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ORIGINAL ARTICLE - NATURAL PRODUCTS AND PHYTOCHEMISTRY

Ameliorative Effects of Aqueous Leaf Extract of *Cola acuminata* on Mercury Chloride-induced Neurotoxicity in Male Wistar Rats

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Abstract

Aim: **The ameliorative effect of aqueous leaf extracts of Cola acuminata (ALECA) on mercury chloride ($HgCl_2$)-induced neurotoxicity in male Wistar rats was investigated.**

Methods: **Thirty-six male Wistar rats weighing 120−150 g were randomly divided into six groups; group I received 0.5 ml normal saline; group II received 5 mg/kg body weight $HgCl_2$; group III received 5 mg/kg $HgCl_2$ 150 mg/kg body weight ALECA; group IV received 5 mg/kg $HgCl_2$ 300 mg/kg ALECA; group V received 5 mg/kg $HgCl_2$ 50 mg/kg N-acetyl cysteine (NAC); and group VI: received 5 mg/kg $HgCl_2$ 500 mg/kg body weight vitamin C. The animals were treated for 21 days through the oral route. The rats were subjected to open field tests and forelimb grip tests on days 14 and 21. The animals were then killed and brain tissues were collected, homogenized, and centrifuged for measurement of biochemical parameters: tumor necrosis factor alpha and nitric oxide.**

Results: **The data obtained from the forelimb grip test showed that muscular strength was higher in the group given ALECA, NAC, and vitamin C when compared with the group given only $HgCl_2$ (P 0.05). The data from the open field test also showed that locomotory activity and exploratory behavior are higher while anxiety levels are lower in the group treated with ALECA, NAC, and vitamin C when compared with the $HgCl_2$ group (P 0.05). Also, the levels of tumor necrosis factor alpha and nitric oxide are lower in the group treated with ALECA when compared with the $HgCl_2$ group (P 0.05). From the results, it was established that ALECA-treated groups compared favorably with the two reference drugs.**

Conclusions: **These findings show the ameliorative effect of ALECA on mercury chloride induced neurotoxicity/ brain damage through modulation of inflammatory pathway.**

Keywords: *Cola acuminate*, Mercury chloride, Neurotoxicity, Nitric oxide, Tumor necrosis factor

1. Introduction

Human exposure to metals is inevitable, and some studies have reported sex differences in the toxicity of metals [1,2]. They may frequently react with biological systems by losing one or more electrons and forming metal cations that have an affinity to the nucleophilic sites of vital macromolecules. There is a growing appreciation of the effects that exposure to heavy metals such as mercury (Hg) may have on the body and, in particular, the brain and nervous system. This is because some of these metals can cross the blood−brain barrier and accumulate in the brain and cause damage [3]. There are many reported cases of mercury food poisoning in Sweden, Mexico, Unites States, and the Minamata Bay that led to the poisoning of more than 800 people. The toxicity of mercury can result from vapor inhalation and ingestion or absorption through the skin. Nervous, digestive, and renal systems are most commonly affected by Hg exposure, while children and pregnant women are most vulnerable to Hg exposure [4]. Hg represents the third most toxic

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element on the planet, according to the US Government Agency for Toxic Substances and Diseases Registry [5]. Three forms of Hg can occur in the environment which are organic Hg, elemental Hg, and inorganic Hg [6]. Inorganic Hg such as mercury chloride ($HgCl_2$) has low lipid solubility, so it is less absorbed and less passed through the blood–brain barrier. Despite its poor ability to cross biological barriers, it is still able to induce motor deficits as it causes apoptosis and decreases the number of neurons and astrocytes in the motor cortex [7]. Its absorption through the blood–brain barrier can be facilitated when there is bacteria methylation and inorganic mercury is converted to organic mercury. Organic Hg is one of the most toxic substances, with the highest number of studies dealing with their side effects on humans [8]. It is known that organic Hg such as methyl mercury has a high capacity to cross cellular membranes such as the blood–brain barrier, accumulates in the hippocampus, and damages its important function regarding learning and memory [9]. After ingestion, it can pass through the blood–brain barrier and be metabolized to inorganic Hg, which is therefore present at low doses in the central nervous system [5]. Accumulation of Hg in the brain can result in disruption to the nervous system, which is the basis of neurotoxicity. This disruption can occur through the increase in inflammatory processes in the brain, and this is evident in the marked increase in inflammatory parameters during neurotoxicity.

