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Aluminium Chloride-induced Prefrontal Cortex Toxicity in Wistar Rats: Evidence From Pretreatment with Aqueous *Psidium guajava* Leaf Extract

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Abstract
Aluminium is reported to be a very common element and one of the most abundant metals on Earth. Although aluminium is neurotoxic and linked to several brain disorders, reports indicate that antioxidants from natural sources can neutralise the toxic free radicals generated by aluminium. Consequently, this study evaluated the protective and antioxidative effects of Psidium guajava (GV) aqueous leaf extract on Aluminium-induced prefrontal cortex toxicity. Thirty (30) adult Wistar rats in six (6) groups were used for this experiment (n=5): Group A (control); Group B rats (Al) were given 100 mg/kg body weight (BW) of Aluminium alone; Group C (Al + GV1) rats were given 100 mg/kg BW of Aluminium and 200mg/kg BW/day of aqueous GV leaf extract; Group D (Al + GV2) was administered 100 mg/kg BW of Al and 400 mg/kg BW of aqueous GV leaf extract; Group E (GV1) was administered 200 mg/kg BW of aqueous GV leaf extract alone and Group F (GV2) was administered 400 mg/kg BW of aqueous GV leaf extract alone. Following the end of administration, neurobehavioral, antioxidant enzymes, and prefrontal cortex histology assessments were done. Findings showed that Group B rats had a significant ($p<0.05$) decrease in final body and absolute brain weights, and antioxidant enzymes activity when compared to control. Likewise, a significant ($p<0.05$) decrease was observed in spontaneous alternation in rats treated with Al alone, thus indicating cognitive dysfunction. Conversely, following pretreatment with Psidium guajava, the alterations induced by Al were mitigated. Taken together, Psidium guajava showed a potent capability as a possible neuroprotective agent that can be useful for inhibiting Al-induced neurotoxicity and cognitive dysfunctions. Further investigations and development of this plant could offer a worthwhile substitute to other orthodox drugs currently used in the treatment and/or management of aluminium-related disorders.

Keywords: Aluminium, *Psidium guajava*, Neurotoxicity, Neuroprotection, Neurobehaviour

INTRODUCTION
Aluminium is the most widely distributed metal in the environment and is extensively used daily, consequently creating a channel for easy exposure to humans (Exley, 2013). Exposure to Aluminium occurs in professions linked to mining and during engagement in Aluminium metal cutting and welding. Animals and humans living in environments contaminated by industrial wastes may also be exposed to high levels of Aluminium (Boran *et al.*, 2013). Intake of Aluminium is by inhalation of aerosols or particles, ingestion of food, water and

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medicaments, skin contact, vaccination, dialysis and infusions (Igbokwe *et al.,* 2019). Reports show that the brain is the most vulnerable to the toxic manifestation of Aluminium and is further linked to other brain disorders such as Alzheimer's and Parkinson's diseases as well as multiple Sclerosis (Inan-Eroglu and Ayaz, 2018). Although the mechanisms underlying Aluminium-induced neurotoxicity are complex, they include oxidative stress, membrane biophysics alterations, deregulation of cell signalling, apoptosis and the impairment of neurotransmission (Dey and Singh, 2022). Aluminium exposure excessively increases reactive oxygen species (ROS) production which ultimately disrupts the lipid bilayer, damages nucleic acid and denatures protein resulting in oxidative damage and ultimately cell death (Achary *et al.,* 2008).

Antioxidants, especially from natural origins, are reported to be fundamental in human health owing to their ability to hunt free radicals and ultimately prevent oxidative damage (Enogieru and Momodu, 2021). *Psidium guajava* (Guava), is an important dietary plant used traditionally for medicinal purposes around the world (Rishika *et al*., 2012). It is very rich in antioxidants and vitamins and also high in lutein, zeaxanthin and lycopene (Rahmat *et al*., 2006). *Psidium guajava* contains both carotenoids and polyphenols, the main classes of antioxidant pigments, giving them relatively high potential antioxidant value among plant foods (Jimenez-Escrig *et al.,* 2001). Different pharmacological experiments in several *in-vitro* and *in-vivo* models have been used to demonstrate the antioxidant potential of *Psidium guajava* against diabetes mellitus, cardiovascular diseases, cancer, and parasitic infections (Díaz-de-Cerio *et al.,* 2017). Other pharmacological studies have demonstrated that *Psidium guajava* exhibits hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, antispasmodic, anti-inflammatory and antinociceptive activities, thereby supporting its traditional uses (Gutiérrez *et al.,* 2008). Quercetin is considered the most active antioxidant in the *Psidium guajava* leaves and is responsible for most of its pharmacological benefits (Naseer *et al.,* 2018). To our knowledge, there is a paucity of information to sufficiently highlight the role of *Psidium guajava* against aluminium-induced prefrontal cortex toxicity in Wistar rats. Therefore, this study aimed to examine such activity in Wistar rats.

