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Allium sativum and Vitamin C Co-administration Attenuates Mercuric Chloride-Induced Neurotoxicity in the Prefrontal Cortex in Wistar Rats

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ABSTRACT
The histological and immunohistochemical assessment of the effects of co-administration of *Allium sativum* ethanolic extract and Vitamin C on the medial prefrontal cortex of mercuric chloride induced neurotoxicity model Wistar rats was investigated. Thirty-five adult Wistar rats weighing 150 - 180 g randomized into 7 groups of 5 animals each were used for this study. Group 1 served as negative control and received 1 ml distilled water daily, group 2 served as positive control and received mercuric chloride 49.8 mg/kg body weight, group 3 received *Allium sativum* ethanolic extract 500 mg/kg body weight. Groups 4, 5, 6 and 7 were initially administered with mercuric chloride 49.8 mg/kg body weight, and treated for 14 days with *Allium sativum* 250 mg per kg body weight, Vitamin C 100 mg per kg body weight, *Allium sativum* 250 mg + Vitamin C 100 mg per kg body weight, and *Allium sativum* 500 mg + Vitamin C 100 mg per kg body weight respectively. Administrations were by orogastric intubation. Twenty-four hours after the last treatment, the rats were sacrificed under chloroform anaesthetic. The brains were harvested, processed for histological studies using H&E for general morphology, Cresyl fast violet for Nissl substance and immunohistochemical method for glial fibrillary acidic protein (GFAP) antibody expression. Results of H&E sections indicated intact pyramidal and granule cells of the prefrontal cortex in groups 6 and 7 compared to group 2. Nissl substance expression was higher in combined treated groups 6 and 7 compared to groups 3 and 4. GFAP-immunopositive astrocytes was mild in combined treated group compared to groups 2, 3 and 4. In conclusion combined administration of ethanolic extract of *A. sativum* and Vitamin C reduced the damaging effects of mercuric chloride on the medial prefrontal cortex of the Wistar rats.

Keywords: mercuric chloride; *Allium sativum*; vitamin C; prefrontal cortex; histology

INTRODUCTION
Man in his accustomed environment is constantly exposed to the potential hazards of heavy metals through their bioaccumulation and biotransformation which can be transferred via food chain as a result of anthropogenic activities [1]. One of such is mercury (Hg), a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immunological, dermatological, reproductive and developmental disorders [2]. It occurs naturally and exists in various forms; elemental or metallic mercury; inorganic (mercuric chloride) and organic (methyl and ethyl mercury), which all have different toxicities and implications for measures to prevent exposure [3].

The World Health Organization advocates the use of traditional and complementary medicine in keeping populations healthy [4]. Medicinal plants are used for treatment of many diseases instead of synthetic drugs [5]. Some plants, such as garlic (*Allium sativum*), due to the contained ingredients, including sulfyhydrl, diallylsulphide and alexin has antitoxic, antimutagenic and anticarcinogenic properties, thus used to improve metal toxicity [6]. The water-soluble organosulfur and cysteine S-allyi compounds of *A. sativum* extract have strong antioxidant potential that can cause free radicals scavenging in lead poisoning comparable with the vitamin C oxidant properties [7, 8].

A. sativum has been investigated in various organs including the hippocampus, frontal lobe, heart, among others and several benefits as well as adverse effects have also been reported. One of its beneficial effects is its neuroprotection against tumours which is achieved via the actions of its constituents, diallyl sulphide, diallyl disulphide and diallyl trisulphide [9]. Other beneficial effects include increase in memory retention [10], learning and cognition [11], and prevention of neuronal damage in the hippocampus [12]. Prevention of degeneration of the frontal lobe [11] and reduction in diabetic levels via its action as an insulin secretagogue by S-allyl cysteine sulphoxide (isolated product from *A. sativum*) have also been reported [13]. Adverse effects of *A. sativum* include loss of body weight and lyses of red blood cells [14].

Antioxidants such as vitamin C may decrease injurious activity of reactive oxygen species (ROS) and free radicals [15] against mercury toxicity. Vitamin C is an antioxidant that prevents the production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues [16, 17] and singlet oxygen [18, 19]. These anti-oxides are generally regarded as primary first line protective agents that nullify free radicals by donating a single electron to yield dehydroascorbic acid [20, 21].

The prefrontal cortex (PFC) is a part of the frontal lobe situated posterior to the forehead and is regarded as one of the most important areas in the brain. The region is responsible for executive functions which include; resolution of conflicting thoughts, determination of choices between right and wrong, good and bad, forecasting future events, governing social and emotional control [22]. The PFC is also known to be important for cognitive control, enabling behavior to be at once flexible yet task focused [23]. The PFC is divided into ventromedial and dorsolateral regions: The ventromedial PFC has reciprocal connections with brain region that are associated with emotional processing (amygdala), memory (hippocampus) and higher-order sensory processing (temporal visual association areas) as well as with dorsolateral PFC. The dorsolateral PFC is involved in representing cognitive action [24].

