO_3) providing stable rationality to block the multifaceted neurotoxicological pathways of $HgCl_2$. Therefore, the present study was carried out to investigate the neuroprotective potential of these nutritional strategies against the $HgCl_2$-induced neurotoxic mice model. Seventy-five albino mice were randomly divided into 5 groups and 15 mice to achieve this objective. Group 1 was kept as a control, and group 2 was administered $HgCl_2$ at a dose 4 mg/kg BW. Groups 3, 4, and 5 were administered $HgCl_2$ in association with garlic and ginger oils and Na_2SeO_3 at doses of 63, 50, and 0.1 mg/kg BW, respectively. $HgCl_2$ and the treatments were supplemented orally through the stomach tube 3 times weekly for 6 weeks. Half of the mice were decapitated from each group after 4 weeks. Moreover, the others decapitated at the end of the experimental period. $HgCl_2$ caused neurological deterioration as indicated by depletion of catalase activity and induction of apoptosis and histopathological afflictions. However, chronic treatment with the three natural therapeutical approaches exerted neuroprotective effects by ameliorating the disturbances mentioned above.

***Keywords**: Mercuric chloride, neurotoxicity, nutrition, antioxidants, apoptosis, histopathology.*

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1. Introduction.

The causative link between massive metal exposure and health burden remains a topic of research for many decades and represents a scientifically attractive area for investigators due to a gradual rise in occupational and environmental poisoning risks. Biomagnification through the food chain, variability in the ways of exposure, and the biochemical ability to interact with proteins direct much attention towards mercuric chloride ($HgCl_2$) as a significant globally hazardous pollutant.

Targeting the nervous system is of particular importance in ranking $HgCl_2$ as a highly neuro-toxicant as it can pass the blood-brain barrier and accumulate in the cerebral cortexes [1], cerebellums [2], and hippocampi resulting in deficits in locomotor activity and short- and long-term memory along with induction of anxiety-associated responses [1]. From the histopathological point of view, $HgCl_2$ is implicated in well manifested histo-architecture alterations on the levels of the cerebrum, cerebellum, and hippocampus as evident by the presence of multiple foci of necrosis, gliosis, and pyknosis, karyolysis of nuclei, congestion of vessels, and increased cellularity of granular and molecular layers [3,4]. The disturbance of antioxidant defensive mechanism and induction of apoptosis and inflammatory responses are the most apparent contributory factors that lie behind the $HgCl_2$-related neurotoxicity [5,6].

Natural therapeutic agents have the utmost significance in alleviating neurotoxicological changes. Garlic possesses a broad spectrum of biological activities giving its position as one of the top-ranked evidence-based herbal strategies because of

its emerging nutritional profile exemplified by the presence of organosulphur compounds, vitamins, amino acids, and minerals [7-9]. There is a growing body of evidence denoting the ability of garlic to protect against several neurological disorders, indicating that some garlic phytochemicals can pass the blood-brain barrier [10,11]. It is considered an up-and-coming candidate owing to its antioxidant, anti-apoptotic, and anti-inflammatory properties [12-14], giving outstanding rationality to block the multifaceted-multifactorial targets of the $HgCl_2$ challenge. Ginger contains a potent free radical scavenger, 6-Gingerol, which can alleviate the increase of oxidant, inflammatory and apoptotic indicators in the brain of chlorpyrifos exposed rats and improve the activities of enzymatic antioxidants and the level of glutathione [15]. Ginger ameliorated reactive gliosis and protected Purkinje cells against oxidative stress in a rat model of induced diabetes [16].

Alzheimer-like changes in the dentate gyrus were improved, as evident by a histological and immunohistochemical study [17]. Earlier research shed light on selenium (Se) alleviating effects on trace elements-induced toxicity models, including $HgCl_2$, due to its free radical scavenging and metal chelating properties [18]. Sodium selenite (Na_2SeO_3) protected against neurodegenerative brain disorders and oxidative toxicity in scopolamine-induced dementia in rats by increasing glutathione peroxidase activity and reducing glutathione, vitamin A and E concentrations, and caspase-3, procaspase-9, and poly (ADP-ribose) polymerase expressions [19].

Therefore, this study aims to assess the potential protective effects of garlic and ginger oil and Na_2SeO_3 on $HgCl_2$-induced neurotoxicity in mice by estimating oxidative stress markers and apoptosis and examination of histopathological changes in the brain.

2. Materials and methods

2.1. Chemicals

$HgCl_2$ and Na_2SeO_3 were purchased from Sigma Chemicals, St. Louis, MO, USA. Garlic and ginger oil were purchased from El- Captain Company, Egypt, and all other chemicals and reagents were of the highest purity commercially available.

2.2. Animals

Albino mice (23-29 g) were purchased from the Animal House, Faculty of Medicine, Assiut University, Egypt, and housed in cages in the Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Egypt. Mice were kept at room temperature (25 ± 5 °C) with an average 12 h light/12 h dark cycle with *ad libitum* access to pelleted diet and water. All experimental protocols were performed according to the Institutional Animal Care regulations and approved by Assiut University.

2.3. Experimental groups

75 Albino mice were randomly and equally divided into 5 groups, 15 mice each. Group 1 was kept as a control, and group 2 was administered $HgCl_2$ at a dose of 4 mg/kg BW [20]. Groups 3, 4, and 5 were administered $HgCl_2$ with the dose as above-mentioned in addition to garlic and ginger oil and Na_2SeO_3 at doses of 63 [21], 50 [22], and 0.1 mg/kg BW [21], respectively. $HgCl_2$ and the treatments were supplemented orally through the stomach tube 3 times weekly for 6 weeks. Half of the mice were decapitated from each group after 4 weeks, and the others decapitated at the end of the experimental period.