METHODOLOGY

Plant Material And Extract Preparation

Psidium guajava leaves were bought from the Oba market in the City of Benin, Edo State. A Botanist authenticated the leaves at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria with UBH-P378 as the authentication number. Subsequently, the leaves were air-dried at room temperature and ground through a laboratory mortar and pestle followed by sieving. One kilogram of the fine powder was saturated in 10 litres of distilled water and was extracted for Twenty-four hours. The water extract was collected and filtered using Whatman filter paper No 42 (125 mm) and was thereafter freeze-dried (LGJ-10, SearchEquipment, UK) to obtain a dried powder which was kept at 4 °C until used.

Phytochemical Screening

Standard methods, previously reported, were carried out to qualitatively assess the chemical composition of *Psidium guajava* leaves (Sofowora, 1993; Trease and Evans, 1983). Compounds such as tannins, phenols, flavonoids, steroids, saponins, terpenoids, carbohydrates, phylobotannins, and alkaloids were tested.

Acute Toxicity Study

This study was carried out as previously reported (Lorke, 1983; Enogieru and Omoruyi, 2022). Three groups (1, 2 and 3) with three rats respectively, given single doses of 10, 100 and 1000 mg/kg body weight of aqueous *Psidium guajava* leaf extract, were observed for seventy-two hours for behavioural changes and death. Afterwards, three new groups (4, 5 and 6), with two rats respectively, were given single dosages of 1600, 2900 and 5000 mg/kg body weight of aqueous *Psidium guajava* leaf extract. They were further monitored for seventy-two hours for likely mortality and behavioural alterations.

Care And Management Of Experimental Rats

Thirty (30) Wistar rats, weighing between 150g-180 g were acquired and kept in the Department of Anatomy, College of Medical Sciences, University of Benin, Nigeria. The rats, fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and water liberally, were allowed to acclimatize for 2 weeks before the commencement of the experiment. The study was approved by the Research Ethics Committee of the College of Medical Sciences, University of Benin (CMS/REC/2023/342).

Experimental Design

The experimental design (Table 1) for the study is shown below:

Table 1: Experimental design for the study.

Group A (control)	Normal saline
Group B (Al)	100 mg/kg body weight (BW) of Aluminium chloride only
Group C (Al + GV1)	100 mg/kg BW of Aluminium and 200mg/kg BW/day of aqueous *Psidium guajava* leaf extract.
Group D (Al + GV2)	100 mg/kg BW of Al and 400 mg/kg BW of aqueous *Psidium guajava* leaf extract
Group E (GV1)	200 mg/kg BW of aqueous *Psidium guajava* leaf extract only
Group F (GV2)	400 mg/kg BW of aqueous *Psidium guajava* leaf extract only

All rats were pretreated with aqueous *Psidium guajava* leaf extract one hour before the administration of Aluminium chloride. After 28 days, the Y-maze neurobehavioural test was carried out.

Evaluation Of Neurobehavioral Activity

Experimental rats were quietly placed individually in the Y-maze apparatus, which had three identical arms (33×11×12 cm each) symmetrically separated at 120° (Dall'igna *et al*., 2007). Each rat positioned at the end of arm A was permitted to discover all three arms (A-B-C) liberally for five minutes. The total number of arm discoveries and order of arm entries (alternation) were assessed. Alternation was recorded as entrances in all three arms at successive times. The percentage of alternation was derived from the total of alternations/(total arm entries –2).

