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Galinsoga parviflora restored associated motor coordination through increased linear distribution of Purkinje Cells in mercury chloride-induced toxicity of mice's cerebellum

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Mercury (Hg) is a poisonous substance associated with diseases, such as ataxia and Joubert syndrome. Therefore, it is important to find a way to disrupt the process of Hg poisoning in the cerebellum, by exploring the medicinal value of local herb such as *Galinsoga parviflora* (GP). This study examined the effects of aqueous leaf extract of GP in $HgCl_2$ induced cerebellar toxicity in adult male mice. Twenty-five adult male mice of an average weight of 25 g were randomly divided into 5 groups (n=5). Animals in Group I received oral administration of 2 ml/kg distilled water for 6 weeks, Group II received 2.3 mg/kg of $HgCl_2$ for 3 weeks, Group III received 2.3 mg/kg of $HgCl_2$ for the first 3 weeks followed by oral administration of 800mg/kg of GP extract for the next 3 weeks, Group IV received 800mg/kg of GP extract for the first 3 weeks followed by 2.3 mg/kg of $HgCl_2$ for the next three weeks, and Group V received 2.3 mg/kg of $HgCl_2$ and 800 mg/kg of GP extract concurrently for three weeks. The test animals were subjected to beam walking tests during the experiment period, followed by euthanasia, perfusion fixation, and tissue sample collection for histological and histochemical analysis. Treatment with the extract of GP showed varying degrees of regeneration in the cerebellar Purkinje cells and myelin sheath of mice in $HgCl_2$ induced toxicity, with corresponding improvements in balance and posture. Cerebellar $HgCl_2$ exposure in the present study was neurotoxic; however, treatment with GP was of therapeutic value.

Key words: Mercury chloride, cerebellum, *Galinsoga parviflora*.

INTRODUCTION

Mercury(Hg) is the third most dangerous element on the earth, and humans are exposed to it in a various forms, including gaseous, organic, and inorganic forms (Aragão et al., 2018). Mercury is found in the environment, following natural processes and human activity (Brooks, 2011). Humans are exposed to Hg in a variety of ways,

including eating Hg-contaminated food, coming into close contact with broken Hg-containing products, and working in mining industries, where Hg is used almost every day and workers are in constant contact with it (Bose-O'Reilly et al., 2010). Inhaling Hg-contaminated air is another effective way via which humans are exposed to Hg (Park and Zheng, 2012); Hg is taken into the bloodstream via the lungs and nervous system (Park and Zheng, 2012). Organic mercury accumulates in CNS-derived cells in the human body, converted to inorganic mercury (Aragão et al., 2018). Niigata Minamata disease and Hunter-Russell syndrome are all caused by methyl mercury, a byproduct of acetaldehyde production (Takahashi et al., 2017). Hg exposure has been known to cause serious health problems, affecting the nervous, immune, and digestive systems and organs such as brain, skin, eyes, lungs, and kidney (Genchi et al., 2017). Clinical signs of methyl chloride intoxication include cerebellar ataxia, sensory and auditory abnormalities, and restriction of visual fields, depending on the location of the lesions (Takahashi et al., 2017). Hg exposure is associated with the following conditions such as behavior changes, fatigue, tremor, headaches, cognitive loss hallucination, incoordination and death (Genchi et al., 2017). Health conditions associated with Hg exposure contribute to the general global and local disease burden. Most of burden from Hg exposure is notable among the developing countries (Budnik and Cateleyn, 2019). In the developing countries, effects of Hg exposure are often not discovered early, therefore making the management of Hg exposure a very difficult task (Bose-O'Reilly et al., 2010).

Cerebellum is an important part of the central nervous system and controls all muscular movements and coordination (Gallucci et al., 2003); made up of the middle vermis and two lateral hemispheres (halves) (Izawa et al., 2012). The cerebellum has three lobes which are anterior, flocculonodular, and posterior lobes (Lunnon et al., 2016). Also, the cerebellar consists of the cerebellar cortex, and an embedded medullary center of white matter (Mihailoff and Haines, 2018). There are layers within the cerebellar cortex which are; outer (molecular layer), Purkinje and the inner layer (granular layer) (Koeppen et al., 2012). The Purkinje cells are the most important neurons in the cerebellar cortex (Jelliffe et al., 2012). The cerebellar cortex is highly vulnerable to the effects of toxic metals, including Hg (Gandhi et al., 2000). Injury or damage to the cells of the cerebellum may lead to conditions like; ataxia, Joubert syndrome, and many others (Jelliffe et al., 2012). Treatment of clinical conditions associated with Hg exposure is also considered to be expensive and not readily available among different populations of the developing world. Therefore, it is necessary to explore other options or

approaches, such as the use of local herbs such as *Galinsoga parviflora* (GP) especially in the management of Hg exposure.