2.4. Sample preparation and estimation of oxidative stress markers

Brains were quickly removed and washed in saline solution (0.9 % NaCl), then stored at -80 °C. A 500 mg of the tissue was homogenized in 5 mL (0.1 M) phosphate buffer (pH 7.4) to prepare 10% w/v of brain homogenate. The homogenates were centrifuged at 8000 rpm for 15 min at 4 °C. The supernatants were kept frozen at -20 °C for the subsequent biochemical assays. Total protein (TP) concentration was determined by the method of Lowry et al. [23]. Lipid peroxidation products (LPO) as thiobarbituric acid reactive substances were estimated according to the method of Ohkawa et al. [24]. Total peroxide (TPX) contents were measured, according to Harma et al. [25]. This method was based on the oxidation of xylenol orange into a purple-colored chromogen that was directly proportionate with peroxide content in the presence of ferrous sulfate as a catalyzer. The activity of superoxide dismutase (SOD) was determined according to its ability to inhibit the autoxidation of epinephrine in the alkaline medium according to the method of Misra and Fridovich [26]. Catalase (CAT) activity was estimated based on its ability to decompose hydrogen peroxide [27].

2.5. Histopathological examination

Tissue specimens from the brain were collected after cervical dislocation of mice and washed in normal saline, fixed in Bouin's solution for about 18 h, and washed several times of 70% ethanol. After fixation, specimens were dehydrated in graded series of alcohol, cleared in xylene, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin [28].

2.6. TUNEL assay

The DNA fragmentation as an apoptotic indicator was examined using TUNEL assay. Tissue specimens from the brain were collected after cervical dislocation of mice, washed in phosphate buffer saline, and fixed immediately in 10% neutral buffer formalin. After fixation, samples were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. TUNEL assay was performed on thin paraffin-embedded sections (5 µm) according to the manufacturer's protocol (in situ cell death detection kit, POD; Roche Diagnostic GmbH, Mannheim, Germany).

2.7. Statistical analysis

The data were presented as mean ± standard error of the mean (SEM). The results were analyzed statistically using column statistics and one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test. These analyses were carried out by the computer Prism program for windows, version 3 (Graph pad software Inc., San Diego, California, USA). Differences between the groups were considered significant when $P < 0.05$.

3. Results and discussion

3.1. Markers of oxidative stress and the number of apoptotic cells

Table 1 shows the effects of garlic and ginger oil and Na_2SeO_3 on the oxidant/antioxidant parameters and the number of apoptotic cells in the brain of $HgCl_2$ challenged rats. An insignificant increase of the LPO level in the $HgCl_2$ and the Na_2SeO_3 groups was observed. While in the other groups, its level insignificantly decreased at the end of 4 weeks. At the end of 6 weeks, the LPO level was insignificantly increased in all groups except the Na_2SeO_3 group, in which it insignificantly decreased. At both time points, the TPX level insignificantly increased in the $HgCl_2$ group compared to the control, but it insignificantly decreased in all protective agent groups. The CAT activity was significantly decreased in the $HgCl_2$ group relative to the control after 4 and 6 weeks ($P < 0.01$ and 0.001, respectively). However, CAT activity was significantly ($P < 0.01$) increased following 4 weeks of dietary supplementations of Na_2SeO_3 along with insignificant increases in the other treatment modalities. Garlic and ginger oil and Na_2SeO_3 succeeded in increasing CAT activity following 6 weeks of dietary supplementation significantly ($P < 0.05$, 0.5, and 0.01, respectively). Except for a significant ($p < 0.01$) decrease of SOD activity in the ginger group at the first time point, SOD activity of all other groups at both first and second time points exhibited no marked change.

As shown in Table 1 and Figure 1 and Figure 2, the number of apoptotic cells showed a high significant ($P < 0.001$) increase in the $HgCl_2$ group at both time points compared to the control group. Relative to the $HgCl_2$ group, a highly significant ($P < 0.001$) decrease was observed in the garlic, ginger, and Na_2SeO_3 groups at both time points and in garlic and Na_2SeO_3 groups at the first time point.

The marked decrease of CAT activity following exposure to the $HgCl_2$ burden occurred previously in the study of [29], probably due to down-regulation through methylation of CAT promoter [30]. A previous study suggested mercury (Hg) ability to form a complex through electrostatic and van der Waals forces and induce a conformational change in CAT using spectroscopic techniques and molecular docking simulation methods [31]. By examining the modulation of kinetic parameters of CAT under the influence of Hg *in vitro*, inhibition of its activity by decreasing its affinity towards hydrogen peroxide was observed [32]. From these activity-relationship studies, it was confirmed that Hg directly altered the physicochemical characters of CAT. Restoration of brain CAT activity following oral supplementation of the three studied dietary formulations is in the same line with the findings of other researchers who studied other neurotoxic models [33-35]. The antioxidant activity of ginger is attributed to its nutritional components like zingerone, shogaols, gingerols, pardols, cineole, curcumene, geranyl acetate, terphineol, terpenes, borneol, limonene, and β-elements [36]. These phytochemicals have a significant role in improving antioxidant capacity, scavenging free radicals, and preventing peroxidative damage [37,38]. Se participates as a functional building block in several enzymatic antioxidants such as glutathione peroxidases and thioredoxin reductase [39] to effectively counteract oxidative damage-induced toxic agents caused by scavenging reactive oxygen radicals and protect membrane integrity [40]. The direct molecular

interaction between Se and Hg provides an effective way to dampen the neurotoxicity of Hg and alleviate its accumulation in the neurological tissues [41]. Garlic has an emerging nutritional profile as phenolic compounds, flavonoids, aglycones, vitamins, and sulfur compounds, and these ingredients may be added to their antioxidant capacity [42]. Present work built a novel suggestion based on the ability of garlic to generate H_2S in the aorta rings to explain the increase of CAT activity following oral supplementation of garlic, [43] and the fact that H_2S up-regulates endogenous antioxidants through the Nrf2-dependent signaling pathway in the myocardial ischemic model [44]. However, whether the same events occur in the brain is remains to be elucidated.

