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RESEARCH ARTICLE

Aluminium-Induced Hepatotoxicity and the Protective Role of *Withania somnifera* (Ashwagandha) in Wistar Rats

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Abstract: Aluminium is a widely distributed environmental contaminant whose chronic exposure leads to accumulation in multiple organs, including the liver, resulting in oxidative stress and tissue damage. When reactive oxygen species production exceeds the body's antioxidant defense capacity, cellular injury ensues — a mechanism central to aluminium-induced hepatotoxicity. *Withania somnifera* (Ashwagandha), an evergreen herb of the Solanaceae family widely cultivated in India, has been reported to exert diverse pharmacological effects, including antioxidant, anti-inflammatory, and hepatoprotective activities. This study aimed to evaluate the protective potential of *Withania somnifera* against aluminium chloride ($AlCl_3$)-induced hepatic damage in Wistar rats. Twenty-four healthy male Wistar rats (150–200 g) were randomly divided into four groups (n = 6 per group): control, Ashwagandha-treated, $AlCl_3$-intoxicated, and $AlCl_3$-intoxicated pretreated with Ashwagandha. All treatments were administered for three weeks. Hepatic oxidative stress was assessed by measuring malondialdehyde (MDA) and glutathione (GSH) levels in tissue homogenates. Serum alanine aminotransferase (SGPT/ALT) and aspartate aminotransferase (SGOT/AST) were analyzed as markers of hepatic function. Histopathological examination of liver tissue was also performed. $AlCl_3$ administration significantly reduced hepatic GSH content and elevated MDA, SGPT, and SGOT levels relative to controls, alongside marked histopathological alterations. Pretreatment with Ashwagandha significantly restored GSH levels, reduced MDA, and normalized SGPT and SGOT, with corresponding improvement in liver histology. These findings demonstrate that *Withania somnifera* effectively mitigates aluminium-induced hepatotoxicity, likely through its antioxidant mechanisms, supporting its potential as a hepatoprotective agent in aluminium-exposed populations.

Keywords: *Withania somnifera*, Ashwagandha, Aluminium chloride, Hepatotoxicity, Oxidative stress, Malondialdehyde, Glutathione, Wistar rats

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Introduction

Aluminium (Al) is frequently present in the environment, making both humans and animals susceptible to its toxic effects under high exposure. Aluminium toxicity is associated with a broad range of pathological manifestations, including oxidative stress, immune dysregulation, genotoxicity, inflammation, and alterations in enzymatic, metabolic, peptide, and protein functions. This metal is also known to induce neurotoxicity [1-3], Hem toxicity [4], hepatotoxicity [5-6], nephrotoxicity [7] and reproductive toxicity [8-11].

Aluminium containing compounds are extensively employed in a range of products utilized in daily human life. Some of these are utilized in the refining of rock oil and of petroleum; in making vessels for cooking preparation of foils, pottery, detergents, cosmetics, pharmaceuticals (drugs), vaccines so on. It also acts as an anticorrosion agent; it is used in food additives and in the preparation of baking powders and colorants [12-13]. An excess of reactive free radicals over antioxidant defenses results in oxidative stress. In other words, it reflects a state in which free radicals are present in excess while antioxidants are insufficient. These free radicals begin to damage the different parts of cells, including lipids (fats) and protein [14]. Aluminium is known to induce oxidative damage through various mechanisms in the body. Aluminium can bind to negatively charged phospholipids on the membrane of various tissue cells which are rich in PUFA that are targeted by reactive oxygen species (ROS) [15]. It is also proved that it can bring about the Fenton reaction, driven by iron, leading to lipid peroxidation and will be the key mechanism underlying oxidative stress. This metal also induces oxidative stress by Neutralizing Superoxide (O2˙-) to form an Al-O2˙- complex [16].

Oxidative damage caused by the release of ROS should be delt seriously. Hence, various antioxidants must be considered in the treatment of this oxidative damage induced by aluminium toxicity. Belonging to the Solanaceae family, Ashwagandha is a key plant in Ayurvedic medicine, indicated for the treatment of several neurological disorders. Ashwagandha is considered one of the most significant herbs in Ayurveda, a traditional Indian system of medicine that emphasizes natural healing. It has been used for centuries to reduce stress, enhance energy, and improve cognitive function. Numerous toxicological studies have confirmed its safety and suitability for human consumption [17].