GP also known as Gallant Soldier, Kofume (Luganda), and Mpunika or Empunika (Runyankore), is an Asteraceae plant that is widely consumed as a vegetable around the world (Ali et al., 2017). These plants are found in almost every part of the world including North America, Europe, Asia, and Africa (Bazylko et al., 2012). According to research, GP contains flavonoids, aromatic esters, caffeic acid derivatives, diterpenoids, alkaloids, and derivatives, as well as vitamin C and phenolic acid (Bazylko et al., 2012). Plants that contain alkaloids have shown to have potential therapeutic effects against a variety of neurodegenerative diseases, including Alzheimer's disease (AD), Huntington disease (HD), Parkinson's disease (PD), Epilepsy, Schizophrenia, and stroke (Hussain et al., 2018). GP has been reported to have analgesic and anti-inflammatory effects, including free radical scavenging activity, reduction of hyaluronidase activity, and inhibition of IL-6 production, all of which can help to reduce the activation of an inflammatory response (Studzińska-Sroka et al., 2018). In addition, earlier study has shown that the herbs of GP are an attractive source of antioxidant-rich preparations (Bazylko et al., 2012). The aim of the present studies was to assess behavioral, histological, and histochemical changes in the cerebellar cortex following treatment with GP in Hg induced cerebellar damage.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the Research and Ethics Committee of Kampala International University-Western Campus, and registered as KIU-2021-14. The study adhered to the international and national ethical regulations outlining use of animals in scientific experiments.

Experimental design

GP leaves (Mbr-10007-2) was collected from a garden in Bushenyi district of Uganda. The leaves were dried at room temperature, powdered with a grinding machine, and extracted. Sixteen grams (16g) of powdered leaves was dissolved in 300 ml of distilled water and allowed to rest for three days, often shaking until the authors extracted all plant elements. They then filtered the extract using filter paper (Dr wax) and solidified in an oven at 70°C for three days (Bazylko et al., 2012). The Mercuric chloride (May and Bakers, England) was purchased from a reputable chemical store in Ishaka town, Western Uganda.

Experimental animals

Twenty-five (25) adult male mice weighing 20-35 g were obtained

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from the Kampala International University Western Campus (KIU-WC) animal facility for this investigation. All mice were placed in a well-ventilated plastic cage and given free access to food and water for two weeks to acclimatize. These animals were further divided into five (5) groups based on their weight (n = 5). Animals in Group (I-V) received oral administration of 2 ml/kg distilled water for 6 weeks, 2.3 mg/kg (Franciscato et al., 2011) of $HgCl_2$ for 3 weeks only, 2.3 mg/kg of $HgCl_2$ for the first 3 weeks followed by oral administration of 800 mg/kg (Yadav and Tangpu, 2008) of GP extract for the next 3 weeks, 800 mg/kg of GP extract for the first 3 weeks followed by 2.3 mg/kg of $HgCl_2$ for the next three weeks, and 2.3 mg/kg of $HgCl_2$ and 800 mg/kg of GP extract concurrently for three weeks, respectively.

Beam walking test

This test was conducted using the method outlined by Luong et al. (2011). The beam walking test can be used to examine fine motor coordination and balance. This is helpful for detecting modest deficiencies in motor skills and balances that other motor test, such as the Rotarod (Loung et al., 2011). The beam apparatus consisted of two poles supporting one-meter beams with a flat surface of 12 mm width that rested 50 cm above the tabletop. The endpoint was represented by a black box put at the end of the beam. The mouse was moved to the room with the beam device around 10 min before the training. The mouse was positioned in the middle of the beam, facing one of the ends, with care. The mouse was permitted to walk to the darkroom's end, and the time it took to get there was recorded. Each mouse was put through three trials through the design that causes the animal to become conscious that a goal box could be reached (Magaji et al., 2012). The timer began when the nose of the mouse entered the center of the beam and ended when the animal reached the end.