Determination Of Relative Brain Weight

Following the end of the Y-maze test, the cerebrum of experimental rats was removed, weighed and the relative weight of the brain (%) was expressed as a percentage of the final body weight at sacrifice (Kim *et al*., 2008). This was done to reduce the individual body weight differences.

Evaluation Of Biochemical Parameters

The cerebrum was homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and centrifuged at 10,000 g for 10 minutes at 4 °C (Montilla *et al*., 2005). The supernatant was collected and

evaluated for malondialdehyde (Buege and Aust, 1978), catalase (Cohen *et al*., 1970), superoxide dismutase (Misra and Fridovich, 1972) and glutathione (Nyman, 1959).

Histological Evaluation
Following appropriate fixation of the cerebrum in 10% buffered formal saline for 72 h, processing through the paraffin wax embedding and the hematoxylin and eosin staining methods were carried out as previously described by Drury and Wallington (1980).

Statistical Analysis
The GraphPad Prism 7.0 Software was used to analyze the data which were presented as mean ± SEM; followed by a one-way analysis of variance (ANOVA). Specific comparisons were carried out using Tukey's multiple comparisons post hoc test for statistical significance at $p < 0.05$.

RESULTS

Phytochemical Screening
Findings showed that aqueous *Psidium guajava* leaf extract contained saponins, phenolic, terpenoids, eugenols, alkaloids, flavonoids and reducing sugar. However, steroids and glycoside were observed to be absent (Table 2).

Table 2: Phytochemical analysis of aqueous *Psidium guajava* leaf extract.

PHYTOCHEMICALS	RESULTS
Glycoside	+
Saponins	+
Phenolics	+
Terpenoids	+
Eugenols	+
Alkaloids	+
Flavonoids	+
Reducing Sugar	+
Steroids	_
Tannins	_

+ Present; - Absent

Acute Toxicity
There were no behavioural alterations and death observed following the administration of aqueous *Psidium guajava* leaf extract at dosages from 10-5000 mg/kg body weight. This suggests that the extract was safe enough for the experimental rats.

Effect of *Psidium guajava* on Weights
For the absolute whole brain weight (Table 3), while a significant decrease ($p<0.05$) was observed in group B (Al) when compared to control, groups C (Al+GV1) and D (Al+GV2) recorded an increase when compared to Aluminium only treated group B. This was, however, not significant ($p>0.05$).

Table 3: Weights across experimental groups.

Groups	Initial BW (g)	Final BW (g)	Absolute whole brain weight (g)	Relative brain weight (%)
Control	157.6 ± 2.249	172.6 ± 1.887	1.840 ± 0.060	1.090 ± 0.022
Al	151.6± 4.057	155.4 ± 3.641	1.620 ± 0.037#	0.880 ± 0.020
Al + CO1	151.6 ± 6.757	172.5 ± 5.867	1.775 ± 0.094	1.029 ± 0.038
Al + CO2	152.6 ± 6.337	172.6 ± 10.670	1.780 ± 0.037	1.050 ± 0.078
CO1	151.0 ± 5.541	182.5 ± 5.979	1.800 ± 0.055	1.026 ± 0.028
CO2	156.2 ± 7.513	173.2 ± 6.476	1.780 ± 0.037	1.030 ± 0.020

Effect of *Psidium guajava* on Neurobehavior

Figure 1 shows the spontaneous alternation (%) during the Y-maze test in experimental group A-F. Results show a significant reduction ($p<0.05$) in group B (Al) when compared to the control. On the contrary, in groups C (Al+GV1) and D (Al+GV2), a significant increase ($p<0.05$) was observed when compared to the Aluminium-only treated group B (Al).