Cola, a tropical African genus of the family sterculiaceae, comprises about 125 species. Cola species are evergreen. *Cola acuminata* has the greatest economic importance among all species. The leaves of cola species are simple, entire, and narrowed or rounded toward the base. The arrangement of the leaves on the stem is alternate in some species and verticulate in whorls of three or more in others [10]. This plant is rich in phytochemicals. Qualitative analysis showed that the leaves of *C. acuminata* contain bioactive components; it shows a high concentration of alkaloid and phenolic acid in young leaves. Flavonoids and carotenoids are in a smaller concentration when compared with alkaloids and phenolic acid. Quantitative analysis of the various components revealed different bioactive components and their composition. The medicinal values of this plant may be related to their constituent phytochemicals because the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value [11].

N-acetylcysteine (NAC) is a sulfhydryl-containing compound, with mucolytic properties, originally patented in 1960, and its use in medicine was first reported in 1967 [12]. Clinically it has been used in cystic fibrosis since 1969 [13]. Since then, NAC use has been expanded to acetaminophen overdose and chronic obstructive lung disease and its role is ever expanding clinically. Cysteine is found naturally in meat, fish, grains, dairy, soybean, and egg products [14]. As a nutritional supplement, NAC is found in small amounts naturally in some fruits and vegetables [15]. The properties of NAC include enhancing glutathione S-transferase activity, repleting glutathione, scavenging free radicals, and stabilizing protein structures by crosslinking cysteine disulfide molecules along with its antioxidant, anti-inflammatory, and mucolytic properties. N-acetylcysteine has been shown to chelate toxic metals in animal studies as well as in human studies with little or no effect on essential metals [16].

Vitamin C, also known as L-ascorbic acid, ascorbic acid, and L-ascorbate is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Sources of vitamin C include tomatoes, kiwi fruit, vegetables, potatoes, green peas, oranges, and orange fruit. Vitamin C is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters; vitamin C is also involved in protein metabolism [17]. It has been shown that mercury exposure can induce degeneration in the cerebrum of adult Wistar rats [18]. Ge'gotek and Skrzydlewska [19] reported that ascorbic acid, as one of the basic exogenous vitamins, occurs in the body in the form of ascorbate, known for its strong antioxidant and anti-inflammatory properties.

Given the lack of scientific evaluation of the aqueous leaf extract of *C. acuminata* (ALECA) in addressing $HgCl_2$-induced neurotoxicity and also the absence of any intervention for mercury poisoning by addressing its inflammatory and reduction of the antioxidant system, the present study was undertaken to evaluate the ameliorating effects of ALECA on $HgCl_2$-induced neurotoxicity. The study investigates the effect of ALECA on $HgCl_2$-induced neurotoxicity in male Wistar rats.

2. Materials and methods

2.1. Identification of plant and preparation of extract

C. acuminata leaves were obtained from a farm in Ilupeju in Ekiti State. It was authenticated by a taxonomist in the Department of Botany, University of Ilorin under voucher number UILH/001/701/2021. The leaves were washed carefully with water

to remove dirt and dust. The washed leaves were air-dried at room temperature for about 2 weeks until they were completely dried. After complete dryness, the leaves were pulverized using an electric blender to give a smooth dry powder. A measure of 200 g of the pulverized *C. acuminata* leave powder was soaked in 1 l of distilled water for 12 h after which it was filtered using a muslin cloth. The filtrate was freeze-dried to produce 20 g of extract. Quantitative and qualitative analyses were done on the extract.