The use of *A. sativum* and vitamin C in this research is of great interest because of its culinary uses and reported effects on related parts of the brain. Available literature has documented various toxicity report of mercuric chloride on the liver, cerebellum and kidney [19]. Documentary evidence on the combined effects of vitamin C and *A. sativum* extract on the histology of the medial PFC is scanty. The aim of the present study was to investigate the possible protective effect of ethanolic extract of *A. sativum* and ascorbic acid on mercuric-induced toxicity on the PFC of adult Wistar rat.

MATERIALS AND METHODS

Experimental animals

Thirty-five adult male albino Wistar rats weighing between 150 – 180 g were were obtained and kept in the Animal House of the College of Health Sciences, University of Uyo. They were housed in 7 cages (40 cm x 35 cm) with adequate space to encourage free movement. Animals were allowed 12 hours' light and dark cycles respectively at a room temperature of 27°C -30°C. They were fed standard rat pelletized diet (Vital Feed®, Grand Cereals Ltd, Nigeria) and water *ad libitum*. The animals were allowed to acclimatize for one week and thereafter randomly allotted into 7 groups consisting of 5 rats. Group 1 was the control and groups 2 to 7 were the test groups. The weight of the animals was taken at the beginning of the experiment and subsequently, weekly. This experiment followed the guidelines for the care and use of laboratory animals [25].

Collection of *Allium sativum*

Whole fresh aged garlic were purchased from Itam market, Uyo, Akwa Ibom State and taken to the University of Uyo Herbarium Unit, and authenticated by a taxonomist. The specimen was deposited with voucher number UUPH44(b).

Preparation of Plant Extract

The fresh and aged bulbs of *A. sativum* were thoroughly washed with tap water to remove dust particles and debris. The *A. sativum* bulbs were peeled and cut into smaller pieces. The bulb was air dried on the laboratory bench for a week. The dried plant materials were ground into powder and 500 g of the powdered sample was macerated in 6000 ml of 70 % ethanol for 24 hours, after which it was filtered and concentrated at 40 °C in a water bath. The yield of crude extract of *A. sativum* (58.40 g) was obtained and stored in a refrigerator at about 8 °C.

Acute Toxicity of *Allium sativum*

Eighteen (18) male Swiss albino mice weighing between 25 – 30 g were obtained from the Animal House, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State. The mice were housed and acclimatized for a period of one week, prior to the commencement of the acute toxicity test. Acute oral toxicity was carried out according to [26]. The test was performed using increasing doses of the extract given orally, 100 mg to 5000mg/kg body weight to different mice group, in different phases. In the initial phase, the mice were divided into three groups of three each, and were treated with 100, 400, 1600 mg/kg body weight of the extract orally. In the final phase, 9 mice were divided into three groups of three each, and were treated with 2900, 4000 and 5000mg/kg body weight of the extract. The mortality, general behavior and toxic symptoms of the mice were observed for 24 hours as well as delayed toxic symptoms for 7 - 14 days.

Phytochemical Screening of *Allium sativum*

Twenty (20) g of the powdered *A. sativum* was dissolved in 100 ml of ethanol for 1 hr. The extract was refluxed in a flask twice for 30min, filtered with a Whatman No.1 filter paper and concentrated to 50 ml. The extract was used for the phytochemical test according to the methods as described by [27, 28].

Administration of *Allium sativum* Extract, Mercury Chloride and Vitamin C

Group 1 served as control and was administered 1 ml/kg of distilled water per body weight. Group 2 were administered mercuric chloride 49.8 mg/kg bwt once on the last day of the experiment, while Group 3 was administered *A. sativum* 500 mg/kg bwt. Groups 4 to 7 were administered mercuric chloride 49.8 mg/kg bwt only on the first day of the experiment. Group 4 was subsequently given *A. sativum* 250 mg/kg bwt alone; Group 5 was administered Vitamin C 100 mg/kg bwt; Group 6 was administered *A. sativum* 250 mg /kg bwt and Vitamin C 100 mg/kg bwt. Group 7 was administered *A. sativum* 500 mg/kg bwt and Vitamin C 100 mg/kg bwt. The *A. sativum* extract and Vitamin C were administered orally for 14 days in the morning.

Termination of Experiment

On the 15th day the animals were anaesthetized by inhalation of chloroform, the thoraco-abdominal wall dissected to expose the heart, and intracardial perfusion of PBS was carried out by means of a cannula and then perfused-fixed using 10 % buffered formalin. The brain of all the animals were excised and fixed in 10 % buffered formalin for 48 hours. The slices of the PFC were obtained and processed for H&E; Cresyl fast violet staining methods and GFAP antibody expression.

