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Research Article

Ameliorative Effect of Vitamin C and N-Acetyl cysteine on Mercury chloride-induced Neurotoxicity in Male Wistar Rats

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ABSTRACT

The present study investigated the ameliorative effects of Vitamin C (Vit. C) and N-acetylcysteine (NAC) on mercury chloride ($HgCl_2$)-induced neurotoxicity in male Wistar rats. Twenty (20) male Wistar rats were randomly grouped into four, n=5. I: control, received 0.5mL normal saline, II: received $HgCl_2$ alone (5mg/kg), III: received $HgCl_2$ (5mg/kg) plus Vit. C (100 mg/kg), IV: received $HgCl_2$ (5mg/kg) and N-acetylcysteine (NAC) (50mg/kg) for fourteen (14) days. All the drugs were administered orally. The rats were subjected to behavioural tests (Morris water maze, novel object recognition, light and dark box, fore limb grip strength and beam walking balance tests). The rats were then euthanized to obtain brain tissues for the determination of catalase activity, total protein and nitric oxide (NO) levels. The result revealed a significant ($P<0.05$) increase in the escape latency, beam walking latency, and footslip scores, and a significant ($P<0.05$) decrease in the recognition ratio, exploration frequency, and drop-off time in the rats that were exposed to $HgCl_2$ only. However, Vit. C and NAC reversed the observed behavioural deficits. Similarly, $HgCl_2$ exposure caused a significant ($P<0.05$) decrease in the brain catalase and total protein, and a significant ($P<0.05$) increase in the NO level. Also, administration of Vit. C and NAC significantly ($P<0.05$) reversed the trend. This study concludes that Vit. C and NAC ameliorated HgCl*2*-induced neurotoxicity via attenuation of behavioural deficits and oxidative stress.

Keywords: *Neurotoxicity; Vitamin C; Mercury chloride; N-acetylcysteine; Behavioural.*

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INTRODUCTION

The neurotoxicity caused by heavy metals, such mercury chloride, continues to be a major global public health concern. It is crucial to look into cutting-edge remedies that can lessen the harmful effects of these toxicants (Pirkle *et al*., 2016), because prolonged exposure to them might cause serious harm to brain tissues and essential biological components (Wani *et al*., 2015). Depending on the precise chemical and the mode of exposure, mercury in particular can have a wide range of harmful effects (Branco *et al*., 2021). Nonetheless, toxicologists are most concerned by neurotoxicity due of its negative health effects.

Animal models of chronic mercury (Hg) exposure have demonstrated neurotoxicity, including cognitive and behavioral abnormalities (Farina *et al*., 2011; Said *et al*., 2021); similar effects have been observed in neurodegenerative illnesses (Caricio *et al*., 2019). According to Stimpson *et al*. (2016), injury to brain serotonergic neurons may have negative repercussions such as increased anxiety-like behavior and memory and learning problems (Meneses, 2015). Primates, including humans, appear to be more

vulnerable to these effects. There are three primary forms of mercury: elemental mercury, which is emitted by nature or by humans and includes coal-fired power plants, waste burning, hazardous waste incinerators, and gold extraction; inorganic mercury, such as mercury chloride ($HgCl_2$), which is used as a fungicide, antiseptic, and medical preservative; and organic mercury, which includes methylmercury (MeHg) and ethylmercury (EthHg). Workers in mines and other industries where mercury is used in manufacturing, including chloro-alkali plants, have long been seen as being at risk, according to Branco *et al*. (2021).

Research in both human and animal models has demonstrated that long-term exposure to HgCl2 might cause deficiencies in learning and memory (Chelini *et al*., 2018; Franco *et al*., 2019). The hippocampus, a part of the brain essential for learning and memory functions, is frequently implicated in these deficiencies (Sui *et al*., 2019). Memory issues may arise from hippocampal injury caused by HgCl2 due to neuronal death, changes in synaptic plasticity, and disruption of neurotransmitter systems (Allen *et al*., 2019).