2.2. Experimental design

The animals were acclimatized for 2 weeks after which they were grouped randomly into six groups. The animals were administered with aqueous leave extract of *C. acuminata*, $HgCl_2$, NAC, and vitamin C. On day 14 of administration, behavioral tests (open field test and forelimb grip test) were carried out. This is followed by 1 week of administration. On day 21, the behavioral tests were repeated and then the animals were killed.

2.3. Experimental animals

In all, 30 male Wistar rats weighing between 120 and 150 g bought from Temilade Investment in Ogbomoso, Oyo State were used for this study. They were all housed in plastic cages at room temperature in the animal house at the Central Research Laboratory, University of Ilorin, Ilorin. The cages were well-ventilated, clean, secured from predators, and lined with animal beddings (sawdust) to make them comfortable and were changed daily to keep them clean and prevent infections. The research was approved by the Ethics Committee of the Department of Physiology, College of Health Sciences, University of Ilorin, and it was carried out in accordance with the guidelines of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The animals were acclimatized for 2 weeks before the beginning of the experiment. They were kept under a 12 h light and dark cycle at normal room temperature and humidity and fed with rat pellets from Ogo Oluwa Feed mill, Sango, Ilorin. Feed and water were provided *ad libitum*. The animals were grouped as follows:

Group 1: control 0.5 ml normal saline.
Group 2: 5 mg/kg $HgCl_2$ [20].
Group 3: 5 mg/kg $HgCl_2$+150 mg/kg ALECA [21].
Group 4: 5 mg/kg $HgCl_2$+300 mg/kg ALECA [22].
Group 5: 5 mg/kg $HgCl_2$+50 mg/kg NAC [23].
Group 6: 5 mg/kg $HgCl_2$+500 mg/kg vitamin C.

2.4. Tissue preparation

Rats were anesthetized with an intraperitoneal administration of 75 mg/kg [24] of ketamine, followed by transcardial perfusion with PBS 7.4 to remove blood from all organs, most especially the brain. The brain was then removed, weighed, and then homogenized. The homogenate was centrifuged and the supernatant was collected. A biochemical assay to determine the level of tumor necrosis factor α (TNF-α) and nitric oxide (NO) was carried out.

2.5. Behavioral test

On experimental days 14 and 21, rats were subjected to behavioral studies which were the open-field test and the forelimb grip strength test.

2.5.1. Open-field test

The animals were placed for 5 min in an open-field arena. The apparatus, made of wood covered with impermeable formica, had a white floor of 100 × 100 cm (divided by black lines into 16 squares of 20 × 20 cm) and high white walls to prevent escape. Each rat was placed at the center of the open field and was free to explore the unfamiliar arena; the total number of squares crossed, rearing, grooming, freezing, and center square duration was measured [23,25]. The quadrant was considered crossed when the animal had four paws in the adjacent square.

2.5.2. Forelimb grip strength test

This test involved the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2 mm in diameter and 1 m in length), placed 1 m above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded. A maximum time of 2 min was given to each rat after which it was removed. The test reflects muscular strength in the animals [23,25].

2.6. Biochemical assay

After the behavioral test, the animals were anesthetized using ketamine after which the thoracic region was opened to expose the heart. The animals were perfused with a solution of phosphate buffer saline (pH 7.4) to remove blood from all organs most especially the brain. The skull was then dissected and the brain was carefully removed. The brain was weighed and then divided into two lobes: one lobe was fixed in formalin for histological

studies, while the other was homogenized. The homogenate was then transferred into a sample bottle and centrifuged for test for inflammatory markers.