Histological and histochemical study

The brain was transcardially perfused using 4% paraformaldehyde fixatives and fixed tissues were processed in a procedure described by (Okesina and Ajao, 2019), for Hematoxylin and Eosin (H and E) Staining to understand the cytoarchitecture of the cerebellum after the various treatments. Subsequently, myelin staining was done as de Also, myelin staning was done as describe by Jouihan (2012).

Statistical analysis

Statistical analysis was performed using SPSS version 25. Data obtained from this study were analyzed using ANOVA to compare mean across the groups followed by Tukey post-hoc test. Statistical significance of $p < 0.05*$ (95% C.I) was used. All results were expressed as mean ± SD or SEM.

RESULTS

Beam walking test

When compared to control group, the group given 2.3 mg/kg of $HgCl_2$ followed by oral administration of 800 mg/kg of GP extract, showed remarkable increase in the time taken to transit the beam to the dark area, notably at the beginning of the test period, but a significant decrease in time at the end of the test time ($P < 0.05$). However, there was a decrease in time during test time

when compared to group 2 animals which were given 2.3 mg/kg of $HgCl_2$. Also, when compared to the control group, group 4 animals which were given 800 mg/kg of GP extract for the first three weeks followed by 2.3 mg/kg of $HgCl_2$ orally for the next three weeks, showed an increase in the time taken to transit the beam at the beginning of the test and a significant reduction in time at the end of the test time ($P < 0.05$). During the test period, Group 5 animals which were concurrently given 2.3 mg/kg of $HgCl_2$ and 800mg/kg of GP extract for three weeks showed a reduction in time when compared to control group (distilled water treated group) and the 2.3 mg/kg of $HgCl_2$ treated group (Table 1).

Histological observation

Normal histo-architecture featuring Purkinje layers, molecular layer (ML), Purkinje cell layer (PL), and granular cell layer (GL) were visible in the photomicrograph of the cerebellum of the adult male mice treated with distilled water (control) (Figure 1A). In the Purkinje cell layer of the cerebellar cortex of group 2, histological examinations based on H and E revealed indications of pyknosis following three weeks of oral administration of 2.3 mg/kg $HgCl_2$ (Figure 1B). In group 3, the section revealed rather normal Purkinje cells with marked reduction of pyknotic cells in mice given 800 mg/kg of GP extract orally for three weeks after receiving 2.3 mg/kg $HgCl_2$ for three weeks (Figure 1C). In the pyramidal cell layer of the cerebellar cortex of group 4 mice, both pyknosis and normal cells were observed in mice given 800mg/kg of GP extract prior to 3 weeks oral administration of 2.3 mg/kg of $HgCl_2$ (Figure 1D). Group 5 demonstrated a more normal pyramidal cell (with an intact nucleus) (Figure 1E).

The photomicrograph of the cerebellum of adult male mice treated with distilled water group 1 (normal control), revealed relatively normal cyto-architecture; having normal myelination (Figure 2A). Section of the cerebellum from the 2.3 mg/kg $HgCl_2$ treated group revealed degeneration of myelin sheath and a region of reduced linear distribution of Purkinje cells (Figure 2B). Section of the cerebellar cortex from Group 3 animals given 800 mg/kg of GP extract orally for three weeks after receiving 2.3 mg/kg $HgCl_2$ for three weeks revealed regions with degenerating myelination (Figure 2C). Sections of the cerebellar cortex from the group 4 animals given 800mg/kg of GP extract prior to 3 weeks oral administration of 2.3 mg/kg of $HgCl_2$ revealed a region of reduced linear distribution of Purkinje cells (Figure 2D). In group 5, the section of the cerebellum shows a region of reduced linear distribution of Purkinje cells (Figure 2E)

DISCUSSION

The beam walking test was employed in this study to

Table 1. Beam walking test of adult male mice during administration of *Galinsoga parviflora* (cav) aqueous leaf extract on the cerebellum after mercury chloride exposure.