Exposure to HgCl2 has been linked to deficits in attention, executive processes, and motor abilities in addition to memory problems (Roos *et al*., 2019). These cognitive impairments can have a substantial negative effect on a person's quality of life by seriously impairing their capacity to carry out daily chores. HgCl2 causes neurotoxicity through a combination of oxidative stress, inflammation, and neurotransmitter system disturbance. Mercury has the ability to combine with thiol groups found in proteins and enzymes to create complexes that can cause oxidative damage to biological components and the release of reactive oxygen species (ROS) (Carocci *et al*., 2014). Increased reactive oxygen species (ROS) can cause DNA damage, lipid peroxidation, and protein oxidation, which can cause neuronal injury and death (Farina *et al*., 2018).

Antioxidants play a vital role in protecting brain cells from oxidative damage. Antioxidant defense systems, which are triggered in response to oxidative damage, stop ROS from being produced as well as block and seize the radicals that are produced (Lobo *et al*., 2010). These systems, which can be enzymatic or non-enzymatic, are found in the membrane and aqueous compartments of cells. An enzymatic antioxidant system comprising glutathione peroxidase, catalase, and superoxide dismutase has been identified as the first line of defense. Reduced thiol and non-enzymatic antioxidants, such as hydro- and lipo-soluble or metabolic substances, constitute the second line of defense (Pisoschi and Pop, 2015).

Water-soluble vitamin C, sometimes referred to as L-ascorbic acid, ascorbic acid, or L-ascorbate, is a nutrient that can be taken as a dietary supplement or found naturally in certain foods. Tomatoes, kiwi fruit, vegetables, potatoes, green peas, oranges, and orange fruit are among the foods high in vitamin C. Vitamin C plays a role in protein metabolism and is necessary for the manufacture of collagen, L-carnitine, and certain neurotransmitters (Li, 2007). The building block of connective tissue, which is crucial for wound healing, is collagen. Another significant physiological antioxidant is vitamin C.

N-acetylcysteine (NAC) is a chemical that contains sulfhydryl and has mucolytic effects. It was first patented in 1960. Since 1969, it has been used clinically to treat cystic fibrosis (Gracey *et al*., 1969). Since then, the use of NAC has been extended to chronic obstructive pulmonary disease and acetaminophen overdose, and its therapeutic utility has continued to grow. In addition to its antioxidant, anti-inflammatory, and mucolytic qualities, NAC also has the ability to increase glutathione S-transferase activity, replenish glutathione, scavenge free radicals, and stabilize protein structures through the crosslinking of cysteine disulfide bonds. Research on both humans and animals has demonstrated that N-acetylcysteine chelates harmful metals, but has little to no effect on necessary metals (Rossignol, 2019).

There is paucity of information on the effect of both Vit. C and NAC on $HgCl_2$-induced neurotoxicity in male Wistar rats, hence this study aimed to evaluate the potential ameliorative effect of Vit. C and NAC against HgCl2-induced neurotoxicity in male Wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals: HgCl2 (catalogue number-104419; Sigma-Aldrich in Steinheim, Germany), ketamine hydrochloride (catalogue number-736, Sigma-Aldrich in Steinheim, Germany) were obtained from Parke-Davies in Freiburg, Germany. Vit. C (catalogue number-10432) and NAC (catalogue number-112422) were purchased from Aromokeye Pharmacy in Ilorin, Nigeria.

Animals: The University of Ilorin Ethical Committee approved the care of laboratory animals on April 13, 2023, with permission number UERC/ASN/2023/2451, after the rules for animal care were followed. Twenty (20) male Wistar rats, weighing between 180 and 220 grams, were used in this study at the University of Ilorin's central research laboratory in Nigeria. The animals were housed in the laboratory for two weeks before to administration so they could become acclimated to their environment. They were placed in cages with unrestricted access to food and water, and they were maintained in environments with consistent humidity, temperature, and 12-hour light/dark cycles.