Tissue Processing

Experimental animals were euthanized via chloroform inhalation then intracardiac perfusion carried out with phosphate buffered saline for 2 minutes and thereafter with 4 % paraformaldehyde until tail stiffness in approx. 15 minutes. Animal brains were dissected out and fixed in

4 % paraformaldehyde and processed for light microscopy 72 hours later. The paraffin wax blocked tissues sectioned at 5 microns with the rotary microtome (Microtome Thermo Scientific – Microm HM 325, England) were stained with haematoxylin and eosin, cresyl fast violet, and immunohistochemical method for glial fibrillary acidic protein antibody (GFAP-Dako, Lot 00083681) and scored using [29], and blindly assessed by 2 other independent histopathologists. Amscope digital camera (MU 1000, China) was attached to a microscope (Olympus - CX31, Japan) to obtain images.

RESULTS

Median lethal dose of *Allium sativum* extract

No signs of acute toxicity or mortality were observed with increasing doses of the extract up to 5000 mg/kg body weight. At this point the process was discontinued and the median lethal dose (LD50) of the ethanolic extract of *Allium sativum* estimated to be over 5000 mg/kg body weight (Tables 1)

Phytochemical analysis

Phytochemical screening of *Allium sativum* indicated it is rich in alkaloids, flavonoids, saponins, glycosides, carbohydrates and protein (Table 2).

Mortality of the animals

There was no mortality in the control and group 3 administered only *Allium sativum* 500 mg/kg bwt. Group 2 administered mercuric chloride had the highest mortality rate, groups 4, 5, 6 and 7 induced with mercuric chloride then administered with garlic + vitamin C, respectively, at different doses had mortality rate ranging from 40 to 60 % (Table 3).

Table 1 Acute toxicity (LD_{50}) of ethanolic extract of *Allium sativum*

Group	No of animals	Dose (mg/kg body weight)	Observation
1	3	100	No fatality
2	3	400	No fatality
3	3	1600	No fatality
4	3	2900	No fatality
5	3	4000	No fatality
6	3	5000	No fatality

Table 2 Phytochemical analysis of ethanolic extract of *Allium sativum*

Chemical constituents	Observations
Alkaloids	++
Flavonoids	+
Saponins	+
Glycoside	+
Carbohydrates	+
Protein	+++
Steroids	++
Anthraquinones	-
Tannins	+
Terpenoids	++
Cardiac glycosides	++

- = not present, + = lowly present, ++ = moderately present, +++ = highly present

Protein were highest followed by alkaloids, steroids, terpenoids and cardiac glycosides. Flavonoids, saponins and tannins were present in minute quantities while there was absence of anthroquinones (Table 4.2)

Table 3: Percentage mortality following co-administration of *Allium sativum* and vitamin C

Group	Number that survived	% Mortality
1	5	0
2	2	60%
3	5	0
4	3	40%
5	2	60%
6	2	60%
7	2	60%

Histological and immunohistochemical analysis
Histological observation
Groups 1 to 7 are represented in the photomicrographs A to G. The PFC photomicrograph of the control (Group 1) showed normal histoarchitecture with well stained granular cells, well arranged pyramidal cells, and blood vessel in the cortical layer (Fig. 1A).

The PFC photomicrograph of group 2 administered single dose of mercuric chloride 49.8 mg/kg bwt showed hypertrophic neurons and granular cells degenerations, scanty and lightly stained pyramidal cells, cortical vacoulations in the cortical layers (Fig. 1B).
The PFC photomicrograph of group 3 administered only *A. sativum* 500 mg/kg bwt showed scanty and degenerated granular cells, pyknosis of granular cells, scanty and loosely spaced pyramidal cells, distorted and vacoulations within the cortical layers (Fig. 1C).
The PFC photomicrograph of group 4 administered mercuric chloride 49.8 mg/kg bwt and *A. sativum* 250 mg/kg bwt had features of hypertrophic neurons, vacuolations, and neuronal shrinkage of pyramidal cells in the cortical layer (Fig. 1D).

The PFC of group 5 administered mercuric chloride 49.8 mg/ kg bwt followed by vitamin C 100 mg/kg bwt had features of neuronal hypertrophy of the granular cells, neuronal shrinkage, numerous blood vessels, and regenerating neurons with numerous pyramidal cells (Fig.1E).
The PFC photomicrograph of ventromedial part of group 6 administered mercuric chloride 49.8 mg/kg bwt followed by *A. sativum* 250 mg/kg bwt and vitamin C 100 mg/kg bwt respectively had features showing numerous granular cells, numerous and evenly spaced pyramidal cells, regenerating granular cells (Fig 1F).
The PFC photomicrograph of group 7 administered mercuric chloride 49.8 mg/ kg bwt followed by *A. sativum* 500 mg/kg bwt and vitamin C 100 mg/kg bwt presented neuronal cells with hypertrophy, degenerated granular and pyramidal cells, atrophied and shrunken neurons (Fig. 1G).

Nissl substance demonstration
The PFC photomicrograph of control (Group 1) showed a normal expression of Nissl substance. Nissl substance were weakly stained (Fig. 2A).
The PFC photomicrograph of group 2 administered a single dose of mercuric chloride 49.8 mg/kg bwt showed increase in Nissl substance with the cells strongly stained (Fig. 2B).
The PFC photomicrograph of group 3 administered *A. sativum* 500 mg/kg bwt showed moderately stained Nissl substance (Fig. 2C).