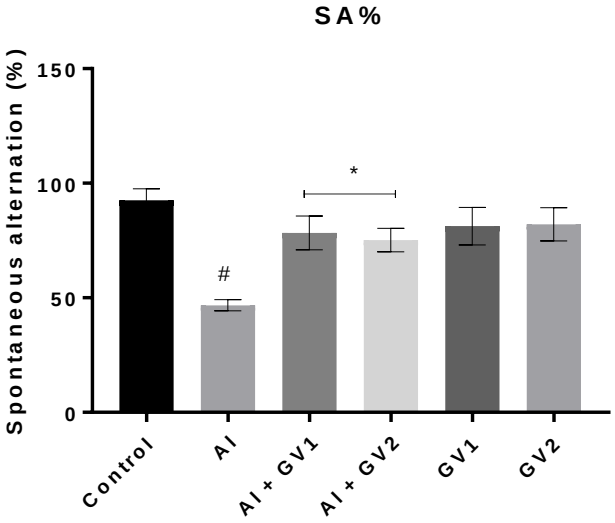
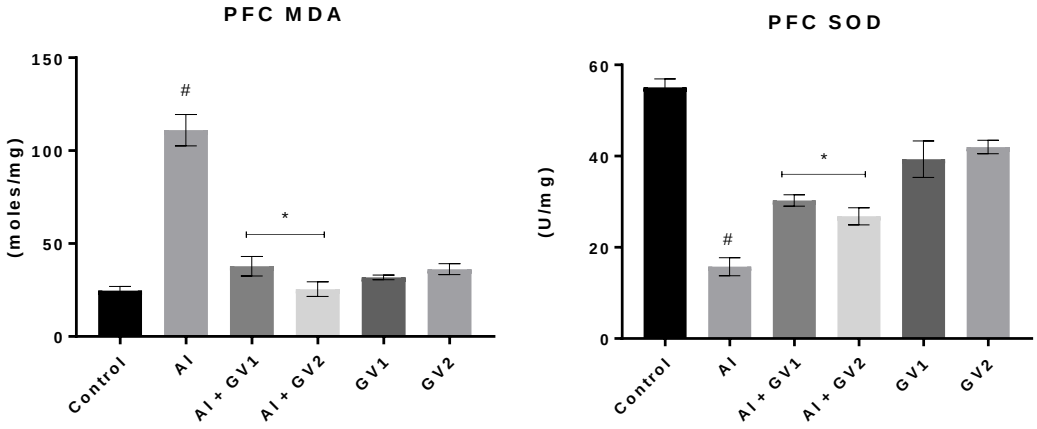


Figure 1: Spontaneous alternation (Y-Maze test) across experimental groups. # and * represents $p< 0.05$ when compared with the control group and Al-alone group respectively.

Effect of *Psidium guajava* on Antioxidant Enzymes and Lipid Peroxidation

Results show a significant reduction in SOD, CAT and GSH ($p<0.05$) in group B (Al) when compared to control (Figure 2). Conversely, in SOD (groups C and D), CAT (groups C and D) and GSH (group C), a significant increase ($p<0.05$) was observed when compared to Aluminium only treated group B. Furthermore, for MDA, a significant increase ($p<0.05$) was observed in group B (Al) following comparison to control. Conversely, a significant reduction ($p<0.05$) was observed in MDA in groups C (Al+GV1) and D (Al+GV2) when compared to the Aluminium-only treated group B.



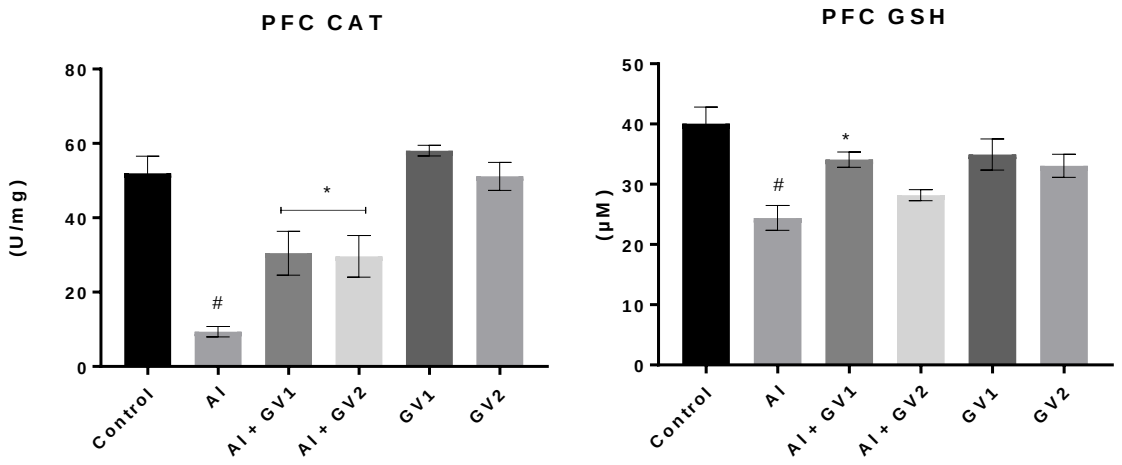


Figure 2: Activity of antioxidants and lipid peroxidation in the prefrontal cortex across experimental groups. # and * represents $p< 0.05$ when compared with the control group and Al-alone group respectively.

