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Original Article

Antioxidant and Neuroprotective Potential of Ashwagandha In Aluminum-Induced Toxicity

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ABSTRACT

Introduction : Aluminium is the most abundant metal and the third most common element in the Earth's crust, following oxygen and silicon. Exposure to aluminium is associated with oxidative damage, primarily due to its ability to disrupt redox balance, generate reactive oxygen species, and impair antioxidant defense mechanisms.This study was aimed to find the potential role of ashwagandha on aluminium induced brain toxicity. **Methods**: In the present study rats were grouped into 4 groups of 6 rats in each. Brain tissue was removed and processed for biochemical and histopathological analysis. **Results**: In the present study, administration of aluminium to rats resulted in a significant decrease in tissue GSH levels and a corresponding increase in MDA levels in the aluminium-treated group compared to the normal control.. Treatment with Ashwagandha showed a significant increase in GSH level and decrease in MDA level. Photomicrographic sections of the Brain in Ashwagandha-treated rats showed normla neuronal Count and exposure to Aluminium has caused significant reduction in the neuronal count. Experimental group pretreated with ashwagandha showed a visible increase in neuronal count in different regions of the rat brain. **Conclusion:** The results revealed that oral administration of aluminium induced adverse oxidative effects in the exposed animals, while treatment with Ashwagandha markedly reduced the extent of aluminium chloride-induced brain injury.

Keywords: Oxidative stress, Aluminum Chloride, Ashwagandha, Reactive Oxygen Species, Reduced glutathione, Malondialdehyde

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INTRODUCTION

Metals are naturally occurring elements, continuously released to the environment by natural events, that easily bioaccumulate in living organisms[1]. Due to the various anthropogenic interventions, such as industrial, domestic and agricultural use of the metal-containing compounds, its human exposure has significantly increased in various regions of the world[2]. Neurotoxic actions of aluminium (Al) have been described in a large number of studies both in vivo and in vitro[3,4]. Aluminium has also been suggested to be involved in neurodegenerative disorders such as dialysis encephalopathy and Parkinson dementia[5,6]. Increasing evidence has demonstrated that oxidative stress is the primary cause of pathogenesis in inflammatory, partial ischemia, metabolic, and denatured cranial nerve disease[7]. Brain tissues are highly susceptible to oxidative damage, probably because of high oxygen consumption rate (20%), the presence of abundant polyunsaturated fatty acids in cell membranes, high iron (Fe) content, and low anti-oxidative enzyme activities[8]. Although it is a relatively low-redox mineral, it can induce oxidative damage through several biochemical mechanisms. Aluminium readily binds to negatively charged brain phospholipids, which are rich in polyunsaturated fatty acids and are highly susceptible to peroxidation by reactive oxygen species (ROS) such as $O_2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, and OH^{-}[9]. Aluminum can also stimulate iron-initiated lipid peroxidation in the Fenton reaction, which causes ROS production and Fe3+ formation. Superoxide ($O2^{\cdot}$-) is neutralized by Al3+ to form an Al-$O2^{\cdot}$- complex, which increases the oxidative capacity of $O2^{\cdot}$[10].

Indian ginseng is also known as Indian winter cherry, Ashwagandha, or the herb Vitania sluggard (*Withania somnifera*). The raw material used in medicine is the root, and the name "Ashwagandha" is derived from the word "ashwa", meaning horse. It is believed that after consuming the root, one gains powers similar to that of a horse. The second part of the name "gandha," means fragrance and refers to the characteristic smell of the fresh root of the plant. Since ancient times, it has been traditionally used in Ayurvedic medicine as a substance that strengthens the nervous system.It contains potent antioxidant compounds like flavonoids and phenolic compounds that help fight free radicals, reduce oxidative damage, and support the body's natural antioxidant enzyme activity. Many toxicological studies have proved the safety and edible nature of Ashwagandha[11]. 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[12] .It is also proved that Ashwagandha has many potent pharmacological role which include restoration of physiological specifications, improvement of mental function in old age as well as recovery from neurodegenerative disorders[13].Alkaloids, steroidal lactones, saponins, and withanolides sitoindosides VII-X and withaferin A are the biological active component of Ashwagandha. Clinical studies conducted in various conditions suggest that

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Ashwagandha root extract, aids in reducing and managing stress and anxiety, improves memory and cognition in healthy adults and patients with bipolar disorder[14]. There are many studies on role of ashwgandha as a neuroprotective agent[12]. However, its role in aluminium-induced brain toxicity has not yet been fully elucidated. Hence, the present study aims to evaluate the potential protective effects of Ashwagandha against aluminium-induced neurotoxicity in rats.