The PFC photomicrograph of groups 4 and 5 administered 49.8mg/kg bwt of mercuric chloride + *A. sativum* 250 mg/kg and mercuric chloride 49.8 mg/kg bwt + vitamin C 100 mg/kg bwt, respectively demonstrated strongly and weakly stained Nissl substance (Figs. 2D and 2E).
The PFC photomicrograph of group 6 expressed moderately stained Nissl substance (Fig. 2F).
The PFC photomicrograph of group 7 had strongly stained Nissl substance (Fig. 2G) compared to the control.

Expression of reactive astrocytes
Section of the PFC of the control showed normal GFAP expression, which were seen in astrocytic processes and cell bodies throughout the cortical layers (Fig. 3A). In group 2 administered single dose of mercuric chloride 49.8 mg/kg bwt there was increased GFAP expression mostly in the processes throughout the cortical layers (Fig. 3B). The group 3 administered *A. sativum* 500 mg/kg body weight showed increased GFAP expression, seen in astrocytic processes and cell bodies (Fig. 3C); while the group 4 showed a marked reduction in GFAP expression. The expression was only in the astrocytic processes (Fig. 3D). The group 5 administered mercuric chloride 49.8 mg/ kg bwt and vitamin C 100 mg/kg bwt showed reduced and focal areas of GFAP expression, seen in the astrocytic processes (Fig. 3E), while Group 6 administered mercury chloride 49.8 m/kg bwt + *A. sativum* 250 mg/kg bwt and vitamin C 100 mg/kg bwt showed a markedly reduced GFAP expression detected in the astrocytic processes in isolated areas (Fig. 3F). The group 7 administered mercuric chloride 49.8 mg/ kg bwt + *A. sativum* 500 mg/kg bwt and vitamin C 100 mg/kg bwt showed highly reduced GFAP expression and absence of GFAP expression in some regions like the multiform layer of the cortex (Fig. 3G).