LPO is a widely used index of lipid peroxidation, indicating damage in the cell membrane and change in the degree of membrane fluidity [42]. Lack of a significant increase of the brain LPO may reflect the higher propensity of Hg to inhibit enzymes than to oxidatively damage lipids in the brain [30]. This pattern of response may indicate tissue specificity regarding lipid peroxidation in response to the $HgCl_2$ burden. One explanation is the actions of non-enzymatic antioxidants, such as cysteine, alpha-tocopherol, and ascorbic acid, whose protective role against metals has been already demonstrated in the brain of rats [45]. An alternative explanation concerns the selective inhibition of specific antioxidants, while others can keep their functionality, resulting in still reasonable defensive mechanisms against an increased rate of lipid peroxidation. Although lipid peroxidation seemed to have been avoided in brain tissues, the occurrence of $HgCl_2$-mediated oxidative damage in other crucial macromolecules like DNA and protein cannot be excluded [29].

Table 1: Effects of garlic and ginger oils and sodium selenite on the oxidant/antioxidant parameters and the number of apoptotic cells in the brain of mercuric chloride challenged rats.

Time point	Control	$HgCl_2$	$HgCl_2$+Garlic	$HgCl_2$+Ginger	$HgCl_2$+ Na_2SeO_3
LPO (nmol/mg protein)					
4 weeks	0.216±0.045	0.237±0.015	0.216±0.005	0.135±0.001	0.269±0.0245
6 weeks	0.141±0.044	0.1908±0.033	0.1712±0.027	0.2004±0.021	0.116±0.009
TPX (nmol/mg protein)					
4 weeks	203±19.69	422.6±22.55	388.1±42.3	169.7±54.89	187±50.15
6 weeks	249.8±21.90	374.1±25.83	274.2±41.42	214.4 ±57.53	280.5±40.64
CAT (U/mg protein)					
4 weeks	23.68±4.282	5.064±0.668^{a**}	13.21±0.5782	4.120±0.3607	20.22±0.3966b**
6 weeks	26.87 ±3.687	7.589 ± 0.7896^{a***}	18.36±0.4027^{b*}	16.67± 0.8919^{b*}	22.37±3.35b**
SOD (U/mg protein)					
4 weeks	6.899±0.387	5.951±0.1842	5.482±0.5488	2.595±0.3794^{b**}	5.182±0.4242
6 weeks	6.899±0.387	6.763±0.8462	4.558±0.5595	7.595±0.4874	6.250±0.9973
Number of apoptotic cells					
4 weeks	7.5± 1.291	15.38±1.302^{a***}	6.333±0.577^{b***}	5±1.155^{b***}	6.750±2.062^{b***}
6 weeks	7.5± 1.291	15.5± 1.761^{a***}	15±1.155	5±0.816^{b***}	10.6±0.894^{b***}

$HgCl_2$, mercuric chloride; Na_2SeO_3, sodium selenite; LPO, lipid peroxidation; TPX, total peroxide; CAT, catalase; SOD, superoxide dismutase. ^{a} represents a significant difference between the control and $HgCl_2$ group. ^{b} represents a significant difference between $HgCl_2$ and protective agent groups. * = p<0.05, ** = p<0.01, *** = p<0.001. Results are expressed as mean ± SEM (one-way ANOVA followed by Newman–keuls post-test).

The significant decrease of SOD activity in the ginger-treated group relative to the intoxicated one represents a special surprise. Compelling evidence has been accumulated, denoting that antioxidants could behave as pro-oxidants under specific conditions [46]. For instance, vitamin C, a well-known antioxidant, generates hydroxyl radicals from hydrogen peroxide secondary to its reductive influence on iron [47]. The dual actions of antioxidants may be relative to their concentrations. Some antioxidants at higher concentrations reverse their beneficial actions by converting themselves to free radicals after their interaction with radicals, and when present in low concentrations, become highly reactive and cause auto-oxidation of fatty acids [48]. The literature is punctuated with a wide range of evidence pointing to the stimulating effect of toxicity-associated oxidative stress on the activity of enzymatic antioxidants indicating a shift of the metabolism from the reductive to oxidative pathway [49,50]. Therefore, the significant decrease of CAT activity following ginger supplementation may be considered a sign of improvement in the redox-sensitive condition. However, this cannot be a cutting-edge conclusion until the estimation of other

enzymatic and non-enzymatic antioxidants, reflecting the rationality behind the recommendation that measurement of a single antioxidant may not reflect the total antioxidant capacity since the expression of some enzymatic antioxidants may be up-regulated as a defensive response [51].

The marked increase in the number of tunnel-positive cells under $HgCl_2$ intoxication is in harmony with the previous nephrotoxic model [52]. This outcome response may be attributed to the ability of $HgCl_2$ to increase the expression of pro-apoptotic factors as Bax and caspase-3 and decrease the expression of anti-apoptotic factors as Bcl-2 [53]. In the present study, garlic, ginger, and Na_2SeO_3 succeeded in protecting against $HgCl_2$-induced cell death in the brain of mice corresponding to previous reports [21,54,55]. The overexpression of Bax pro-apoptotic protein was decreased following administration of garlic to rats suffered from cisplatin-induced nephrotoxicity and aspirin-induced gastric ulcer [56,57]. A genetic study focused on the effects of ginger on hepato- and nephrotoxicity in rabbits revealed a decrease in mRNA expression of caspase-3: Glutathione-S-transferase and increased expression of Bcl-2 in the liver and kidney [54]. The anti-apoptotic activity of Se is excreted by inhibition of oxidants generation, mitochondrial membrane collapse, cytochrome c release, cytosolic calcium release, calcium entry through TRPM2, and TRPV1 channels and caspase-dependent pathway, and modulation of pro-and anti-apoptotic regulators and glutathione redox cycle [55,58-60].