Animal Grouping: Twenty Wistar rats were grouped in to (4) groups of (5) rats each, they were placed in individual cages and classified as follows:

Table 1:
Animal grouping and the administered dosages

S/No	Group	Dosages that were administered once daily for 15 days
1	Control	0.5 mL of normal saline orally
2.	$HgCl_2$ only	5 mg/kg body weight orally (Agbon *et al*., 2016)
3.	$HgCl_2$ + Vit.C	5 mg/kg body weight of $HgCl_2$ orally + 100 mg/kg body weight of vit. C orally (Agbon *et al*., 2016)
4.	$HgCl_2$ + NAC	5 mg/kg body weight of $HgCl_2$ orally + 50 mg/kg body weight of NAC orally (Farombi *et al*., 2018)

Behavioural Tests

Morris water maze Test: Morris water navigation task is another name for this process (Morris *et al*., 1982). In behavioral neuroscience, this behavioral assessment test is frequently used to examine spatial learning and memory in rodents such as rats, mice, and others. A round plastic tank with a diameter of around 180 cm and a depth of approximately 90 cm made up the assessment setup. White powdered milk was added to the tank along with water to create a murky watery media. A platform made of cream-colored iron is submerged in the water, roughly one centimeter below the surface. One of the tank's hypothetical quadrants is where the platform is kept in the middle. This is the rats' escape platform, and it was kept in situ at a specific location during the experimental training process with a picture cue next to it. The rat's training sessions with the Morris water maze were captured on camera using a webcam, and the observations were also entered into the lab record book for documentation's sake. Four trials made up each training session, with a brief pause to allow the animals to settle down before the next one. Before administration, this happens every day for seven days. During the training-learning phase of the first experiment, all groups of animals learned how to flee to the platform in less than 60 seconds. The rat was gently directed to the platform if, after sixty seconds, it could not find it. The rats were then taken off the platform to rest for thirty minutes after being let to stay on for an additional sixty seconds. Then, in the second, third, and fourth trials, the entire procedure was repeated. Essentially, on the fourteenth day following the administration of medications and test substances, the animals were assessed (memory test post-administration) for all groups.

Novel Object Recognition Test: The animals' capacity to recognize a known object over a varied period of time is evaluated using the Novel Object Recognition Test (NORT); this capacity is known as recognition memory (Brown *et al*., 1999). Three stages comprise the NOR tasks: the familiarization phase, the test phase, and the habituation phase. Rats were allowed to run freely in an open field for five minutes during the habituation period. Two items that were positioned in the arena were used for the training (familiarization phase) experiment. Double-sided tape was used to secure objects so they are counterbalanced in the arena, six by six centimeters from two non-release corners. For ten minutes, rats were free to explore the stadium and its contents while video records were being made. After 20 minutes, the rats were put back into their spotless holding cages, and ethanol was used to clean the arena. In order to execute the NORT, a new object was inserted in place of one of the old ones, and double-sided tape was used to secure the new object six centimeters away from the corner's two walls. The experimental animals in the vicinity were captured on camera for ten minutes. The recognition ratio (RR) was used to measure the performance of the NOR task.

As a percentage of the animal's overall exploration time (Ttotal), RR represents the length of time spent exploring the new object (Tnew) as opposed to the old object (Told). In the process, methylated spirit was used to properly clean all items and arenas in between uses.