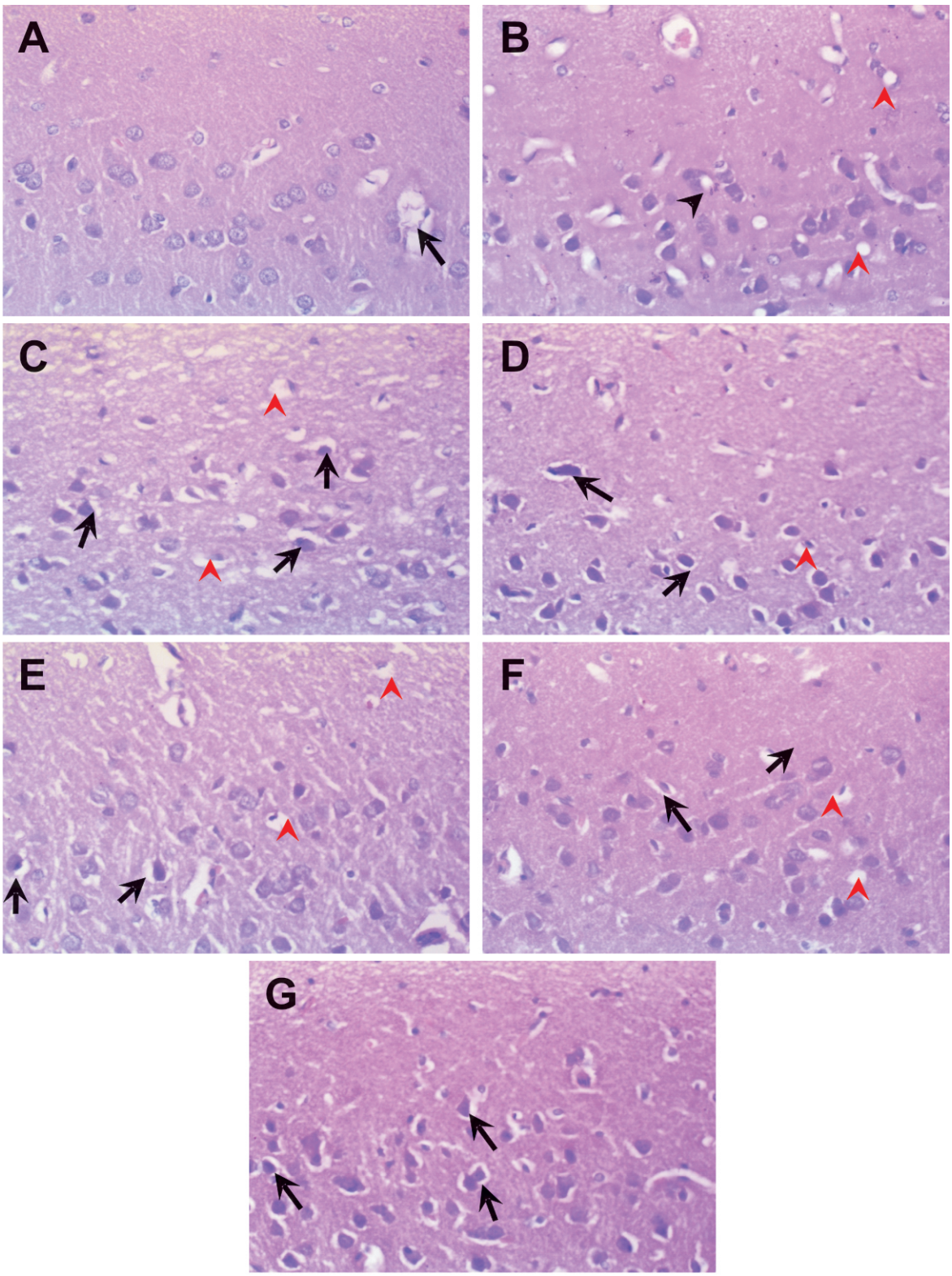

Figure 1: Photomicrograph of medial prefrontal cortex of NC (A) showing good cytoarchitecture, and the test groups (B - G) with dense atrophic neurons in red arrow; severe distortions in B, D, E and F, and moderate distortion in G and H. H&E stained x400

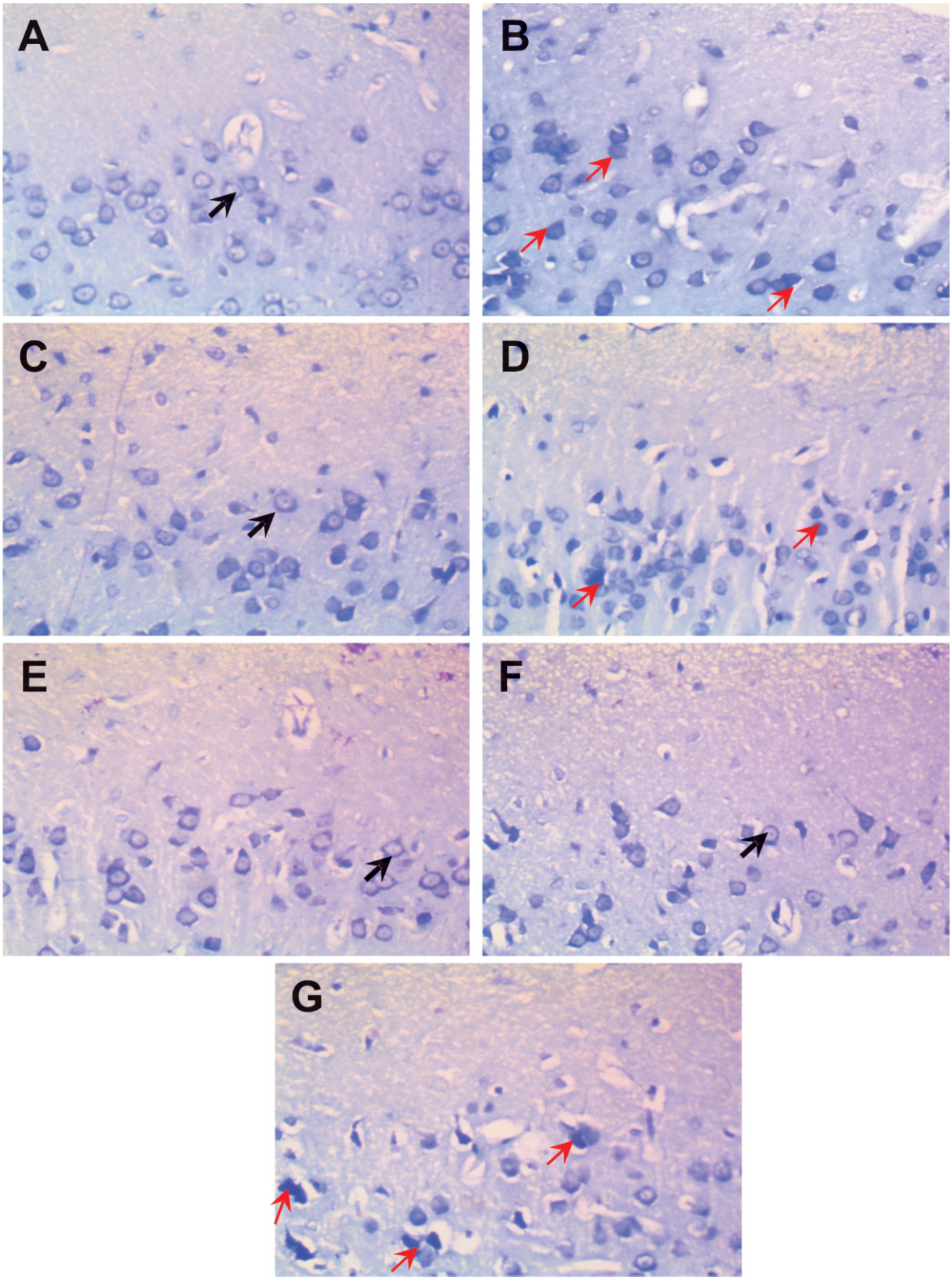

Figure 2: Photomicrograph of medial prefrontal cortex (A and E) showing lowly-stained Nissl substances, moderate (C and F), while (B, D and G) have high Nissl substances; red arrow – dark stained Nissl substances. Cresyl fast violet stained x400