MATERIAL AND METHODS

Collection of rats and ethical clearance

The Institutional ethics committee approval was secured before starting the experiment (KMC/MNG/IAEC/14-2023). Healthy adult male wistar rats weighing between 150 and 200 grams was taken for this study from our institution's central animal house. Rats were kept in the laboratory's central animal housing, which had regulated lighting and temperature as well as regular rat food and water.

Collection of chemicals

Aluminium chloride was from Sigma company and Ashwagandha was purchased from Saptam veda company**.**

Experimental design and animal grouping

Animals were segregated into the following groups of 6 rats in each group. Twenty-four rats were randomly allocated into four groups (6 rats each) as followed:

Group I: Normal rats were given water (2 ml/kg bw) via oral gavage for 3 week and acted as the negative control (Figure 1).

Group II: Rats were treated with Ashwagandha (60mg/kg bw dissolved in distilled water) via oral gavage for 3 weeks and acted as the positive control (Figure 1).

Group III: Rats were treated with aluminium (100mg/kg bw dissolved in distilled water) via oral gavage for 3 weeks (Figure 2).

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

On completion of the desired protocol (after 3 weeks in each groups), rats were sacrificed, and the brain samples were excised, and parts of the brain samples were stored at – 80 ◦C for the subsequent biochemical analysis of MDA and GSH. Moreover, parts of brain samples were fixed in 10% buffered formalin for histopathological investigations.

Malondialdehyde(MDA)estimation

The lipid peroxidation product in brain homogenate was measured by the estimation of malondialdehyde (MDA), through the estimation of thiobarbituric acid reactive substances (TBARS) by the method. 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 2ml of the supernatant added and then 0.67% of thiobarbituric acid (TBA) was added. Then the test tube was kept in boiling water bath for 10 min and cooled. The pink chromogen color that was developed was read immediately at 532nm. Thiobarbituric acid reactive substances (TBARS) concentration was calculated using the molar extinction coefficient of chromophore (1.56×105 $(mol/L)^{-1}cm^{-1}$ and the values were expressed in nmoles/L.

Reduced Glutathione Estimation

For the estimation of GSH concentration, the tissue supernatant(1ml) was precipitated with 1ml of metaphosphoric acid and cold digested at 4ºC for 1h. The samples were centrifuged at 1,200g for 15 min at 4ºC. To1ml of this supernatant +2.7ml of phosphate buffer+0.2ml of 5, 5' dithio-bis-2-nitrobenzoic acid (DTNB) were added. Then the OD of the yellow color developed in the fluid was read immediately ata wavelength 412nm using visible spectrophotometer (Systronic-117 UV).

Histological analysis

Formalin-fixed liver and brain specimens were subjected to routine histological sample preparation protocol. Briefly, tissue samples were passed in different grades of alcohols, xylene, and embedded in paraffin. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H&E) and Masson trichrome stain (MTC). Stained slides were examined using Olympus BX43 light microscope (Olympus, Tokyo,