Group	TN 1(s)	TN 2 (s)	TN3 (s)	TN 4 (s)	TN 5 (s)	TS 1 (s)	TS 2 (s)	TS 3 (s)	TS 4 (s)
Group 1	15.67±1.87	10.92±0.47	12.67±1.35	20.10±2.34	11.10±1.93	15.80±1.71	10.81±0.98	15.80±1.71	10.81±0.98
Group 2	13.81±1.88	11.79±1.30	11.43±1.62	20.75±2.60	16.02±1.29	19.80±3.25	15.68±3.40		
Group3	17.50±1.96	12.70±1.84	11.50±1.19	21.77±3.69	20.42±2.48[a]	26.72±5.73	25.23±5.06[a]	10.30±2.39	20.99±4.43[a]
Group 4	17.18±3.59	12.10±2.26	10.41±1.12	17.46±3.53	12.28±1.86	14.27±4.29	11.87±1.75	23.41±1.22a	9.87±1.22
Group 5	12.29±1.36	12.67±1.34	15.63±2.36	19.50±3.26	13.90±1.67	14.04±3.12	8.74±1.73		
F value	0.969	0.225	1.596	0.265	3.809	1.907	4.507	11.175	8.681
P value	0.446	O.921	0.214	0.897	0.018	0.149	0.009	0.002	0.005

n=5, TN=Training time, TS=Test time. The value was considered significantly different at $p < 0.05$. "a and b": indicates significant difference when compared to the distilled water and the 2.3 mg/kg of $HgCl_2$ treated group respectively. Group 1: Distilled water treated group, Group 2: 2.3mg/kg body weight of $HgCl_2$, Group 3: 2.3 mg/kg $HgCl_2$ + GP extract (800mg/kg), Group 4: GP extract (800mg/kg) + 2.3 mg/kg $HgCl_2$, Group 5: GP extract (800mg/kg) + 2.3 mg/kg $HgCl_2$ concurrently.
Source: Author

examine fine motor coordination, and the time it took the mice to get to the darkroom. The present showed that the administration of Hg was neurotoxic and affected motor coordination among the test animals, as indicated by the increase in the time taken to transit the beam to the dark box; this could possibly be attributed to a cortical impact lesion. the authors observation align with Buccafusco et al. (2009) findings, who reported earlier that mice with cortical impact lesions frequently exhibits contralateral slippage on the beam in an earlier investigation. The poor fine motor coordination and balance seen in the beam walk test suggest that CNS lesions may have caused motor impairments as a result of mercury damage to the CNS. On the other hand, treatment with GP was associated with improved coordination in all the treatment groups. This finding may support the previous report on the possible anti-inflammatory effects, free radical scavenging activity, reduction of hyaluronidase activity, and inhibition of IL-6 production associated with GP (Studzińska-Sroka et al., 2018).

Hg exposure was associated with degenerative alterations in the Purkinje layer of the cerebellar cortex, as evident in the depletion of Purkinje cells population and the presence of pyknotic Purkinje cells. The finding may support the previous report by Ibegbu et al. (2014) revealed degenerative and necrotic changes in the Purkinje cells of the Purkinje cell layer of the cerebellar cortex following aluminum chloride exposure. Pyknosis seen in cerebellar cortex in this study is a sign of neuronal death, with its consequent effects on fine motor coordination. Alteration of fine motor function, balance, posture, and motor learning are often affected in cerebellar injury (Gray's Anatomy, 2008). Damage to the Purkinje cells in the cerebellum can obstruct fine motor performance and learning (Gandhi et al., 2000); this could have happened as a result of chromatin condensation, which causes cell death (Bianchi and Manfredi, 2004). Because the cerebellum possesses a blood brain barrier (BBB) that is susceptible to mercury intoxication (Takahashi et al., 2017). Methyl mercury has the potential to cause damage to the BBB through the production of RECA-1 and extravasation of endogenous IgG (Takahashi et al., 2017). Our findings are in line

with those of (Olivier et al., 2021), who found glial cell multiplication and granular cell loss beneath the Purkinje cell layer. Furthermore, our findings using a histochemical stain (Kluver-Barrera) demonstrated myelin component depletion in the Purkinje layer. This could be due to oligodendroglia cell loss or disruption, as well as an immune-mediated response (Duncan and Radcliff, 2016). The processes of immunotoxicity, on the other hand, are claimed to be depending on the amount of Hg exposed to and the type of Hg (Eagles-Smith et al., 2018). Higher doses can cause the production of cytokine signals, whilst smaller doses only cause the production of cytokine signals without affecting cell numbers (Eagles-Smith et al., 2018).