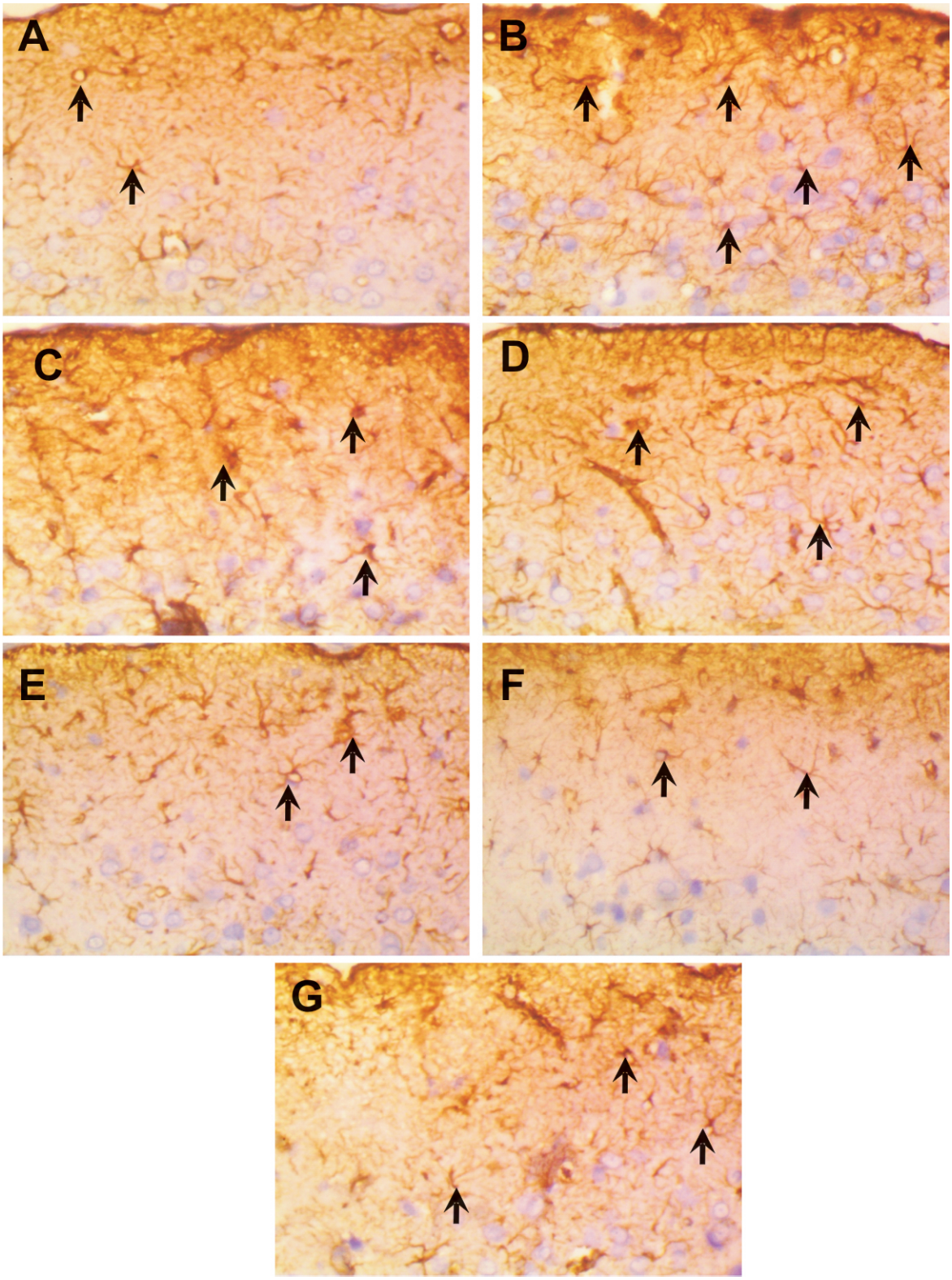

Figure 3: Photomicrograph of medial prefrontal cortex showing lowly expressed GFAP antibody in (A and F), moderate expression in (D and E) while strong expression was in groups (B, C and G). GFAP antibody stained x400

Table 4: Effect of HAARTs on Prefrontal cortex GFAP expression

Group	(A) % of IHC	(B) Intensity of IHC	(A+B) Final score	Expression
1. NC	30 - 60 %	Mild	4	Low
2. MC	> 60%	Strong	6	High
3. AS_{500}	30 - 60 %	Mild	4	Low
4. MC+ AS_{500}	> 60%	Strong	6	High
5. MC+$VitC_{100}$	30 - 60 %	Mild	4	Low
6. MC+ AS_{250}+Vit C_{100}	30 - 60 %	Mild	4	Low
7. MC+ AS_{500}+Vit C_{100}	> 60%	Strong	6	High