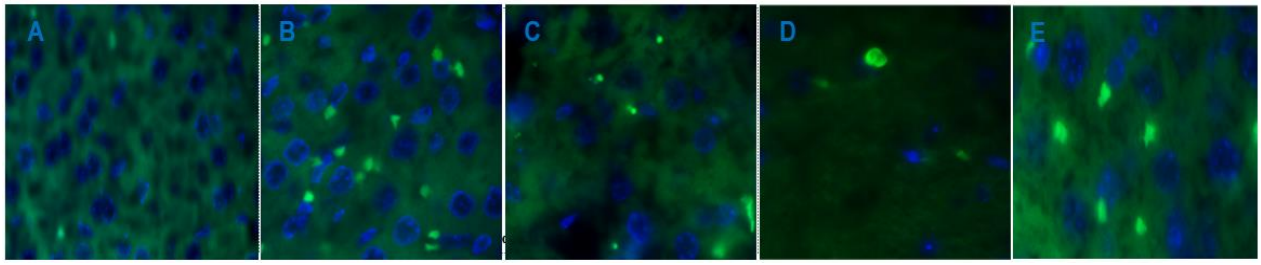

Figure 1. Photomicrographs obtained by fluorescent microscope after TUNEL assay from the brain of different groups after 4 weeks' post-treatment showed apoptotic nuclei displaced by green fluorescent while viable nuclei stained blue (x40). Here, **A**: Control, **B**: $HgCl_2$, **C**: Garlic, **D**: Ginger, **E**: Na_2SeO_3.

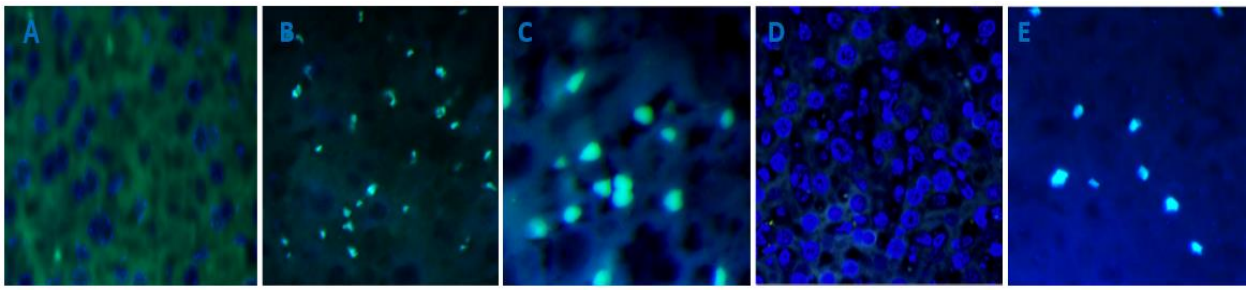

Figure 2. Photomicrographs obtained by fluorescent microscope after TUNEL assay from the brain of different groups after 4 weeks' post-treatment showed apoptotic nuclei displaced by green fluorescent while viable nuclei stained blue (x40). Here, **A**: Control, **B**: $HgCl_2$, **C**: Garlic, **D**: Ginger, **E**: Na_2SeO_3.

3.2. Histopathological findings

Four weeks following $HgCl_2$ intoxication, the cerebrum mice showed diffuse ischemic neuronal injury, meningitis, the focal area of malacia in grey matter, vacuolation of neurons and neuropils, and severe pericellular edema. Severe astroglia cell reaction, perivascular edema, blood vessel with perivascular cuff, and diffuse chronic ischemic neuronal injury were also found (Figure 3). Severe vacuolation of neurons, along with diffuse chronic ischemic neuronal degeneration and moderate to severe astroglia cell reaction, was observed in the hippocampus (Figure 4). The cerebellum showed necrosis of molecular, Purkinje cell, and granular layers together with intracerebellar hemorrhage and vacuolation of neuropils (Figure 5). At the second time point, the cerebrum displayed extensive and diffuse ischemic neuronal degeneration in some areas, spongiosis, and perivascular and pericellular edema. The photomicrograph of the hippocampus illustrated severe ischemic neuronal injury with moderate astrogliosis and perivascular and pericellular edema. Ischemic neuronal injury in molecular and Purkinje cell layers, along with

pyknotic changes, astrogliosis, prominent hyperemia, and multiple focal areas of hemorrhage in the granular layer, was found (Figure 6).

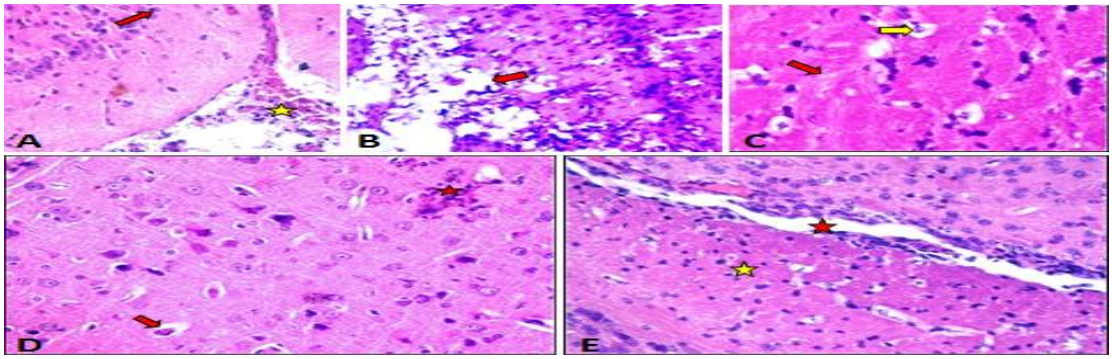

Figure 3. Photomicrograph of cerebrum from $HgCl_2$ group after 4 weeks showing (**A**) diffuse ischemic neuronal injury (red arrow) and meningitis (yellow star), (**B**) focal area of malacia in grey matter(red arrow), (**C**) vacuolation of neurons and neuropils (red arrow) and pericellular edema of severe degree (yellow arrow), (**D**) severe astroglia cell reaction (red star), perivascular edema of severe degree (red arrow) and (**E**) large blood vessel with perivascular cuff (red star), diffuse chronic neuronal injury of ischemic type and severe astrogliosis (yellow star) (H&E, x40)