Ashwagandha is known to have many valuable and protective properties like antioxidant, antibacterial, adaptogen. Some prefer to have it as liver tonic, aphrodisiac, and anti-inflammatory drug [18]. It has been shown to possess several potent pharmacological effects, including the restoration of physiological functions, enhancement of cognitive performance in aging, and support in recovery from neurodegenerative disorders [19]. Alkaloids, saponins, and Withanolides, Sitoindosides VII-X and withaferin A are the biological active component of Ashwagandha. Their antioxidative activity has been confirmed through enhanced levels of antioxidant enzymes (superoxide dismutase and GSH), an increase in vitamin C, and a reduction in lipid peroxidation [20]. Accordingly, the objective of this study was to assess the role of Ashwagandha in mitigating aluminium-induced hepatic oxidative damage in Wistar rats.

Materials and Methods

The study was conducted after securing approval from the Institutional Ethics Committee (KMC/MNG/IAEC/14-2023). Healthy adult male Wistar rats weighing between 150 and 200 grams aged one to one & half month was procured from our institution's central animal house. They were kept in the central animal house, which had regulated lighting and temperature as well as regular rat food and water. Aluminium chloride was procured from Sigma company and Ashwagandha was purchased from Saptam Veda company.

Experimental Design and Animal Grouping

Twenty-four rats were randomly allocated into four groups, with six rats per group, as described below:

Group I: Normal rats were given water (2 ml/kg body weight) via oral gavage for 3 weeks and acted as the negative control.

Group II: Rats were treated with Ashwagandha (60 mg/kg body weight dissolved in distilled water) via oral gavage for 3 weeks and acted as the positive control.

Group III: Rats were treated with aluminium (100 mg/kg body weight dissolved in distilled water) via oral gavage for 3 weeks.

Group IV: Rats treated with Ashwagandha (60 mg/kg bw) & aluminium (100mg/kg body weight) via oral gavage for 3 weeks.

The dose selection of aluminium chloride was based on previously published literature. Following the completion of the 3-week experimental protocol, rats were anesthetized, and blood sample was drawn by cardiac puncture to measure serum SGPT and SGOT levels. Following blood collection, the rats were sacrificed, and liver tissues were excised. Portions of the liver were stored at −80 °C for subsequent biochemical analysis, while other portions were fixed in 10% buffered formalin for histopathological examination [21]. Serum levels of SGPT & SGOT were estimated in all the groups by commercially available kits using spectrophotometer (Brand-Elico company). The procedures for the assays were carried out as per the manufacturer’s guidelines.

Malondialdehyde (MDA) Estimation

The extent of lipid peroxidation was determined by estimating MDA through the TBARS method, following the procedure [22]. Tissue homogenate (1 mL) was precipitated with 2.5 mL of ice-cold trichloroacetic acid (TCA). The samples were centrifuged at 3000 g for 10 min. To the obtained homogenate added 2ml of the supernatant and then 0.67% of Thiobarbituric acid (TBA) was mixed and after this the test tube was kept in boiling water bath for 10 min and cooled. The intensity of the pink chromogen was read at 532 nm immediately after development. MDA levels were determined using a molar extinction coefficient of 1.56×10^5 (M^{-1} cm^{-1}) and expressed as nmol/L.

Reduced Glutathione Estimation

Ellman method was used for the estimation of GSH concentration in different tissues. One milliliter of tissue supernatant was precipitated with an equal volume of metaphosphoric acid and incubated at 4 °C for 1 hour. The mixture was then centrifuged at 1,200 × g for 15 minutes at 4 °C. To 1 mL of the resulting supernatant, 2.7 mL of phosphate buffer and 0.2 mL of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were added. The intensity of the yellow chromogen was measured immediately at 412 nm with a visible spectrophotometer (Systronic-117 UV).

Histological Analysis

Tissue samples of liver fixed in formalin were processed for routine histology, involving dehydration through graded alcohols, clearing in xylene, and paraffin embedding. Sections (5 µm) were stained with H&E and MTC, examined under an Olympus BX43 light microscope, and images were taken using the Olympus DP27 digital camera.

Statistical Analysis

Statistical evaluation was carried out with SPSS statistical software, version 17. All values were examined for normal distribution and equal variance before statistical evaluation. Data are expressed as mean ± SD, and intergroup comparisons were performed using an unpaired parametric t-test. p-value < 0.05 was regarded as significant.