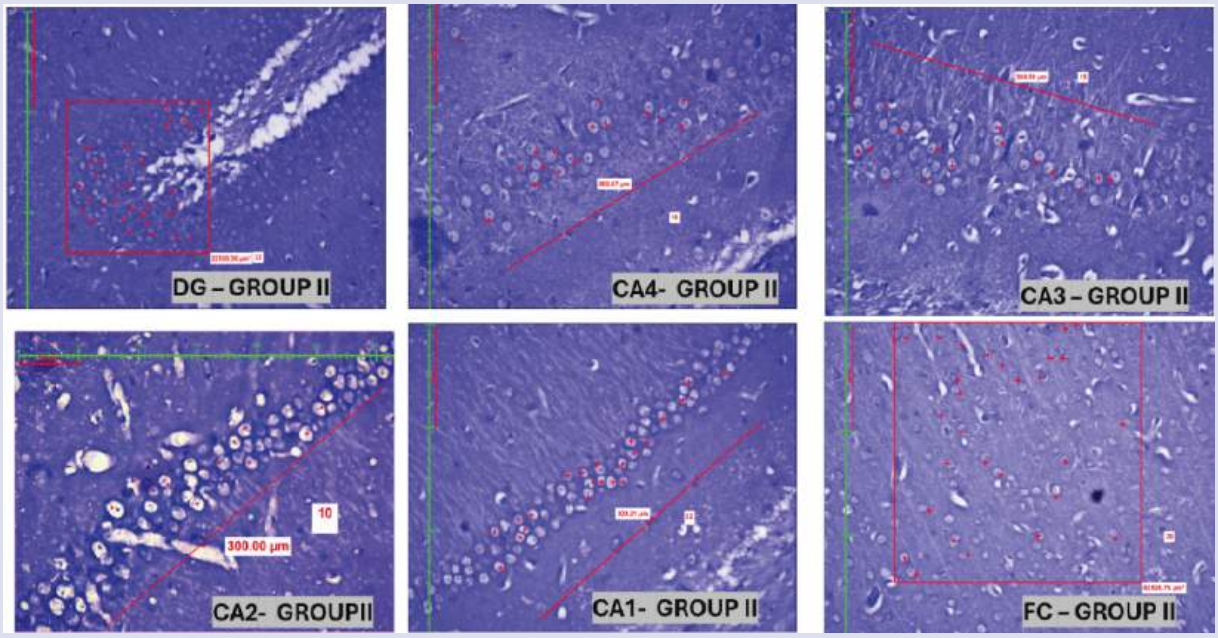


Figure 1. Figure represents the Group 1& 2:AS group- dentate gyrus (150 x 150 sq µm² area), the Cornu ammonis (300 sq µm length area - CA 1 to CA4) parts of the hippocampus, and Frontal Cortex (250 x 250 sq µm² area FC) respectively

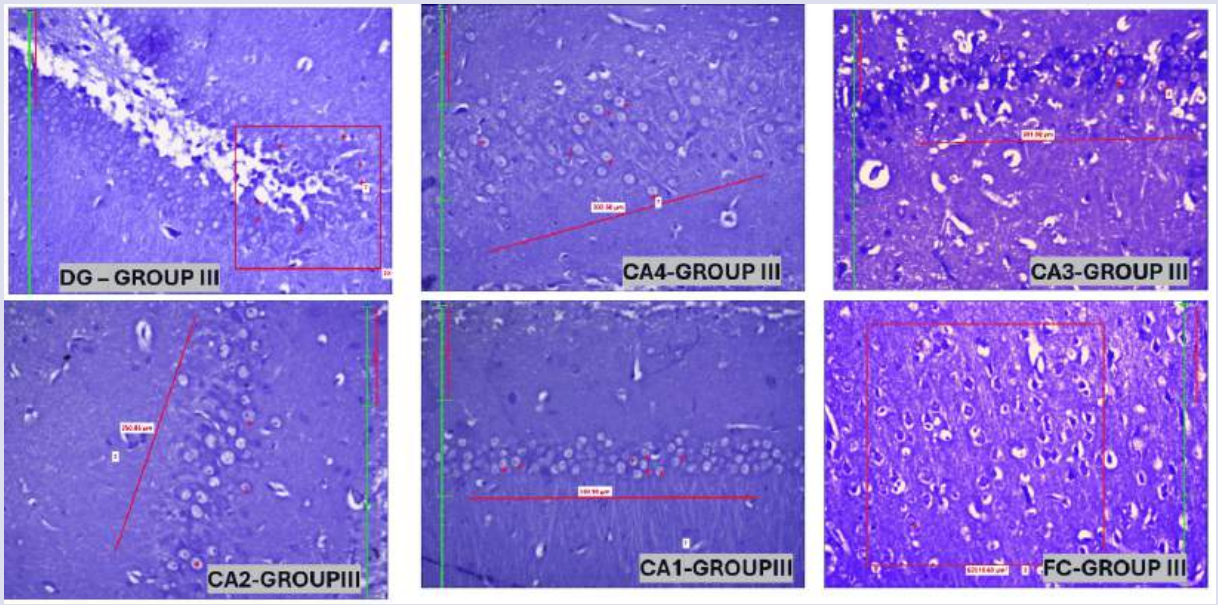


Figure 2. Figure represents the Group 3:Al Intoxicated - dentate gyrus (150 x 150 sq µm² area),the Cornu ammonis (300 sq µm length area - CA 1 to CA4) parts of the hippocampus, and Frontal Cortex (250 x 250 sq µm² area FC) respectively