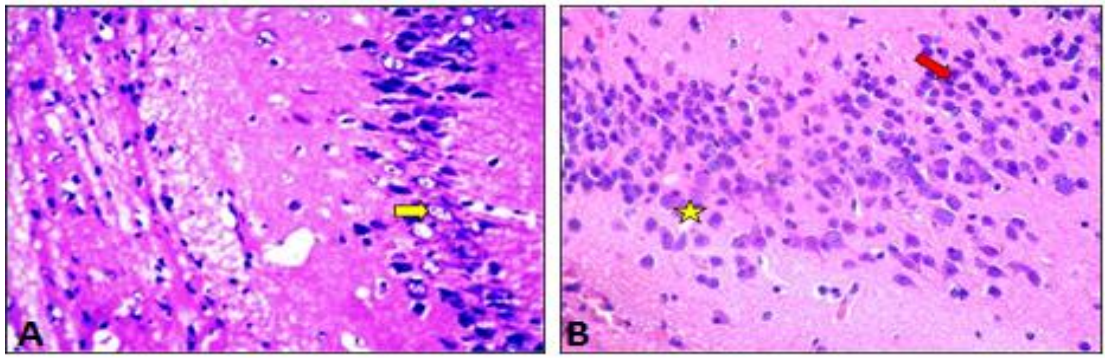

Figure 4. Photomicrograph of hippocampus from $HgCl_2$ group after 4 weeks showing (**A**) severe vacuolation of neurons (yellow arrow) (**B**) diffuse chronic neuronal degeneration of ischemic type (star) and moderate to severe astroglia cell reaction (red arrow) (H&E, x40)

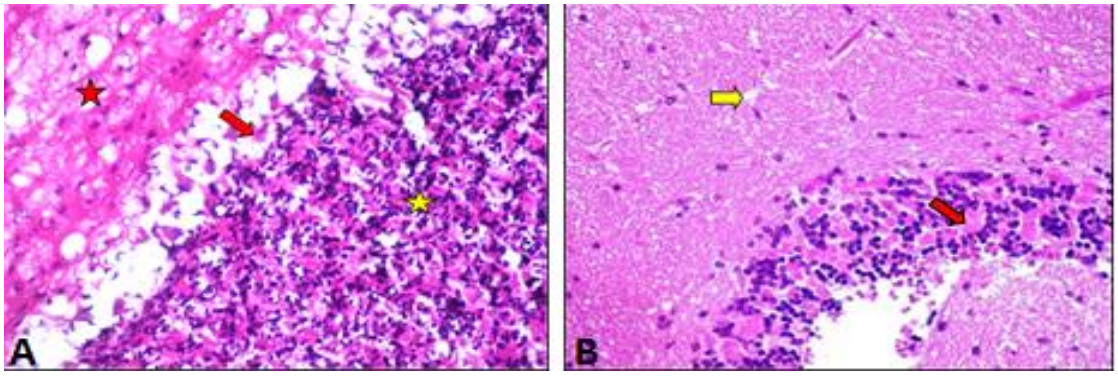

Figure 5. Photomicrograph of the cerebellum from $HgCl_2$ group after 4 weeks showing (**A**) necrosis of molecular layer (red star), Purkinje cell layer (red arrow) and granular layer (yellow arrow) (**B**) intracerebellar hemorrhage (red arrow), and vacuolation of neuropils (H&E, x40)

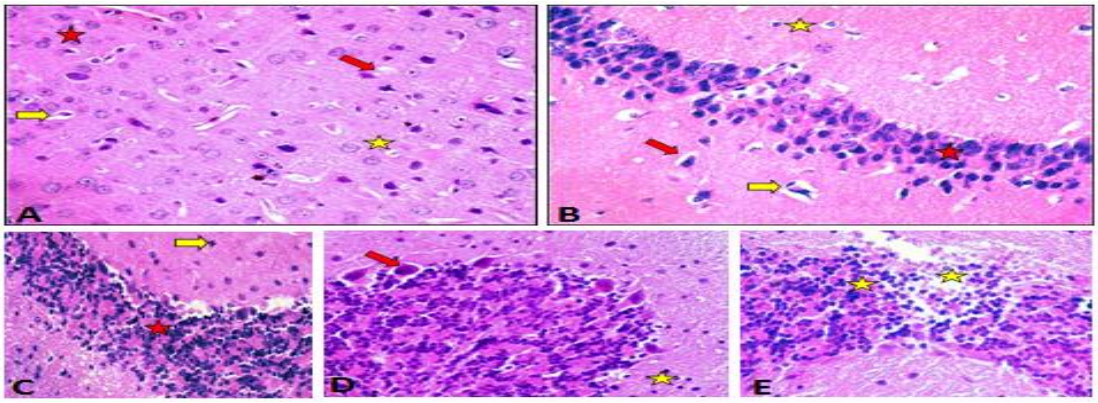

Figure 6. Photomicrograph of the brain from $HgCl_2$ group after 6 weeks showing (**A**) cerebrum with extensive and diffuse ischemic neuronal degeneration in some areas (red star), spongiosis (yellow star), perivascular (yellow arrow) and pericellular (red arrow)edema, (**B**) hippocampus with severe degree of ischemic neuronal injury (red star) with moderate astrogliosis (yellow star), perivascular (yellow arrow) and pericellular (red arrow) edema, (**C**) cerebellum with ischemic neuronal injury in molecular layer (yellow arrow), pyknotic changes in granular layer (red star), (**D**) cerebellum with ischemic neuronal injury in Purkinje cell layer (red arrow) and astrogliosis (yellow star) and (**E**) cerebellum with prominent hyperemia and multiple focal areas of hemorrhage in granular layer(yellow star) (H&E, x40)