2.6.1. Tumor necrosis factor-alpha assay

The sandwich-enzyme-linked immunosorbent assay (ELISA) principle was used. The ELISA well was coated with an antibody that is specific to the antigen it is designed for; this is known as the primary antibody. The homogenate was added into the well and left for about 4 h, to allow all the TNF-α present in the homogenate to binds to the primary antibody, after which the wells were rinsed. The kit also comes with a secondary antibody, which is coupled to an enzyme known horseradish peroxidase to form the ELISA. The secondary antibody was then added which resulted in a color change. The well was then rinsed off, and the wells were placed in the microplate reader.

2.6.2. Nitric oxide assay

The supernatant was added to the well. NO concentration in the supernatant was determined by assaying for the concentration of nitrite. Vanadium trichloride converted nitrate to nitrite. After which the Griess reagent was added which determined the concentration of nitrite. The concentration of nitrite was used to infer the concentration of NO using a microplate reader.

2.7. Statistical analysis

The data obtained at the end of the experiment were presented as mean ± SD and statistical analysis for multiple comparisons conducted by using one-way analysis of variance, followed by a post hoc Newman–Keuls using the GraphPad prism, version 6.0 for Windows, GraphPad Software, San Diego, California USA. The data values were defined to be statistically significant at P value less than 0.05.

3. Results and discussion

3.1. Phytochemical analysis result

The result of the phytochemical screening conducted on the extract is shown in Table 1. The phytochemical result shows that *C. acuminata* contains alkaloids, flavonoids, saponin, glycosides, etc. However, phenol and tannins were absent in the plant extract.

The quantitative analysis of the extract is shown in Table 2. The result shows that the plant extracts contain a high amount of alkaloids and flavonoids but a low amount of saponin and triterpenes.

3.2. Behavioral results

Tables 3 and 4 shows the effect of ALECA leaf on muscular strength, exploratory behavior, and locomotor activity of the animals on day 14 and day 21, respectively.

3.2.1. Forelimb grip and open-field tests on day 14

On day 14, $HgCl_2$ significantly decreased the muscular strength of animals that were not given the intervention drugs when subjected to the forelimb grip test. The result shows that there is a significant decrease (78%) in the muscular strength of animals given $HgCl_2$ when compared with the control group, while there is a significant increase in the muscular strength of animals treated with ALECA, NAC, and vitamin C when compared with the $HgCl_2$ group. Administration of the three intervention drugs significantly improves muscular strength of the animals; however, there is no significant difference in the effect of ALECA, NAC, and vitamin C.

Also, $HgCl_2$ significantly decreases locomotor activity and exploratory behavior, and increases anxiety levels in animals that were not given the intervention drugs when subjected to the open-field test. The result shows a significant decrease (75%) in locomotor activity, exploratory behavior, and increased anxiety levels in animals given $HgCl_2$ when compared with the control group, while there was a significant increase in locomotor activity, exploratory behavior, and decreased anxiety levels in animals treated with ALECA, NAC, and vitamin C when compared with the $HgCl_2$ group. Administration of the intervention drugs improves locomotor activity in animals. There is no significant difference between the line crossing, grooming, center square duration, and grooming of animals

Table 1. Qualitative analysis result.

Phytochemical	Saponin	Alkaloid	Phenol	Tannins	Triterpenoid
Results	+	+	−	−	+
Phytochemical	Steroid	Flavonoid	Protein	RS	Glycosides
Results	+	+	+	−	+

+ shows presence.
− shows absence.

Table 2. Quantitative analysis result.

Alkaloids (mg/100 g)	Steroids (mg/100 g)	Glycoside (mg/100 g)	Flavonoid (mg/100 g)	Saponin (mg/100 g)	Triterpenes (mg/100 g)	Nitrogen %
183.4	9.251667	0.57918	29	0.000878	0.701667	2.09958
183	9.468333	0.578392	28	0.000983	0.718333	

Table 3. Behavioral tests (forelimb grip strength and open-field tests) for day 14.