Results

Biochemical Analysis

Effect of Ashwagandha treatment on liver tissue level of GSH in aluminium treated rats is depicted in “Fig. 1”. It showed that the GSH level in Group I (720±22.23 ug/g tissue) and Group II (708.36±20.564 ug/g tissue) respectively and it was found that there was no significant difference between these two groups. In the present study administration of aluminium for three weeks in rats showed a significant ($P<0.0001$) fall in the GSH level in the liver tissue (524.089±22.3 ug/g tissue) when compared to normal control group (720.940±20.564 ug/g tissue). Administration of Ashwagandha significantly elevated liver GSH content (800.008±24.764 µg/g tissue) (Fig. 1). MDA levels in the treated group (Group II, 0.1083±0.0255 µg/g tissue) were comparable to those of the normal control group (Group I, 0.1067±0.025 µg/g tissue), with no significant differences. Aluminium administration significantly increased the level of MDA (0.4150±0.0367 µg/g; $P < 0.0001$) (“Fig. 2”). Findings from the present study showed that treatment with Ashwagandha (Group IV) resulted in a decrease in MDA levels compared to the aluminium-intoxicated rats (Group III). In the aluminium-treated group (Group III), SGPT and SGOT levels were significantly elevated (81.24±4.52 IU/L and 105.6 ± 7.55 IU/L, respectively; $P < 0.001$) compared to the normal control (Group I, 52.34± 3.30 IU/L) and Group II (62.14±7.07 IU/L). Treatment with Ashwagandha alongside aluminium intoxication led to a marked reduction in SGPT and SGOT levels (37.49 ± 3.99 IU/L and 82.41±1.12 IU/L, respectively) (“Figs. 3-4”).

Histological Analysis

“Figs. 5-6” shows Photomicrographic sections of rat liver of normal control (Gr.I) and Ashwagandha treated (Gr.II) groups, showed the normal arrangement of the hepatocytes with no ballooning of hepatocytes and normal sinusoidal capillaries and there was no macrophage infiltration observed. In Fig. 7 aluminium administration showed that normal arrangements of hepatocytes were disrupted with central vein enlargement and ballooning of the hepatocytes were maximum, macrophagic infiltration and apoptosis of hepatocytes is also observed (Gr.III). Sections of the rat liver treated with Ashwagandha with aluminium exposure showed decrease in the ballooning of the hepatocytes and reduced infiltration and apoptosis as well as better arrangement of hepatocytes and sinusoids as shown in Fig. 8 which indicates that the treatment of the Ashwagandha has been beneficial.

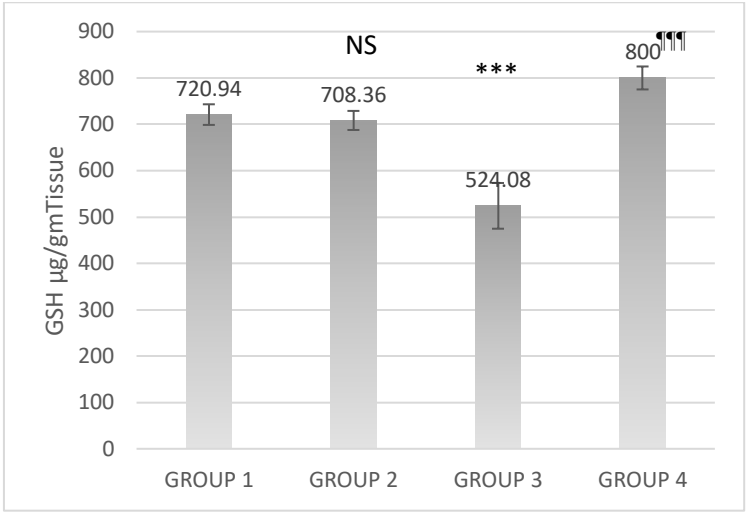

Fig. 1: Effect of Ashwagandha treatment on Liver tissue level of GSH in aluminium treated rats. Values are express as Mean±SD. Number of animal (n) = 6. P <0.05 is taken as significant. NS (not significant) Gr.I versus Gr.II ***P<0.0001, Gr.I versus Gr.III,¶¶¶P<0.0001, Gr.III versus Gr.I