Light and Dark Box Test: Rats are utilized to assess the unconditioned anxiety response in a light-dark box. It is predicated on rodents' intrinsic aversion to light and their impulsive exploration activity in reaction to minor stressors, such as light and open spaces. The rats' spatial and associated kinds of learning and memory were assessed using the light and dark box test (Ayinla *et al*., 2020). With an external size of 46 x 27 x 30 cm, the dimensions of the light and dark box compartment are typically one-third for the dark compartment and two-thirds for the light compartment. The device was set up in a quiet, isolated room with a low light intensity that was free from outside distractions. Methylated spirit was used to clean the apparatus's two compartments. The animals were placed inside the box and given five minutes to roam around the two chambers at will throughout the training session. After five minutes of training, the animals' short- and long-term memories were examined. The animals were deposited into one of the box sections, and the passage between the light and dark boxes was blocked. The webcam video recorder was used to record video of the equipment during the procedure, and scores of frequency at which the rats visited the barricade between the light and dark boxes were calculated. Just before sacrifice, on the fourteenth day, the last day of administration, this experiment was conducted.

Forelimb Grip Strength Test: A modified version of Olopade *et al*. (2012)'s protocol was employed to assess sensory motor function using the forelimb grip strength test. The metal wire, measuring 2.5 mm in diameter and 1 m in length, was suspended horizontally, and the rat's forepaws were placed upon it. One meter above a landing area with soft bedding covering it, the wire was placed. The longest amount of time that each rat could be hanged before falling off the wire was two minutes, and this duration was noted for every rat. Two days before the test, or before treatment, the rats were gradually acclimated to the setup every day. Prior to testing, rats were administered medication for 14 days.

Beam Walking Balance Test: The animals were made to traverse a thin beam to reach an enclosed safety platform in order to test their balance and motor coordination (Fan *et al*., 2008). For this, a modified version of the beam walking apparatus (BWA) developed by Puurunen (2001) and Carter *et al*. (2001) was used. The BWA consisted of a raised platform connected by a 100 cm long by 3 cm wide wood beam. The beam was set up horizontally at a height of 50 cm, with one end fastened to a goal box measuring 20 by 20 by 20 cm and the other end fastened to a small support measuring 10 by 10 cm that was attached to the start platform. At the start, a powerful light source was placed to entice the rats to cross the beam. A sawdust-filled chamber at the base kept the rats safe from the fall. A video of the performance was taken. Prior to the test, the rats were pre-trained by being placed on the beam in front of their home cage and told to return there. Three training repetitions were conducted. The time it took for the animal's snout to enter the goal box was measured (Abou-Donia *et al*., 2008), and it may take up to 60 seconds. A 60-

second latency was applied to rats that fell off the beam or failed to enter the goal box. The beam-walk score was calculated using an average of three trials, cleaned with methylated spirit in between trials. Foot slip scores, or the number of times the hind feet slipped off each beam, were recorded for each trial. We used a webcam to record. Before the test, or before treatment, the rats were habituated to the BWA once a day for three days. Prior to testing, rats were administered medication for 14 days.

Euthanasia and Brain tissue Collection: On the fifteenth day of administration, the animals were offered as sacrifices. This came about following the completion of the behavioral testing. An intraperitoneal injection of 0.5 ml of ketamine solution was used to put each animal to sleep. Subsequently, the animal brain tissues were removed, weighed, and homogenized in a 0.1 M phosphate buffer solution (pH 7.4) at a ratio of 1 g tissue to 4 ml. After centrifuging the homogenate for ten minutes at 3000 rpm, the supernatant was removed and used for biochemical analysis. Between 0 and 40C, centrifugation and homogenization were carried out.

Biochemical Assay: Using the Clairbone (1995) method, catalase activity was measured by monitoring the rate of hydrogen peroxide clearance at pH 7.0, 25oC, and 240 nm wavelength. Based on the reaction of nitrite content with Griess reagent to generate a colored molecule that absorbs light at 540 nm, the level of nitric oxide was measured (Green *et al*., 1982). Using bovine serum albumin, the Bradford technique of protein determination (Bradford, 1976) was used to quantify the total protein concentration.

Statistical Analysis: The mean ± standard error of the mean (S.E.M.) were used to express all the results. Version 6.0 of Graph Pad Prism was utilized, and one-way ANOVA was applied to the data. The Newman-Keuls post hoc test was used. At P<0.05, the data were statistically significant.