Behavioral parameters	Control	$HgCl_2$	$HgCl_2$+ALECA (150 mg/kg)	$HgCl_2$+ALECA (300 mg/kg)	$HgCl_2$+NAC	$HgCl_2$+Vit. C
Forelimb (s)	32.20 ± 20.18	7.20 ± 1.09[a]	28.80 ± 9.23[b]	24.40 ± 2.30[b]	19.00 ± 0.71[b]	24.80 ± 9.55[b]
Line crossing (frequency)	36.00 ± 22.5	9.80 ± 5.68[a]	51.20 ± 7.12[b]	47.00 ± 8.97[b]	44.80 ± 9.68[b]	33.80 ± 8.73[b]
Center square crossing (s)	0.75 ± 0.15	3.94 ± 1.17[a]	1.69 ± 0.67[b]	1.54 ± 0.36[b]	1.79 ± 0.45[b]	1.92 ± 0.16[b]
Rearing test (frequency)	8.80 ± 1.79	2.40 ± 1.14[a]	19.00 ± 7.48[b]	19.20 ± 4.49[b]	13.20 ± 3.96[b]	9.00 ± 0.71[b]
Grooming test (s)	42.82 ± 17.82	5.20 ± 2.49[a]	27.11 ± 12.47[b]	34.62 ± 8.35[b]	33.95 ± 18.09[b]	18.47 ± 0.91[b]
Freezing test (s)	33.97 ± 27.11	138.0 ± 21.75[a]	44.45 ± 17.15[b]	65.24 ± 5.32[b]	98.99 ± 0.38[c]	93.50 ± 5.24[c]

Data are presented as mean ± SD, $n = 6$.
ALECA, aqueous leaf extract of *Cola acuminata;* $HgCl_2$, mercury chloride; NAC, N-acetylcysteine; Vit. C, vitamin C.
[a]P value less than 0.05 versus control.
[b,c]P value less than 0.05 versus $HgCl_2$.

Table 4. Behavioral tests (for limb grip strength and open-field tests) for day 21.

Behavioral parameters	Control	$HgCl_2$	$HgCl_2$+ALECA (150 mg/kg)	$HgCl_2$+ALECA (300 mg/kg)	$HgCl_2$+NAC	$HgCl_2$+Vit. C
Forelimb (s)	25.00 ± 3.08	4.00 ± 0.70[a]	21.60 ± 10.97[b]	34.80 ± 15.34[b]	32.20 ± 11.23[b]	30.20 ± 8.44[b]
Line crossing (frequency)	36.60 ± 10.71	7.00 ± 0.71[a]	32.60 ± 5.86[b]	29.00 ± 9.98[b]	22.80 ± 0.45[c]	40.20 ± 7.76[b]
Center square crossing (s)	0.96 ± 0.17	2.70 ± 1.57[a]	1.29 ± 0.02[b]	1.29 ± 0.04[b]	1.16 ± 0.06[b]	0.91 ± 0.21[b]
Rearing test (frequency)	13.80 ± 7.95	2.00 ± 1.23[a]	12.20 ± 1.79[b]	10.80 ± 2.68[b]	6.80 ± 1.09[b]	9.00 ± 2.55[b]
Grooming test (s)	54.92 ± 17.80	10.68 ± 4.64[a]	32.08 ± 7.33[b]	49.09 ± 7.58[b]	33.03 ± 4.26[b]	60.11 ± 9.11[c]
Freezing test (s)	56.08 ± 13.49	190.40 ± 12.46[a]	92.72 ± 15.83[b]	102.00 ± 45.37[b]	114.20 ± 21.90[b]	51.62 ± 12.85[c]

Data are presented as mean ± SD, $n = 6$.
ALECA, aqueous leaf extract of *Cola acuminata*; $HgCl_2$, mercury chloride; NAC, N-acetylcysteine; Vit. C, vitamin C.
[a]P value less than 0.05 versus control.
[b,c]P value less than 0.05 versus $HgCl_2$.

treated with ALECA, NAC, and vitamin C; however, there is a significant difference in the effect of ALECA, NAC, and vitamin C on the freezing time of the animals. ALECA reduces the freezing time of the animals more than both NAC and vitamin C. This is also an indication that there is reduced anxiety in those animals.