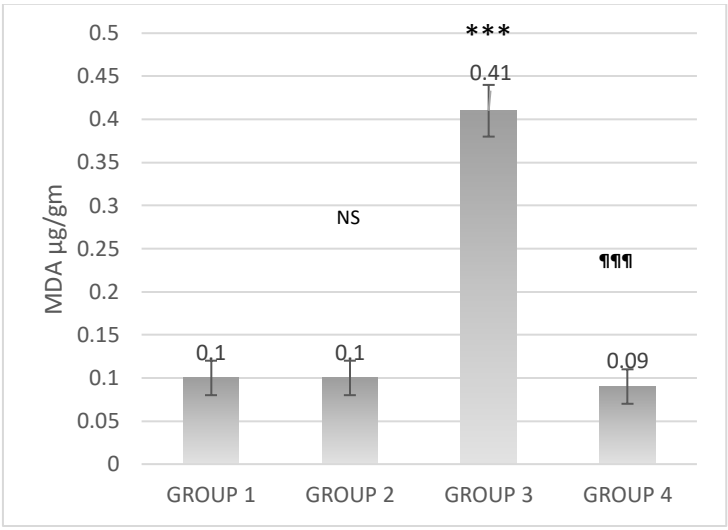

Fig. 2: Effect of Ashwagandha treatment on Liver tissue level of MDA in Aluminium treated rats. Values are express as Mean±SD. Number of animal (n) = 6. P <0.05 is taken as significant. NS (not significant) Gr.I versus Gr.II,*P<0.0001, Gr.I versus Gr.III,¶¶¶P<0.0001, Gr.III versus Gr.IV**

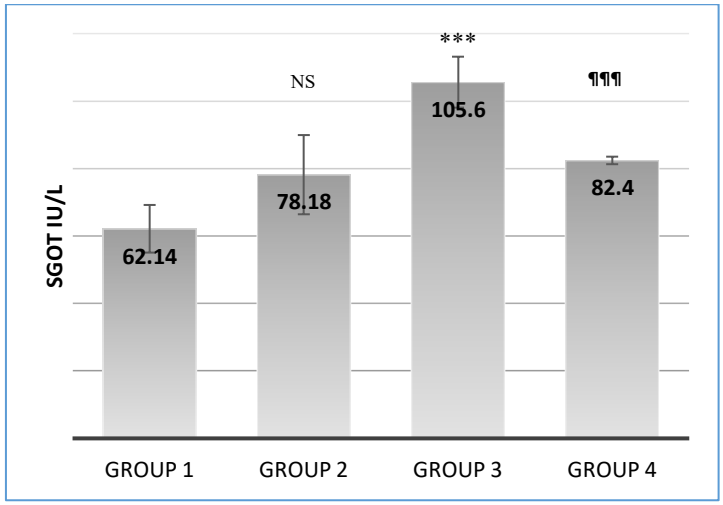

Fig. 3: Effect of Ashwagandha on serum biochemical hepatic markers-SGOT in aluminium intoxicated rats. Values are express as Mean±SD. Number of animal (n) = 6. P <0.05 is taken as significant. NS (not significant) Gr.I versus Gr.II ***P<0.001,Gr.I versus Gr.III , ¶¶¶P<0.001 Gr,III Vs Gr.IV