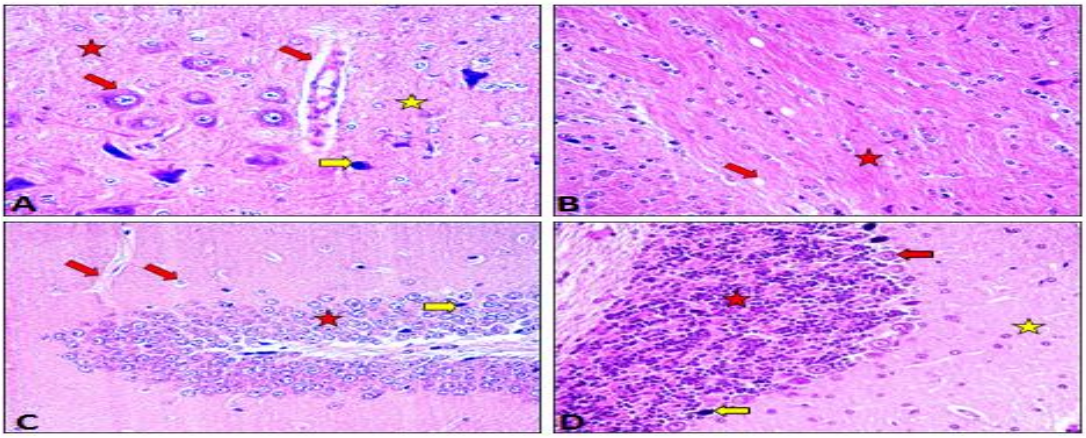

Figure 7. Photomicrograph of the brain from the garlic group after 4 weeks. (**A**) Showing deep cerebral cortex with very mild pericellular and perivascular edema (red arrow), few cells with ischemic neuronal injury (yellow arrow), mild astrogliosis (yellow star), and insignificant vacuolation (red star). (**B**) Showing cerebral white matter with insignificant spongiosis (red star) and a very mild degree of edema (red arrow). (**C**) Showing hippocampus with a very mild degree of perivascular and pericellular edema (red arrow), very few degenerated neurons (yellow arrow) while most of the neurons were healthy (red star). (**D**) Showing cerebellum with the healthy molecular layer (yellow star), healthy Purkinje cell layer (red arrow) with few necrosed cells (yellow arrow), healthy granular layer (red star) very mild perivascular edema and moderate astrogliosis (H&E, x40)

The histopathological lesions in the brain of $HgCl_2$ intoxicated mice in the current study are in harmony with previous cytomorphological investigations showing multiple foci of necrosis and congestion of vessels in the cerebellum, cerebrum, and hippocampus, degenerative changes in cortical neurons, dentate gyrus, and cornu ammonis 3 and loss of nuclear material in Purkinje cells following oral administration of $HgCl_2$ in rats [3,4].

However, the histopathological changes in the brain following administration of garlic oil for 4 weeks were shown in Figure 7. Very mild pericellular and perivascular edema, few cells with ischemic neuronal injury, mild astrogliosis, and insignificant vacuolation were observed in the deep cerebral cortex. Insignificant spongiosis and a very mild degree of edema were found in the cerebral white matter, and the hippocampus showed a very mild degree of perivascular and pericellular edema, with very few

degenerated neurons. In contrast, most of the neurons were healthy. The cerebellum displayed healthy molecular and Purkinje cell layers with few necrosed cells, a healthy granular layer, very mild perivascular edema, and moderate astrogliosis. Figure 8 showed the photomicrograph of the brain from the garlic group after 6 weeks. The cerebral cortex exhibited insignificant pericellular and perivascular edema, completely healthy neurons, and mild vacuolation. Insignificant astrogliosis, perivascular, and pericellular edema and completely healthy neurons were found in the hippocampus. The cerebellum showed healthy molecular, Purkinje cell, and healthy granular layers.

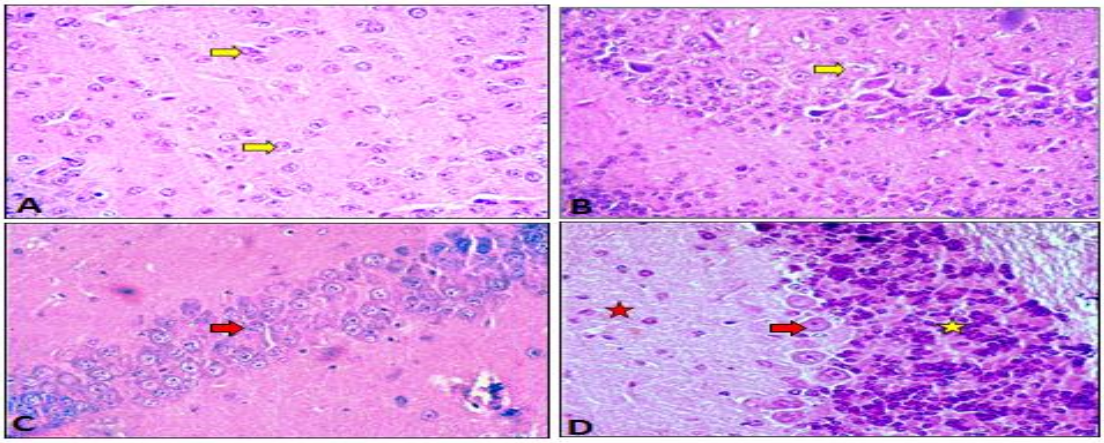

Figure 8. Photomicrograph of the brain from the garlic group after 6 weeks. (**A**) Showing cerebral cortex with insignificant pericellular and perivascular edema, and completely healthy neurons (yellow arrow). (**B**) Showing cerebral cortex with mild vacuolation (yellow arrow). (**C**) Showing hippocampus with insignificant astrogliosis, perivascular and pericellular edema, and completely healthy neurons (red arrow). (**D**) Showing cerebellum with the healthy molecular layer (red star), healthy Purkinje cell layer (red arrow), and healthy granular layer (yellow star). (H&E, x40)

The well-marked histoprotective effect of garlic on $HgCl_2$ neurotoxic affliction is parallel to the results of [61], demonstrating its highly beneficial role in alleviating lead-related maternal and fetal cerebellar injury during gestation period in rats. It not only provides neuroprotection by attenuation of oxidative stress but also suppresses the rise in tumor necrosis factor α level and cyclooxygenase-2 expression and attenuating the activation of caspase-3, DNA fragmentation, and Poly (ADP-ribose) polymerase cleavage [62,63].

Figure 9 illustrated the photomicrograph of the $HgCl_2$ poisoned brain supplemented ginger for 4 weeks. The cerebral cortex exhibited a high proportion of healthy neurons to other necrosed ones, astroglia cell reaction, and moderate perivascular and pericellular edema. Perivascular cuff and moderate perivascular and pericellular edema were observed in the cerebral cortex. The hippocampus showed healthy cells, very mild astrogliosis, and pericellular edema, and the cerebellum displayed healthy molecular, granular, and Purkinje cell layers. The photomicrograph of the brain from the ginger group after 6 weeks was shown in Figure 10. The cerebral cortex exhibited a high proportion of healthy neurons and few cells suffering from chronic ischemic neuropathy and mild pericellular edema. Healthy cells rarely observed chronic neuronal injury, and mild perivascular and pericellular edema was observed in the hippocampus. The cerebellum showed a healthy molecular layer, granular, and Purkinje cell layers.