To test the curative therapeutic potential of GP extract, mice were administered 800 mg/kg orally after being exposed to $HgCl_2$. When compared to the control group, the histoarchitecture of the cerebellar cortex revealed a reduction in Purkinje cells, with the myelin and Nissl staining suggesting possible recovery. Plants containing alkaloids have potential therapeutic effects against a variety of neurodegenerative diseases, according to Hussain
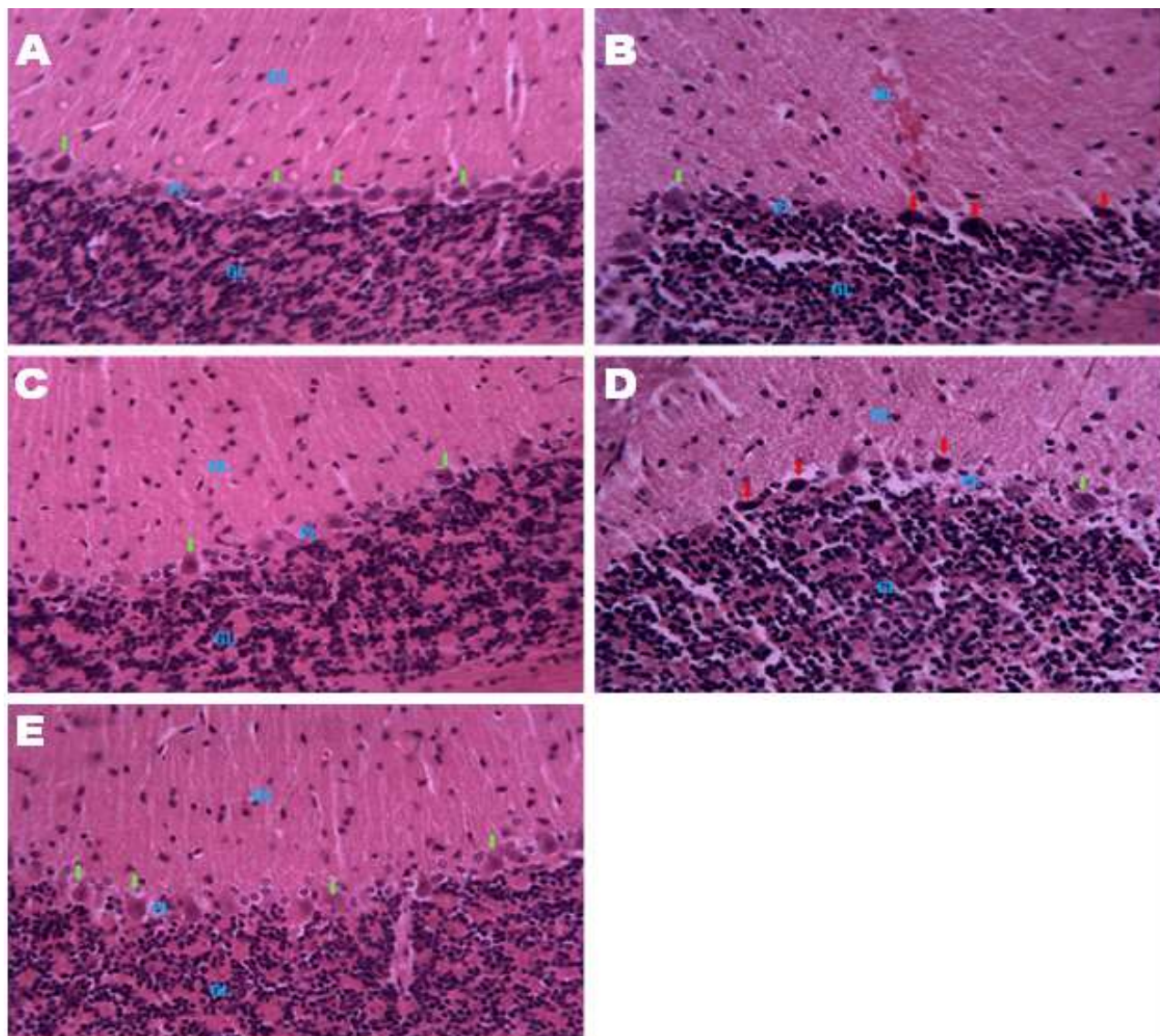
Figure 1. Photomicrograph from the cerebellum mice from the 2 ml/kg bw of H_2O TREATED GROUP (1A), THE 2.3 ml/kg $HgCl_2$ treatment group (1B), the mg/kg $HgCl_2$ followed by 800 mg/kg GP (1C), 800 mg/kg GP followed by 2.3 mg/kg $HgCl_2$ treated group (1D), and the 800 mg.kg GP and 2.3 mg/kg $HgCl_2$ concurrently (1E). Molecular layer (ML), Pyramidal cell layer (PL), Granular cell layer (GL), relatively normal pyramidsl cells (Green arrow), pyknotic pyramidal cells (Red arrows) (H and E; x 250).
Source: Author