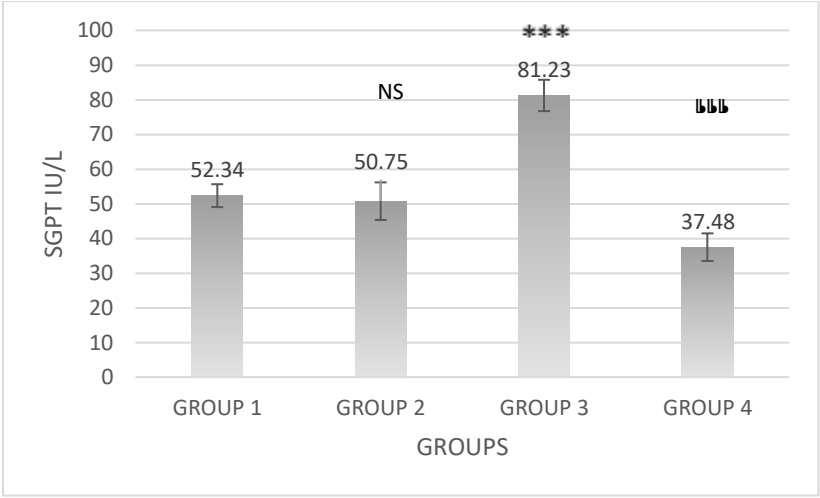

Fig. 4: Effect of Ashwagandha on serum biochemical hepatic markers-SGPT in aluminium intoxicated rats.Values are express as Mean±SD. Number of animal (n) = 6. P <0.05 is taken as significant. NS (not significant) Gr.I versus Gr.II ***P<0.001,GR.I versus Gr.III, P<0.001 Gr,III Vs Gr.IV

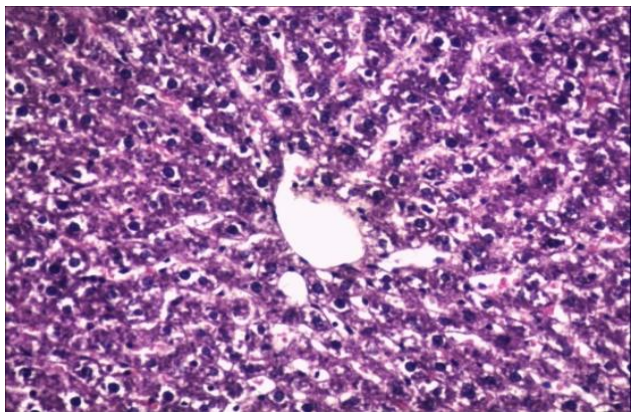

Fig. 5: Photomicrograph of rat liver shows normal histological features in normal control group (Group I) (Hematoxylin & eosin)

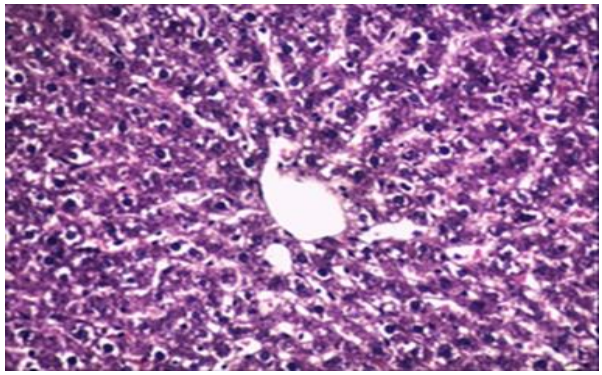

Fig. 6: Photomicrograph of rat liver shows normal histological features (Group II) (Haematoxylin & eosin)

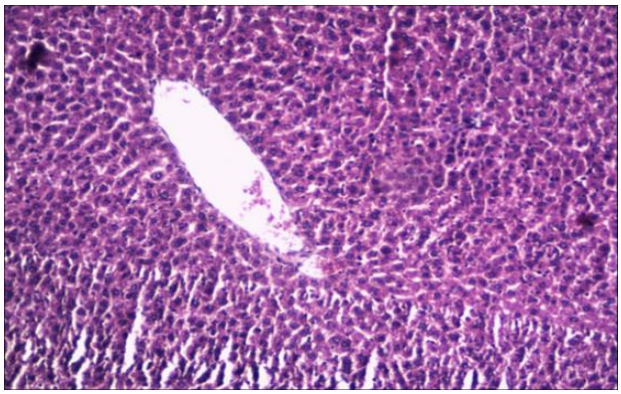

Fig. 7: Normal architecture of hepatocytes and sinusoidal arrangements are seen as disrupted. Ballooning of the hepatocytes is seen (Gr.III) (Hematoxylin & Eosin)

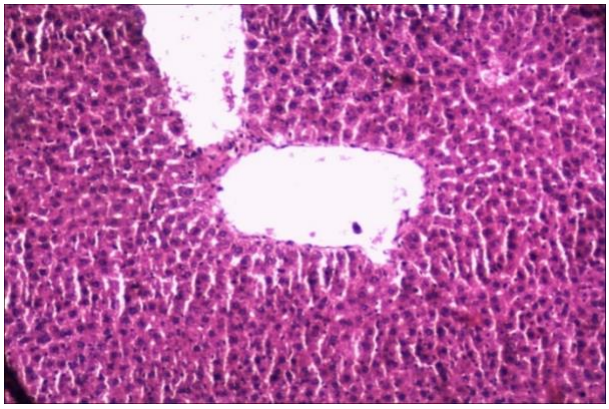

Fig. 8: (Gr. IV) (Haematoxylin & eosin .Ballooning of the hepatocytes is minimal. The arrangement of hepatocytes and sinusoids is better in structure. (Gr. IV) (Haematoxylin & eosin)