Effect Of Treatment On The Histology

Plate 1 shows the histology of the prefrontal cortex stained with Haematoxylin and Eosin. Photomicrographs of the experimental rats in the control group revealed normal prefrontal cortex histology. However, multiple neuropil vacuolation and degenerating pyramidal cells due to the administration of aluminium were observed in rats in group B. In Plate 1 (C-D), relatively normal Pyramidal cells were observed in the *Psidium guajava* pretreated groups when compared to the aluminium-only treated group. Plate 1 (E-F) shows relatively normal Pyramidal and neuroglial cells having similar tissue morphology to control.

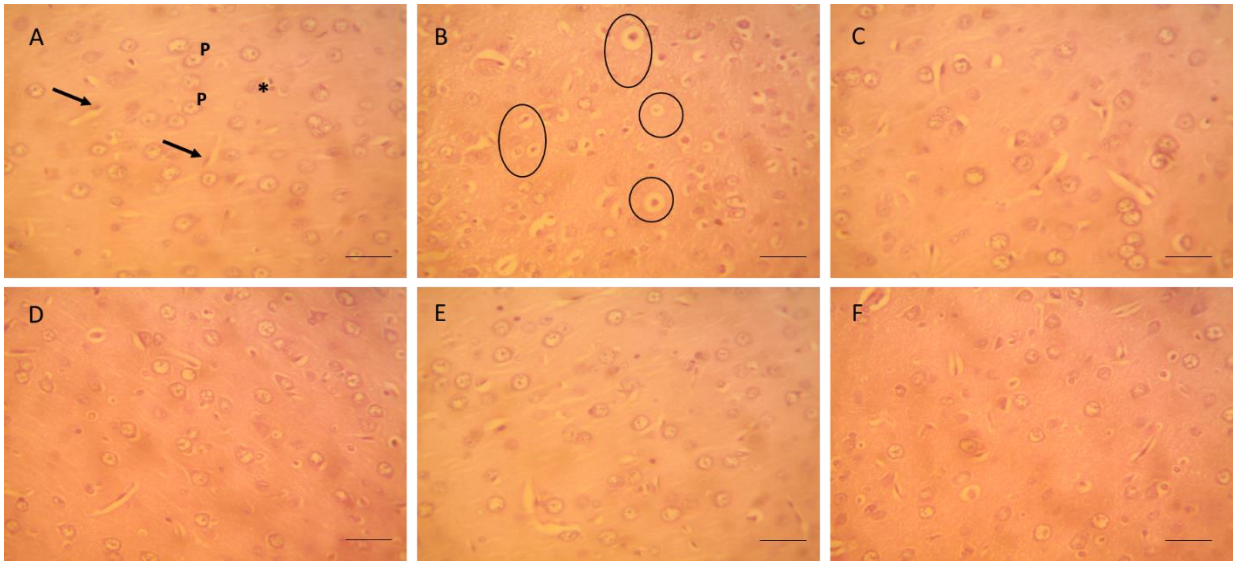


Plate 1: Representative histology of the Prefrontal cortex across experimental groups. (A) Control rats revealed typical Pyramidal cells with open-face nuclei and basophilic cytoplasm (P), vacuolated neuropil (asterisk) and normal neuroglial cells (arrow). (B) Group B rats showed multiple neuropil vacuolation and degenerating pyramidal cells (circles). (C-D) Group C and D rats revealed relatively normal pyramidal cells (E-F) Group E and F rats revealed normal pyramidal and neuroglial cells. H&E, Scale bar: 25µm

DISCUSSION

Aluminium toxicity is a prominent metal poisoning that affects all human body functions. However, since oxidative stress is one of the key mechanisms of Aluminium-induced toxicity, reports indicate that antioxidants can counteract and mitigate the toxicity of Aluminium (Sumathi *et al*., 2011). Hence, this study evaluated the protective activity of aqueous *Psidium guajava* leaf extract on Aluminium-induced prefrontal cortex toxicity.