RESULTS

Behavioural

Vit.C and NAC significantly ameliorated behavioural and cognitive deficits caused by HgCl2 by significantly improves the escape latency, recognition ratio, frequency of exploration, drop-off time, beam walking latency and footslip score in the Morris water maze, novel object recognition, light and dark box, forelimb grip strength and beam walking tests respectively.

In fig. 1 administration of $HgCl_2$ causes a significant (P<0.05) increase in the escape latency of the $HgCl_2$-treated group compared to the control, however, administration of both Vit.C and NAC significantly (P<0.05) reduces the escape latency in both Vit.C- and NAC-treated groups compared to the $HgCl_2$-treated group

In fig. 2, administration of $HgCl_2$ significantly decreases the recognition ratio (RR) in the $HgCl_2$-treated group compared to the control, however, administration of Vit. C and NAC significantly increase the RR of the Vit. C- and NAC-treated groups compared to the $HgCl_2$-treated group.

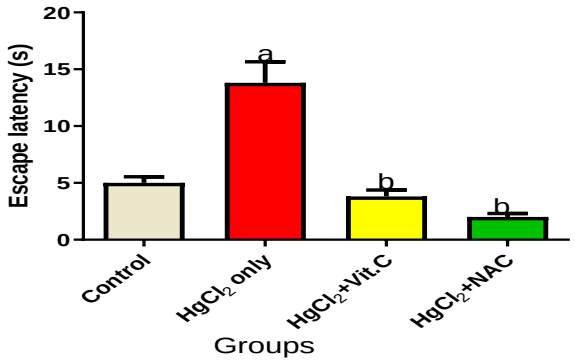

Fig. 1:
Effect of vitamin C and N-acetylcysteine on Morris water maze test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine.

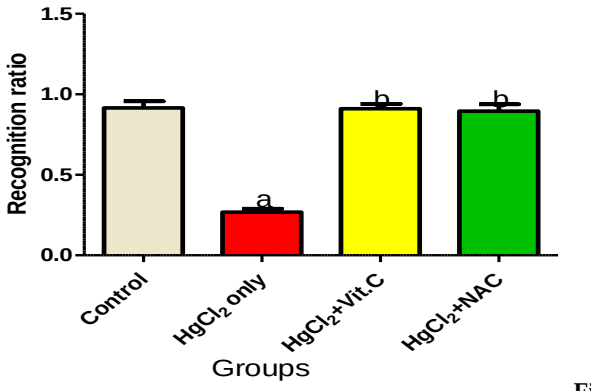

Fig. 2:
Effect of vitamin C and N-acetylcysteine on novel object recognition test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine.

In fig. 3, there is a significant decrease in the exploration frequency of the $HgCl_2$-treated group compared to the control; however, there is a significant increase in the exploration frequency of both Vit. C- and NAC-treated groups when compared to the $HgCl_2$-treated group.

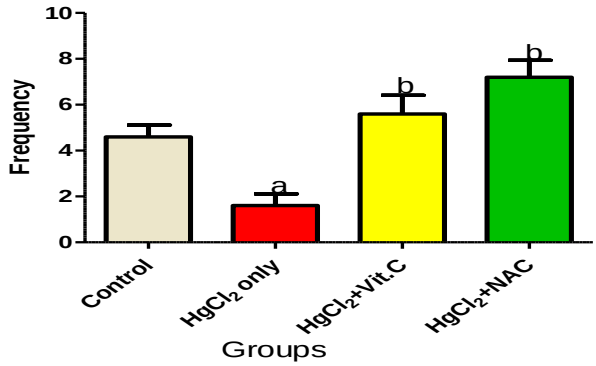

Fig. 3:
Effect of vitamin C and N-acetylcysteine on light and dark box test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine.

In fig. 4, there is a significant decrease in the drop-off time of the $HgCl_2$-treated group compared to the control; however, there is a significant increase in the drop-off time of both Vit. C- and NAC-treated groups when compared to the $HgCl_2$-treated group.