In consistent with the histopathological findings of ginger-treated brain in this study, [17] and [16] observed improvements in the neurodegenerative changes in aluminum chloride-induced Alzheimer and streptozotocin-induced diabetic rat models. Inhibition of peroxidative damage, apoptosis and inflammation, modulation of astroglial response towards injury, reduction of AChE expression, and enhancement of neurogenesis [64] could be the most excellent tools by which ginger maintains the cellular integrity in the brain.

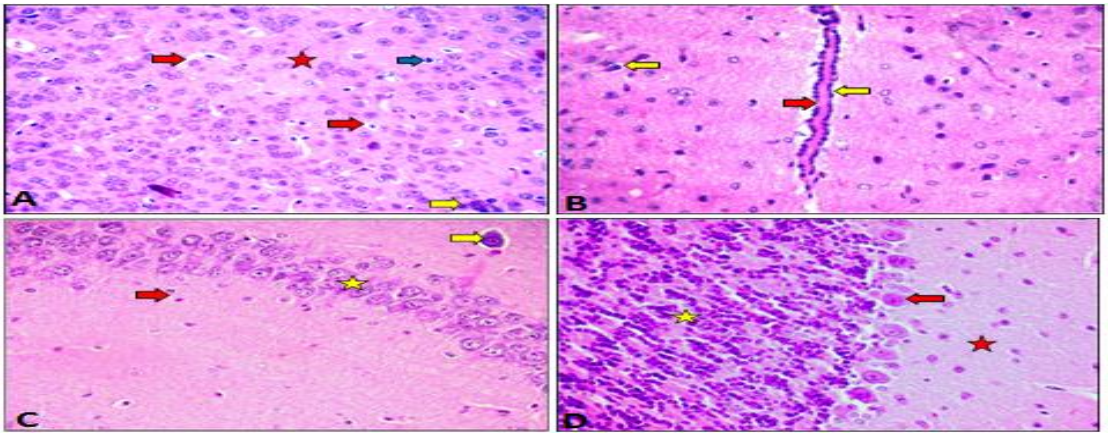

Figure 9. Photomicrograph of the brain from the ginger group after 4 weeks. (**A**): Showing cerebral cortex with a high proportion of healthy neurons (red star) to other necrosed ones (blue arrow), astroglia cell reaction (yellow arrow), moderate perivascular, and pericellular edema (red arrow). (**B**): Showing cerebral cortex with perivascular cuff and moderate perivascular and pericellular edema (yellow arrow). (**C**): Showing hippocampus with healthy cells (yellow star), very mild astrogliosis (red arrow), and pericellular edema (yellow arrow). (**D**): Showing cerebellum with a healthy molecular layer (red star), healthy Purkinje cell layer (red arrow), and healthy granular layer (yellow star). A, B, C and D (H&E, x40)

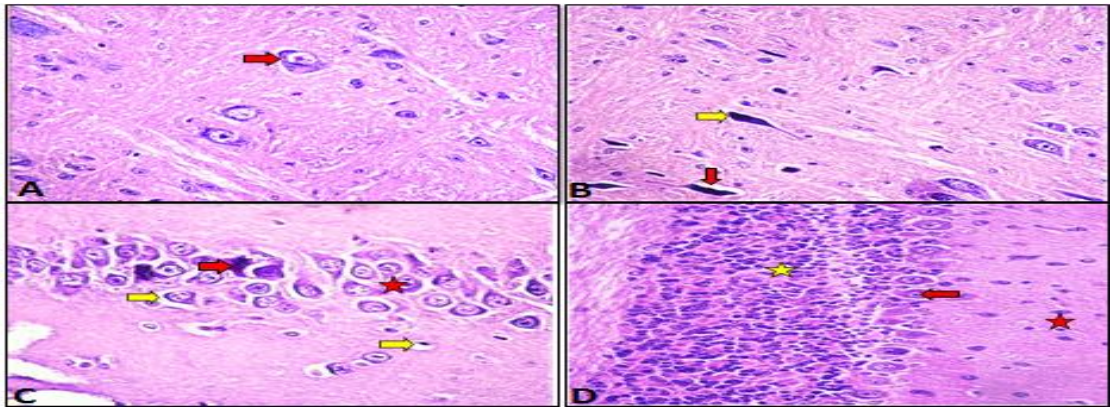

Figure 10. Photomicrograph of the brain from the ginger group after 6 weeks. (**A**): The cerebral cortex showing a high proportion of healthy neurons (red arrow). (**B**): Cerebral cortex showing a few proportions of cells suffering from chronic ischemic neuropathy (yellow arrow) and mild pericellular edema (red arrow). (**C**): Hippocampus showing healthy cells (red star), rarely observed chronic neuronal injury (red arrow), and mild perivascular and pericellular edema (yellow arrow). (**D**): Cerebellum showing, healthy molecular layer (red star), healthy Purkinje cell layer (red arrow), and healthy granular layer (yellow star). A, B, C and D (H&E, x40)

Figure 11 demonstrated the photomicrograph of the brain from the Na_2SeO_3 group after 4 weeks. The cerebral cortex showed infrequent vacuolation of neurons, perivascular and pericellular edema, astroglia cell reaction of mild to a moderate degree, and few proportions of cells suffering from chronic ischemic neuropathy. The hippocampus displayed healthy cells, a small number of neurons suffering from ischemic neuronal injury, and perivascular and pericellular edema. The cerebellum exhibited congestion of some blood vessels, necrotic changes in the granular cell layer, and a small proportion of Purkinje cells suffering from ischemic neuronal degeneration. The photomicrograph of the brain from the Na_2SeO_3 group after 6 weeks was demonstrated in Figure 12. Moderate to severe degree of perivascular and pericellular edema, astrogliosis, and chronic ischemic neuropathy was found in the cerebral cortex. The cerebellum exhibited a mild to moderate degree of chronic ischemic neuropathy in the Purkenji cell layer and necrotic changes in the granular layer.