Legend: NC – normal control; MC – mercuric chloride; AS – Allium sativum; Vit C - vitamin C

Key: % IHC	Intensity of IHC	Final Score	
0 = 0 %	0 = No reaction	A+B	= Range from 0 to 6
1 = < 30 %	1 = Weak	0/6	= Negative Reaction
2 = 30 - 60 %	2 = Mild	1/6, 2/6, 3/6	= Low expression
3 = > 60 %	3 = Strong	4/6, 5/6, 6/6	= High expression

DISCUSSION

Mercury intoxication is commonplace amongst miners and industrial workers. Mercury have been detected in the hippocampus (medium 0.04 μg/g) and cortex (medium 0.06 μg/g) of rat brains, suggesting that mercury has a slightly higher affinity for the cerebral cortex than the hippocampus [30]. Hence this study was designed to evaluate the effect of co-administration of *A. sativum* and vitamin C against mercury toxicity in the prefrontal cortex of adult Wistar rats.

The result of acute toxicity of the ethanol extract of *A. sativum* showed a median lethal dose of over 5000 mg/kg body weight. This value is similar to those obtained by [31] and indicates that garlic has a low acute toxicity, and is relatively safe for consumption.

The phytochemical screening of the extract showed that *A. sativum* is rich in proteins, alkaloids, steroids, terpenoids, glycosides, tannins, carbohydrates, flavonoids, saponins, and cardiac glycosides, but not in anthroquinones. These chemicals may account for the biological activities associated with this extract. Several of these phytochemicals have been shown to have antioxidant effect on heavy metals toxicity. Flavonoids help in preventing atherosclerosis and neoplasmatic changes [32]. In living cells proteins are directly involved in the cellular defense mechanism against oxidants [33]. Alkaloids generally provide protection to plants against microbial or herbivore attack and UV-radiation [34]. The phytochemical screening showed that garlic extract had more proteins and this may suggest that protein is the main constituent of garlic which acts as cellular defense mechanism against mercury toxicity.

In this study, section of the PFC stained with H&E showed neuronal shrinkage in group 2 administered mercuric chloride 49.8mg/kg bwt. There was evident vacuolated lesions across the layers of the cortex.

The section of PFC cortex in group 3 administered *A. sativum* extract 500 mg/kg bwt also showed a lesser cellular density in the cortical layers. The section of the PFC of group 4 administered mercuric chloride 49.8 mg/kg and *A. sativum* 250 mg/kg bwt showed a decrease in cellular density, although groups supplemented with vitamin C showed regenerative tendencies compared to other treatment groups.

The group administered *A. sativum* 250 mg/kg bwt + vitamin C 100 mg/kg bwt showed an increase in cellular density and the group treated with *A. sativum* 500 mg/kg bwt and of vitamin C 100 mg/kg bwt showed decrease in cellular density compared to control, and the group administered only mercuric chloride These observations suggest that co-administration of *A. sativum* with vitamin C gives a better protective tendency to the metallic-chloride poisoning and cause a regenerative potential to toxicity-exposed neural cells. However, neural degenerations observed in a higher dose co-administered *A. sativum* suggest that it may also induce oxidative stress to tissue cells at higher concentration by increasing free radical deposit, which may be due to an incomplete metabolism of the *A. sativum* within the biological system.

The PFC of mercuric chloride-induced, untreated group indicated neuronal distortions with signs of neurodegeneration, poor expression of Nissl substance and neuroinflammation. In a sub-chronic study lasting 4 weeks, mercuric chloride at 4 mg/kg body weight of mice caused ischaemic neuronal degeneration with foci to diffuse alterations in the cerebrum, perivascular and pericellular oedema were severe, astroglial reactivity were severe, with perivascular cuff, congestion and thrombosis [35]. Mercuric chloride initiates multiple additive or synergistic disruptive mechanisms,

principal of which is the mitochondrial disruption directly linked to reactive oxygen species production. The over activation of N-Methyl-D-aspartate receptor is likely the initial trigger for the downstream of cascades in $HgCl_2$-induced toxicity, as this injurious effect in the brain can be blocked and effectively mitigated by the inhibition of N-methyl-D-aspartate receptor activity [36]. The PFC in the co-administered groups showed better ameliorative potential than in groups.

Section of the PFC stained with GFAP antibody for astrocytes, showed gliosis in the group 2, and administered only mercuric chloride 49.8 mg/kg bwt without the extract (Fig 3B). This was evident by increased GFAP expression in the astrocytic processeses and cell bodies, with score in (Table 4). The control group expressed normal immuno-positive astrocytes. The section of the PFC in group 3 administered *A. sativum* extract 500 mg/kg bwt also showed an increased GFAP expression as this was evidence by the presence of gliosis in the pyramidal and granular cells. There was a marked reduction in the expression of the astrocytic protein in the rats which were given 49.8 mg/kg body weight of mercuric chloride followed by 250 mg/kg body weight of the extract, indicating that the extracts possess a better neuroprotective effect than vitamin C. Astrogliosis was seen in the section of the PFC administered mercuric chloride 49.8 mg/kg bwt + vitamin C 100 mg/kg, as this resulted into abnormal increase in number of astrocytes due to destruction of neurons from the central nervous system and neurodegenerative changes. The group administered *A. sativum* 250 mg/kg bwt + vitamin C 100 mg/kg bwt showed a moderate GFAP expression, while the group administered *A. sativum* 500 mg/kg body weight and vitamin C 100 mg/kg bwt showed less GFAP expression compared to control, and the group administered only mercuric chloride, indicating that the extract possesses more neuroprotective effect than vitamin C as evident by decreased astrogliosis.