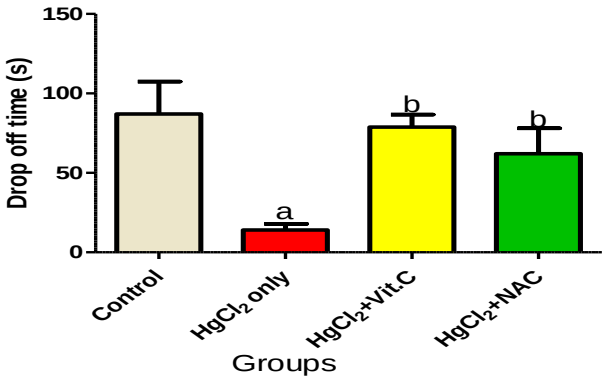

Fig. 4:
Effect of vitamin C and N-acetylcysteine on fore limb grip strength test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine

In fig. 5, there is a significant increase in the beam walking latency of the $HgCl_2$-treated group compared to the control; however, there is a significant decrease in the beam walking latency of both Vit. C- and NAC-treated groups when compared to the HgCl2-treated group.

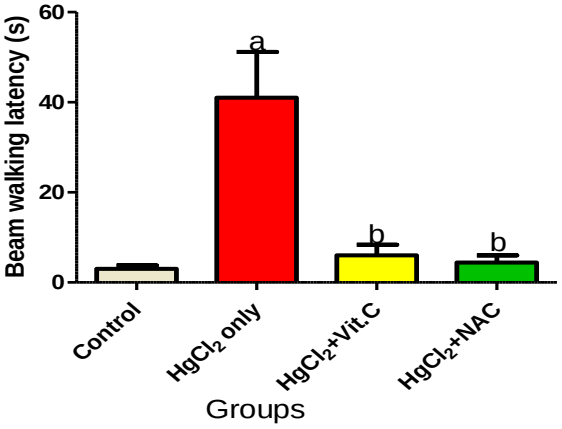

Fig. 5:
Effect of vitamin C and N-acetylcysteine on beam walking test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine

In fig. 6, there is a significant increase in the foot slip score of the $HgCl_2$-treated group compared to the control; however, there is a significant decrease in the foot slips score of both Vit. C- and NAC-treated groups when compared to the $HgCl_2$-treated group.

Biochemical Results

Vit. C and NAC significantly increases the antioxidant enzyme activity (catalase), decreases an inflammatory marker nitric oxide (NO) and increases total protein level.

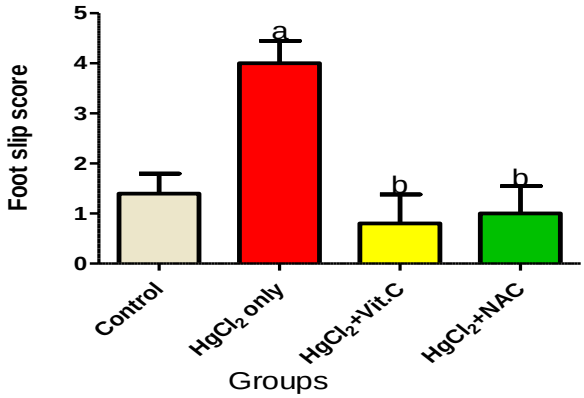

Fig. 6:
Effect of vitamin C and N-acetylcysteine on beam walking test. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine

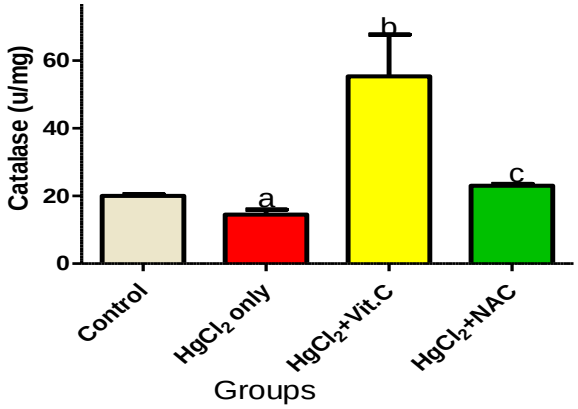

Fig. 7:
Effect of vitamin C and N-acetylcysteine on catalase activity. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b,c}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine

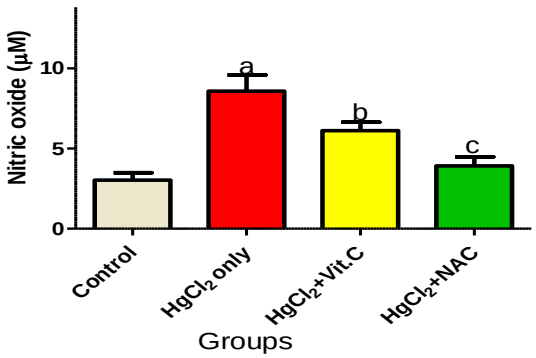

Fig. 8:
Effect of vitamin C and N-acetylcysteine on NO level. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b,c}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine.

Fig. 7 shows the effect of Vit. C and NAC on catalase activity. It is observed that $HgCl_2$ causes a significant decrease in the catalase activity of the $HgCl_2$-treated group when compared to

the control, however Vit. C and NAC significantly increase the catalase activity in the Vit. C- and NAC-treated groups compared to the $HgCl_2$-treated group. Also there is a significant difference in the catalase activity of Vit. C- and NAC-treated groups.

Fig. 8 shows the effect of Vit. C and NAC on nitric oxide (NO) level. It is observed that $HgCl_2$ causes a significant increase in the NO level of the $HgCl_2$-treated group when compared to the control, however Vit. C and NAC significantly decrease the NO level of the Vit. C- and NAC-treated groups compared to the $HgCl_2$-treated group. Also there is a significant difference in the No levels of Vit. C- and NAC-treated groups.

Fig. 9 shows the effect of Vit. C and NAC on total protein level. It is observed that $HgCl_2$ causes a significant decrease in the total protein level of the $HgCl_2$-treated group when compared to the control, however Vit. C and NAC significantly increase the total protein level of the Vit. C- and NAC-treated groups compared to the $HgCl_2$-treated group. Also there is a significant difference in the total protein levels of Vit. C- and NAC-treated groups.

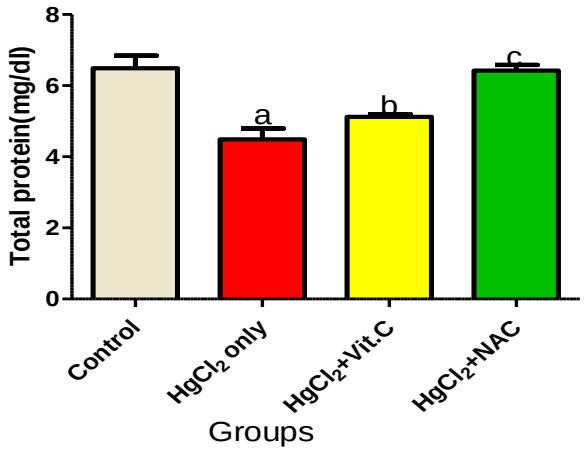

Fig. 9:
Effect of vitamin C and N-acetylcysteine on total protein level. Data are presented as Mean ± SEM, n = 5; $^{a}P < 0.05$ vs. control; $^{b,c}P < 0.05$ vs. $HgCl_2$. $HgCl_2$, mercury chloride; Vit.C, vitamin C; NAC, N-acetylcysteine.