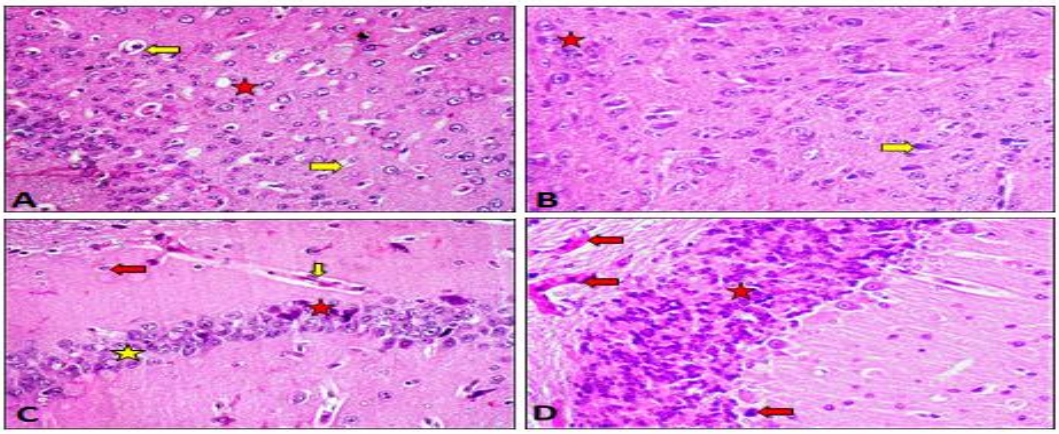

Figure 11. Photomicrograph of the brain from the Na_2SeO_3 group after 4 weeks. (**A**): Cerebral cortex showing infrequent vacuolation of neurons (red star) and perivascular and pericellular edema (yellow arrow). (**B**): Cerebral cortex showing astroglia cell reaction of mild to moderate degree (red star) and few proportions of cells suffering from chronic ischemic neuropathy (yellow arrow). (**C**): Hippocampus showing healthy cells (yellow star), a small number of neurons suffering from an ischemic neuronal injury (red star), and perivascular (yellow arrow) and pericellular (red arrow) edema. (**D**): Cerebellum showing congestion of some blood vessels (red arrow), necrotic changes in granular cell layer (red star), and a small proportion of Purkinje cells suffering from ischemic neuronal degeneration. A, B, C and D (H&E, x40)

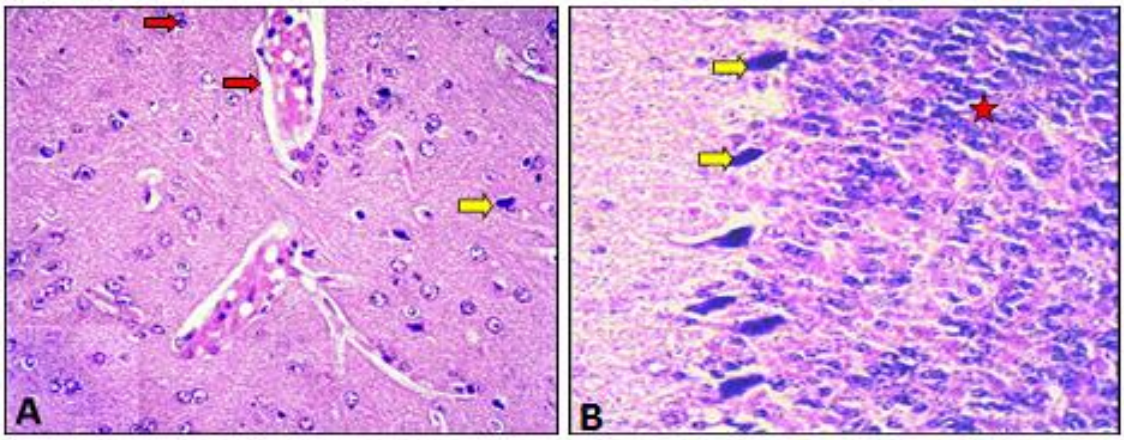

Figure 12. Photomicrograph of the brain from the Na_2SeO_3 group after 6 weeks. (**A**): Cerebral cortex showing moderate to severe degree of perivascular and pericellular edema, astrogliosis (yellow star), and chronic ischemic neuropathy (yellow arrow). (**B**): Cerebellum showing mild to moderate degree of chronic ischemic neuropathy in purkenji cell layer (yellow arrow) and necrotic changes in the granular layer (red star). A, B, C and D (H&E, x40)

In Na_2SeO_3 treated group, the low frequencies of toxic, pathological lesions, mostly of mild degree at the first sampling point, are matched with prior findings in sliver nitrate and chromium-induced neurotoxicity in rats [65]. Several peer-review publications suggested Se acts as a gate housekeeper of the brain by its involvement in the synthesis of highly potent antioxidant selenoproteins, limitation of cytosolic Ca^{2+} release, and regulation of cytokine balance [19,60,66]. Surprisingly, the brain of mice slaughtered later on after 6 weeks' post-treatment showed an advanced degree of damage than $HgCl_2$. This finding gives insight into the double-faced effect of Se, which swifts from neuroprotection [19] to neurodegeneration [67] based most probably on its supplemented dose. Overload of Se seemed neurotoxic, indicating the necessity to determine the Se dose/duration exposure protocol [68].

4. Conclusion

Apoptosis, cytological deterioration, and inhibition in catalase activity were observed in the brain of $HgCl_2$ induced neurotoxicity in mice in the time window of 4 and 6 weeks. On the other hand, garlic, ginger, and Na_2SeO_3 represented promising therapeutic modalities and offered new opportunities to treat associated neurotoxic affections. However, further studies involved dose-time responses are warranted to provide the most optimum therapeutic protocol.

Conflict of interest. The authors declare that they have no conflict of interest.

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