Astrocytes, constituting the majority of resident glial cells, are about 10 times more in number than neurons in normal adult brain. Evidence suggests that astrocytes play an active role in central nervous system function by influencing or even directing the neuronal activities [37]. The GFAP is the principal intermediate filament protein of mature astrocytes in the central nervous system and is probably involved in controlling the shape and movement of astrocytes. Although GFAP is not required for the morphogenesis of the central nervous system [38], this protein does play other important roles. The GFAP positive mice display enhanced long-term potentiation of both population spike amplitude and excitatory post-synaptic potential slope in the hippocampus after tetanic stimulation [39]. The report by Shibuki *et al.,* [40] also indicated that GFAP negative mice are deficient in long-term depression at distinct sites in the brain as well as in eye blink conditioning. Therefore, it has been suggested that GFAP is important for astrocyte-neuronal interactions, and that GFAP-mediated astrocytic processes play a vital role in modulating synaptic efficacy in the central nervous system. In addition, GFAP expression is essential for normal white matter architecture and blood–brain barrier integrity in aged mice and its absence may be responsible for a late onset of CNS demyelination [41]. The section of the PFC group administered *A. sativum* extract 500 mg/kg bwt also showed an increased GFAP expression of as this was evident by the presence of gliosis in the pyramidal and granular cells.

Vitamin C administration has been demonstrated to ameliorate the mercuric chloride-induced degenerative changes in the cerebellum of Wistar rats [19]. Vitamin C is a vital antioxidant molecule in the brain; it also has a number of other important functions, participating as a co-factor in several enzyme reactions including catecholamine synthesis and collagen production. Ascorbic acid is transported into the brain and neurons via sodium dependent vitamin C transport-Z (SVCTZ) which causes accumulation of ascorbate within the cells against concentration gradients [42]. In comparison with the single dose groups administered with *A. sativum* along with vitamin C had a better neuro-protective effect than those given only vitamin C. Farooqui and Farooqui, [43] also reported that garlic is an effective scavenger of ROS and inhibits low-density lipoprotein (LDL) oxidation; prevents endothelial cell damage by increasing superoxide dismutase, catalase and glutathione peroxidase activities.

These changes may be transient, but permanent abnormalities may be induced only by sustained exposure of this chemical in an excessive quantity [44]. Decreased pyramidal cellularity and parenchymal hypochromasia seen in the rats administered mercuric chloride in this study is indicative of potential decline in the functional anatomy of related area such as those responsible for skilled movement, executive function, decision making and thought processing. The loss of movement and a diminished response to noise are attributable to systemic perturbations from mercury chloride [45]. The abnormal activity in the ventro-medial prefrontal cortex has been consistently linked in neuropsychiatric disorders. This result corroborates [46], who reported that acute doses of mercuric chloride-induced alterations in the cytoarchitecture of the cerebral cortex, with significant reduction in the frequency of transitions, rearing and geotaxis latency.

Mercuric chloride is toxic to astrocytes and microglia [47], causes *myelin disintegration*, *cell organelle alterations and neuronal loss* [48]*, and it is* either causal or contributory in the brain pathology of autism spectrum disorder [49]. Histopathological examination of the brain revealed that normal cytoarchitecture of all

the three areas of cerebrum, cerebellum and hippocampus were distorted resulting in various neurological disorders following mercuric chloride exposure [50].

A. sativum administration has been investigated against mercuric chloride-induced toxicity in the cerebrum, hippocampus and cerebellum of the brain, and had promising and a great improvement towards the tendency of histoarchitectural normalization of this brain structure. In mercuric chloride-induced animals spongiosis and focal areas of necrosis were seen with prominent reduction in the number of neurons showing ischemia neuronal injury and these effects were improved following garlic and ginger [36]. Moreover, astrogliosis, perivascular and pericellular oedema were very mild and were infrequently observed. This prominent improvement was due to antagonistic effect of *A. sativum* against mercuric chloride-induced toxicity [51].

CONCLUSION

In conclusion the combined administration of ethanolic extract of *A. sativum* at low doses, and together with Vitamin C reduced the damaging effects of mercuric chloride on the medial PFC of the Wistar rats.

Conflict of Interest

Authors declare that there is none.

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Author's Contributions

BEU performed the experiments, BEU and AUE, IAE analyzed the data and wrote the paper, AUE and IAE designed and supervised the research.

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