Findings from this study showed a decrease in the final body weight of the Aluminium only treated group following comparison to the control. This is consistent with other studies which reported that exposure to Aluminium decreased rats' body weight (Buraimoh and Ojo, 2014; Bekhedda *et al*., 2020). In addition, Golub and Germann (2001) showed significant decreases in mice pup body weight after Aluminium exposure during the mother`s gestation/lactation and offspring exposure.

The prefrontal cortex plays a vital role in short-term, long-term, and learning memory formation and is highly vulnerable to oxidative damage (Woo *et al*., 2021). The Y-maze test is a spatial working memory test, used to measure cognitive dysfunction (learning and memory) by measuring the spontaneous alternation of the animals as an index of cognitive function (Ben-Azu *et al*., 2016). In this study, a significant decrease in the spontaneous alternation of the Aluminium-treated-only group following comparison to the control signified cognitive dysfunction. This is in line with previous studies reporting memory impairment, particularly for short-term memory, as a characteristic of Aluminium-associated encephalopathies (Kawahara and Kato-Negishi, 2011; Kandimalla *et al*., 2016). Conversely, there was a significant increase in the spontaneous alternation of the *Psidium guajava* pretreated groups. This infers that *Psidium guajava* leaf extract was able to produce a cognition-enhancing effect, being able to reverse Aluminium-induced cognitive deficit. This is in line with reports demonstrating that *Psidium guajava* leaf extract improves memory as well as cognitive impairments (Penido *et al.,* 2017).

Aluminium exposure increases reactive oxygen species (ROS) production which disrupts lipid bi-layer, damages nucleic acid, disrupts antioxidant enzymes and denatures protein, ultimately resulting in oxidative damage (Achary *et al*., 2008). Results from this study showed that there was a significant decrease in SOD, CAT and GSH in the prefrontal cortex of Aluminium only treated group. This highlights the ability of Aluminium to alter antioxidant activities through the inhibition of antioxidant enzymes and generation of reactive oxygen species (ROS). In addition to ROS, oxidative stress may also induce uncontrolled lipid peroxidation, which in turn, can result in cell injuries via DNA damage and directly inhibit proteins (Spiteller 2006; Enogieru *et al*., 2018). Malondialdehyde (MDA) is often utilized as an indication of lipid peroxidation and the findings showed a significant increase in prefrontal cortex MDA levels indicating a high level of lipid peroxidation. Conversely, a significant reduction was observed in the prefrontal cortex MDA levels of the *Psidium guajava* extract pretreated groups when compared to Aluminium only treated group. This implies that the extract was able to protect against Aluminium-induced oxidative stress. This is in line with previous studies demonstrating that *Psidium guajava* boosts the activities of antioxidant enzymes and inhibits lipid peroxidation (Manikandan and Anand, 2016; Fatoki *et al*., 2022).
Histological findings of the control group showed normal histology of the prefrontal cortex. Conversely, the Aluminium only treated group showed altered morphology with multiple neutrophil vacuolations and degenerating pyramidal cells in the prefrontal cortex. This agrees with the histological data from previous reports demonstrating neuronal degeneration or pyknosis and vacuolation in the prefrontal cortex of aluminium chloride-induced Alzheimer-

like dementia in Wistar rats (Elgendy *et al*., 2008; Olanrewaju *et al*., 2018). In *Psidium guajava* pretreated groups, the histology of the prefrontal cortex remains relatively normal. Thus, the mitigation of Aluminium-induced histological alterations by *Psidium guajava* indicates its significant neuroprotective effect. In addition, findings from this study showed that experimental rats treated with *Psidium guajava*-alone had a similar appearance to the control signifying that aqueous Psidium guajava leaf extract had no toxic effect on the prefrontal cortex of the experimental rats.

CONCLUSION

The findings of this study suggest a possible therapeutic role of Psidium guajava owing to its protection against Aluminium toxicity, mediated probably, through its potent antioxidant properties and active phytochemical constituents.

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