DISCUSSION

The present study examined the impact of Vit. C and NAC administration on HgCl2-induced neurotoxicity in male Wistar rats. The effects of the drugs on learning and memory, sensory motor function, motor coordination, and balance were examined using behavioral investigations. The considerable increases in escape latency, beam walking latency, and foot slip scores, as well as the significant decreases in recognition ratio, exploration frequency, and drop-off duration of the $HgCl_2$-treated group relative to the control, demonstrate that $HgCl_2$ causes behavioral deficits. It is commonly known that oxidative stress and excessive free radical generation are linked to behavior changes brought on by $HgCl_2$ (Mello-Carpes *et al*., 2013). Due to $HgCl_2$'s capacity to bind to antioxidants' sulfhydryl groups, glutathione levels are reduced and thiols are depleted, which raises reactive oxygen species (ROS) and eventually causes oxidative stress and enhanced neurotoxicity (Ahmed, 2015). However, it's possible that the antioxidant qualities of Vit. C and NAC contributed to the reversal of the behavioral deficits seen in this study (Verma *et al*., 2007; Martensson *et al*., 2019). Vitamin C is essential for the proper operation of the immune system and is needed for the action of numerous enzymes. Because Vit. C can neutralize ROS and lower oxidative stress, it has an antioxidant effect (Verma *et al*., 2007). NAC is essential for preserving redox equilibrium in cells because it functions as a precursor to glutathione (GSH), the body's major endogenous antioxidant (Martensson *et al*., 2019). NAC lessens oxidative stress-induced cellular damage by increasing GSH levels, which improves the cell's capacity to neutralize dangerous ROS and reactive nitrogen species (RNS). Moreover, peroxynitrite radical (ONOO-), an aggressive and powerful oxidant that causes nitrosylation of sulfydryl group, DNA, and brain damage, is created when NO is synthesized by inducible nitric oxide synthase (iNOS) in reaction with superoxide anion (Abdel-Salam *et al*., 2016; Famurewa *et al*., 2019). This study clearly indicates that HgCl2-induced increases in NO may worsen oxidative damage to the brain. The current study's elevated NO level following exposure to HgCl2 could be the result of nuclear factor-kappa B (NF-kB) signaling and iNOS activation (Moneim, 2015).

Research suggests that elevated NO signaling is linked to NF-kB/iNOS neuroinflammatory signaling activation, which may result in cell death (Mahmud *et al*., 2017). However, Vit. C and NAC both reduced the amount of oxidative damage to the brain, inhibited the neuroinflammatory signaling of NF-kB/iNOS, and reduced the amount of cell death caused by $HgCl_2$.

Since proteins are involved in many important physiological processes, measuring proteins can be used as a diagnostic technique to identify an organism's physiological stages. Proteins are extremely vulnerable to poisoning from heavy metals. Most proteins become inactive after they are coupled to mercury (Islam *et al*., 2018). The oxidative stress situation and excess intermediate metabolism may be responsible for this study's observed decrease in total protein level in the $HgCl_2$-treated group (Islam *et al*., 2018). Nonetheless, by increasing total protein in this investigation, Vit. C and NAC mitigated the $HgCl_2$-induced oxidative stress situation; this may be because of their strong antioxidant qualities.

Our findings support earlier research (Mesquita *et al*., 2016; Adedara *et al*., 2019) that found acute exposure to HgCl2 results in a decrease in anti-oxidative enzyme activity.

Changes in oxidative status, either through excess oxidant production or deficits in antioxidant activity, may be one of the direct impacts of $HgCl_2$ toxicity and poisoning in living organisms (Owoeye and Arinola, 2017). In the brain tissues of rats exposed to $HgCl_2$, a decrease in catalase activity was found in the current investigation. However, this was mitigated by Vit. C and NAC, demonstrating their antioxidant properties.

In conclusion, this study concludes that Vit. C and NAC ameliorated behavioural deficits and oxidative stress in a $HgCl_2$-induced neurotoxicity in a male Wistar rats through the regulation of anti-oxidant defense system. Hence, the use of

either or both drugs as standard drugs in the oxidative/antioxidative experiments or research involving animals should be upheld.

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