3.2.2. Forelimb grip and open-field tests on day 21

At day 21, $HgCl_2$ significantly reduced the muscular strength of animals that were not given the intervention drugs when subjected to the forelimb grip test. There is a significant decrease (84%) in the muscular strength of animals given $HgCl_2$ when compared with the control group, while there is an increase in muscular strength of animals treated with ALECA, NAC, and Vit C was observed when compared with the $HgCl_2$ group. Administration of the three intervention drugs significantly improves the muscular strength of the animals; furthermore, there is no significant difference in the effect of ALECA, NAC, and vitamin C.

Also, $HgCl_2$ significantly reduces locomotor activity, exploratory behavior and increases anxiety levels in animals that were not given the intervention drugs when subjected to the open field. From the result, there is a significant decrease (80%) in locomotor activity, exploratory behavior, and increased anxiety levels in the $HgCl_2$ group when compared with the control group. However, there is a significant increase in locomotory, exploratory behavior, and decreased anxiety levels in animals treated with ALECA, NAC, and vitamin C when compared with the $HgCl_2$ group. Although there is no significant difference between the rearing, center square duration, and grooming of animals treated with ALECA, NAC, and vitamin C, there is a significant difference in the effect of ALECA, NAC, and vitamin C on the freezing time of the animals. The ALECA effect on the freezing time of the animals is similar to the effect of NAC. Also, there is a significant difference in the effect of ALECA, NAC, and vitamin C on line crossing as the effect of ALECA is similar to the effect of

vitamin C. The reduction in anxiety-related behaviors in the group treated with the extract also buttresses the neurotoxicity mitigating effects of the extract.

3.3. Biochemical results

3.3.1. Nitric oxide level

Figure 1 shows the effect of ALECA on NO levels in the brain. The result shows that the NO level in the brain is significantly increased (62.5%) in the $HgCl_2$ group when compared with the control group. Administration of ALECA, NAC, and vitamin C causes a significant decrease (38.5%) in the level of NO level when compared with the $HgCl_2$ group. However, there is no significant difference in the effect of ALECA, NAC, and vitamin C.

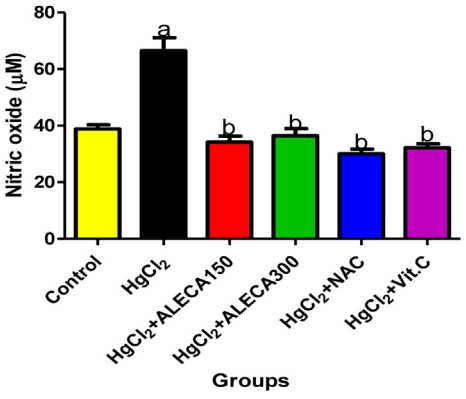


Fig. 1. Effects of ALECA on the nitric oxide level. Data are presented as mean ± SD, n = 6; [a]P value less than 0.05 versus control; [b]P value less than 0.05 versus $HgCl_2$. ALECA, aqueous leaf extract of Cola acuminata; $HgCl_2$, mercury chloride; NAC, N-acetylcysteine; Vit. C, vitamin C.

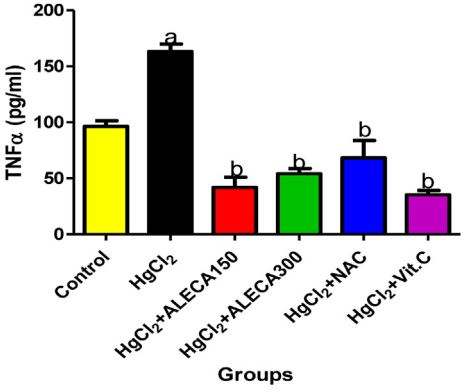


Fig. 2. Effects of ALECA on the tumor necrosis factor-α level. Data are presented as mean ± SD, n = 6; [a]P value less than 0.05 versus control; [b]P value less than 0.05 versus $HgCl_2$. ALECA, aqueous leaf extract of Cola acuminata; $HgCl_2$, mercury chloride; NAC, N-acetylcysteine; Vit. C, vitamin C.