et al. (2018), who studied the function of plant-derived alkaloids and their processes in neurodegenerative diseases. The presence of phytochemicals found in GP, such as alkaloid, carbohydrate, flavonoid, polyphenol, and glycoside, could explain the observed therapeutic potential of GP. This shows that the presence of such phytochemicals could be responsible for the observed recovery of cells.

In comparison to the control group, further investigation into the preventative impact of GP revealed a dramatic and considerable loss of cells in the Purkinje layer. The Purkinje layer has few cells as a result of cell death. A postmortem analysis found a drop in Purkinje cell numbers in the brains of patients with essential tremor who did not have Lewy bodies, according to Axelrad et al., (2008). This shows that a decrease in the linear distribution of Purkinje cells in the cerebellum may be caused by cell death or Lewy bodies. When compared to the control group, histochemical demonstration with Kluver-Barrera revealed a considerable reduction in the content of the myelin sheath in the Purkinje layer. Due to its improved histoarchitecture and lower number of
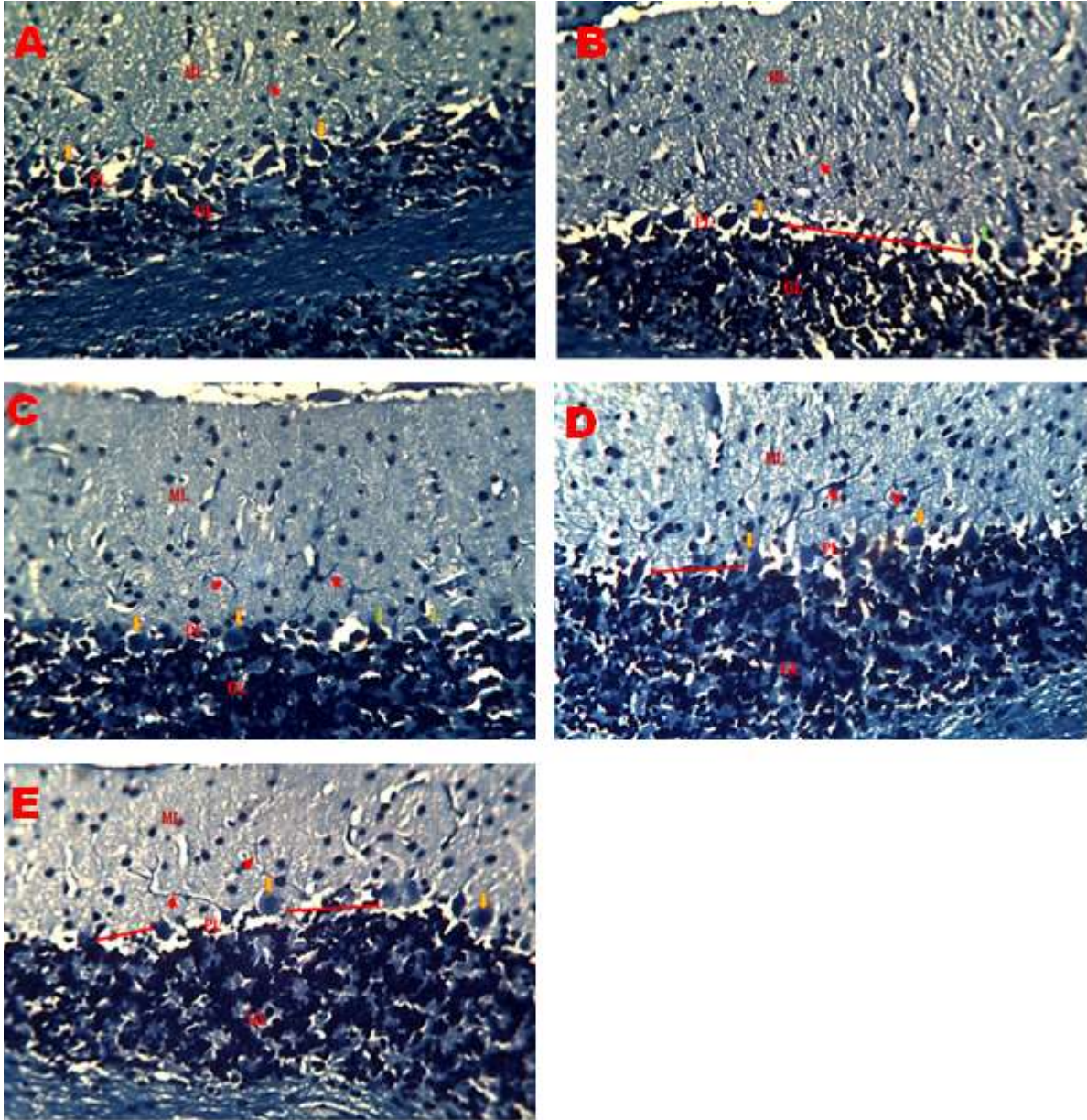
Figure 2. Photomicrograph from the cerebellum mice from the 2 ml/kg bw of H_2O treated group (2A), the 2.3 ml/kg $HgCl_2$ treatment group (2B), the mg/kg $HgCl_2$ followed by 800 mg/kg GP (2C), 800 mg/kg GP followed by 2.3 mg/kg $HgCl_2$ treated group (2D), and the 800 mg.kg GP and 2.3 mg/kg $HgCl_2$ concurrently (2E). Showing; the molecular layer (ML), Pyramidal cell layer (PL), Granular cell layer (GL), normal pyramidal cells (Orange arrow), nerve fibres (red arrowhead) degenerating myelin (Green arrows), and region of reduced linear distribution of purkinje cells (red line) (Kluver Barrera; × 250).
Source: Author