Discussion

Being the main site of metabolism, the liver is especially prone to damage from harmful metabolites formed during metabolic processes. The dual aspects of aluminium-induced hepatotoxicity and ashwagandha's potential hepatoprotective effects present a complex interplay. Numerous studies have established the neurotoxic nature of Aluminium Chloride [23]. Even though it is considered as a metal with relatively low redox activity, it acts as a causative agent of oxidative stress by various mechanisms [24]. Aluminium has been implicated in several neurological disorders, including Alzheimer's disease, and multiple sclerosis, according to various clinical studies. Aluminium toxicity causes liver damage, apoptosis, and cellular

necrosis leading to OS and excess ROS production via Fenton reaction [25]. The current study demonstrated that $AlCl_3$ treatment caused a disturbance in antioxidant defenses, with decreased GSH, elevated MDA, and significant reductions in SGOT and SGPT. This observation supports prior studies showing that overproduction of ROS leads to damage of cellular macromolecules, such as lipids, proteins, and DNA [26]. The study by Yu et al. demonstrated that aluminium preferentially accumulates in the liver and kidney during the initial eight weeks post-exposure [27]. By decreasing the activity of glutathione-synthase, aluminium can induce oxidative stress in soft tissues, such as the kidney and liver, leading to reduced GSH content [28, 29]. In consistent with the mentioned data Ashwagandha has significantly increased the level of Glutathione and a decrease in MDA which indicates the hepatoprotective and antioxidant action of Ashwagandha. Such effects could be linked to the antioxidant and anti-inflammatory activities of Ashwagandha, as reported in previous studies by Kumar and Patnaik, and Sajida and Prabhu [30, 31]. These protective actions occur through decreased ROS production, inhibition of the accumulation of oxidative damage products, and regulation of biochemical alterations arising from inflammatory cascades. In addition to that, results of our study showed that in aluminium treated group with high level of SGOT and SGPT and has declined in the pre-treated group with Ashwagandha consumption. SGPT and SGOT are considered sensitive biomarkers of liver damage because they are released from the cytoplasm of hepatocytes into the serum when hepatic injury occurs. The observed antioxidant properties of Ashwagandha may result from both the neutralization of free radicals and ROS and the inhibition of ROS-producing pathways activated after radiation exposure. The biochemical parameters of this study were supported by histological examination of liver, where macrophagic infiltration and apoptosis of hepatocytes are diminished in the observation of tissue. In summary, our findings demonstrate that Ashwagandha effectively reduces aluminium induced oxidative stress in the liver by restoring antioxidant balance. This study is novel in that it models chronic low-dose exposure and highlights Ashwagandha's potential as a natural hepatoprotective agent against environmentally relevant aluminium toxicity, thus, our data provide preliminary evidence showing the hepatoprotective effect of Ashwagandha.

Conclusion

This study revealed that aluminium administration led to oxidative damage in the exposed animals, indicated by abnormalities in key biochemical parameters. Moreover, significant histopathological modifications were detected in the organs under study. Ashwagandha treatment provided protection against oxidative stress-induced damage in the study. These results highlight the pervasive and detrimental impact of aluminium on the hepatic tissue, emphasizing the need for careful evaluation and reduction of its use and of such chemicals to mitigate their potential risks and Ashwagandha offers potential therapeutic benefits due to its antioxidant and anti-inflammatory properties. This study shows that even at a lower dose, Ashwagandha significantly mitigates aluminium-induced oxidative stress in the liver, in contrast to earlier studies that relied on much higher doses.

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Author's Contributions

Anchana Babu: Responsible for obtaining ethical approval, collecting the data, and performing the analyses.

Rekha D Kini: Participated in data collection, designed the study, and wrote the initial manuscript draft.

Nayanatara Arun Kumar: Reviewed and approved the final manuscript.

Megha Gokul: Reviewed and approved the final manuscript.

Ethics

The Institutional Ethics Committee granted approval for this study.

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