3.3.2. Tumor necrosis factor-alpha level

Figure 2 shows the effect of ALECA on TNF-α levels in the brain. The result shows that the TNF-α level in the brain is significantly increased in the $HgCl_2$ group when compared with the control group (62.5%). Administration of ALECA, NAC, and vitamin C causes a significant decrease in the TNF-α level when compared with the $HgCl_2$ group (68.8%). However, there is no significant difference in the effect of ALECA, NAC, and vitamin C.

Medicinal plants have always been used in unorthodox medicine and their medicinal values have been attributed to various phytochemical components, which produce definite physiological actions in the human body. These phytochemicals that are naturally occurring and biologically active plant compounds have been reported to have potential disease-inhibiting capabilities believed to be effective in combating or preventing many diseases due to their antioxidant and anti-inflammatory effects [26].

Several substances have been used to induce neurotoxicity in animal models such as colchines, methamphetamine ochratoxin, acrylamide, methyl mercury, ethyl mercury, $HgCl_2$, acrylonitrite, etc. $HgCl_2$ used in this study has been reported to have prooxidant and neuro-inflammatory properties, which result in neurotoxicity. Accumulating evidence has shown that neuroinflammation is closely related to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, etc. Extensive research is being conducted into finding a cure for these neurodegenerative diseases. Vitamin C and NAC used in this study are standard antioxidants and anti-inflammatory drugs used in the treatment of different disease conditions.

In this study, the significant increase in muscular strength in the group treated with ALECA when compared with the untreated group shows that ALECA can restore the motor deficit caused by $HgCl_2$. The phytochemical analysis of the ALECA revealed the presence of alkaloids, glycosides, terpenoids, and flavonoids [27–31]. The quantitative screening of the aqueous leaf extract of *C. acuminata* shows an abundance of alkaloids. Alkaloids play a positive role in ameliorating the pathophysiology of neurodegenerative diseases by functioning as muscarinic and adenosine receptor agonists, antioxidants, anti-amyloids, and acetylcholinesterase inhibitors [32]. Alkaloids are well known for their anti-inflammatory properties. It fights inflammation by causing a reduction in TNF-α levels. Flavonoids such as anthocyanins ameliorate neuroinflammation by decreasing TLR4 expression,

inactivating nuclear factor kappa B, and reducing proinflammatory mediators like TNF-α [33]. Generally, these phytocompounds collectively inhibit the expression of TLR4 receptor and block the activation of nuclear factor-kappa B transcription factor, thus inhibiting the generation of downstream proinflammatory cytokines such as TNF-α, IL-6, and IL-1β, and the free radicals, such as NO and ROS. This is evident in the decreased levels of inflammatory markers in the group treated with ALECA contrary to the elevated level of NO and TNF-α in the $HgCl_2$ group.

The ameliorative effect of ALECA on $HgCl_2$-induced neurotoxicity could be attributed to the secondary metabolite reported to be present in it.

3.4. Conclusion

Results from this study revealed that ALECA can ameliorate neurotoxicity induced by $HgCl_2$. This is believed to occur due to the abundant phytochemicals, for example alkaloids, flavonoids, etc., present in it, which decrease neuroinflammation as evident in the reduced TNF-α and NO levels. Therefore, the increase in exploratory behavior, locomotor activities, and a reduction in anxiety levels observed in this study may be attributed to the anti-inflammatory and neuroprotective properties of ALECA. In light of the above, it may be worthwhile to explore the potential of this plant in the management of mercury poisoning and diseases related to neurotoxicity.

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Conflicts of interest

None declared.

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