pyknotic nuclei, the therapeutic treatment to Hg poisoning appears to be better when compared to group three. This could be because the GP extract boosted the immunological response produced by early $HgCl_2$ exposure. To confirm that GP has a neuromodulatory effect, more study is needed in this area. Future study may need to concentrate on figuring out how GP works and investigating the impact of the phytochemicals

involved in the process. The molecular interplay between the neuron-glia triad may aid in elucidating the therapeutic role of GP extract.

Concurrent administration of $HgCl_2$ and GP extract in group 5 revealed normal histoarchitecture of the cerebellar cortex when compared to the control group. However, histochemical demonstration of the myelin sheath revealed reduction in the content of the Purkinje

cells which seems to be recovering. Overall, myelin sheath degeneration occurs when a neuron dies or if its axons have been severed, the myelin sheath surrounding the degenerating axon breaks up and is phagocytosed (Burnett and Zager, 2004). The conduction of signals in the affected nerves is hampered by myelin degradation. As a result, the reduction in conduction ability causes deficiency in sensation, movement, cognition, or other functions depending on which nerves are involved (Swanson, 2017). Degradation of myelin in cerebellar cortex neurons, as found in the study, may have disrupted the conduction of electrical signals in neurons, impairing normal cerebellar cortex functioning. The pyknosis and degenerating myelin seen in histomorphological investigations of the cerebellum could be implicated in the motor deficiencies seen in neurobehavioural studies.

Conclusion

The study revealed that Hg exposure in the cerebellum of mice caused pyknosis, reduced myelination in the axons, loss of neuromuscular function, loss of balance, and coordination. However, the administration of GP was of therapeutic value against Hg induced cerebellar toxicity in adult male mice.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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