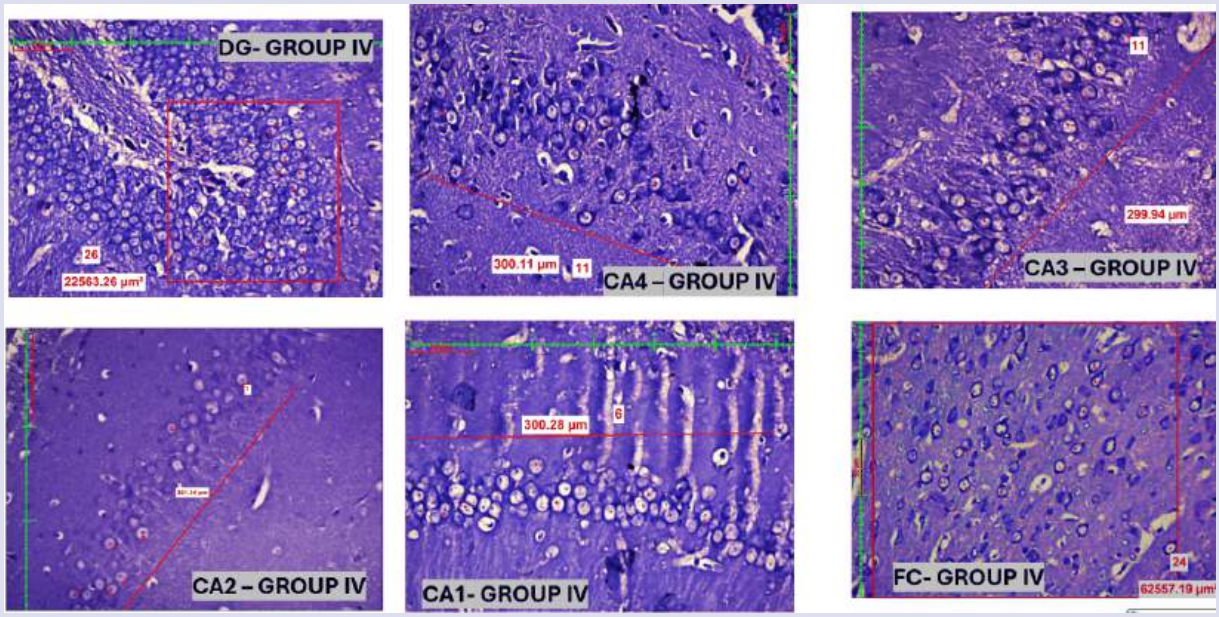
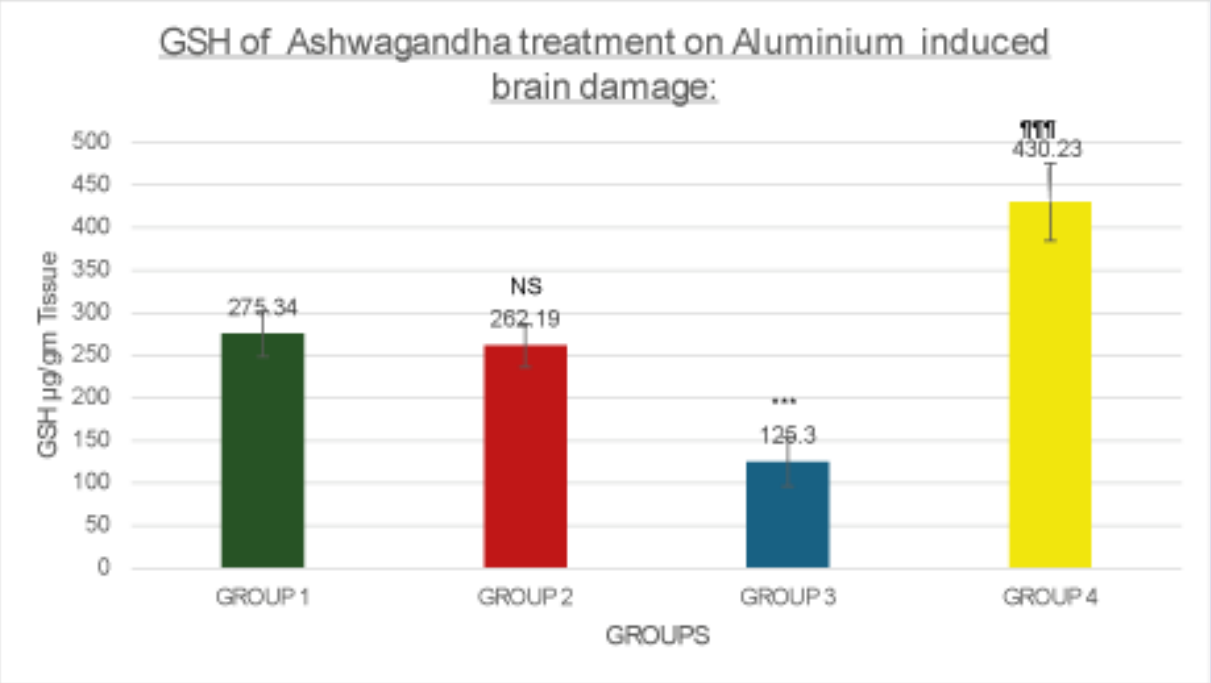


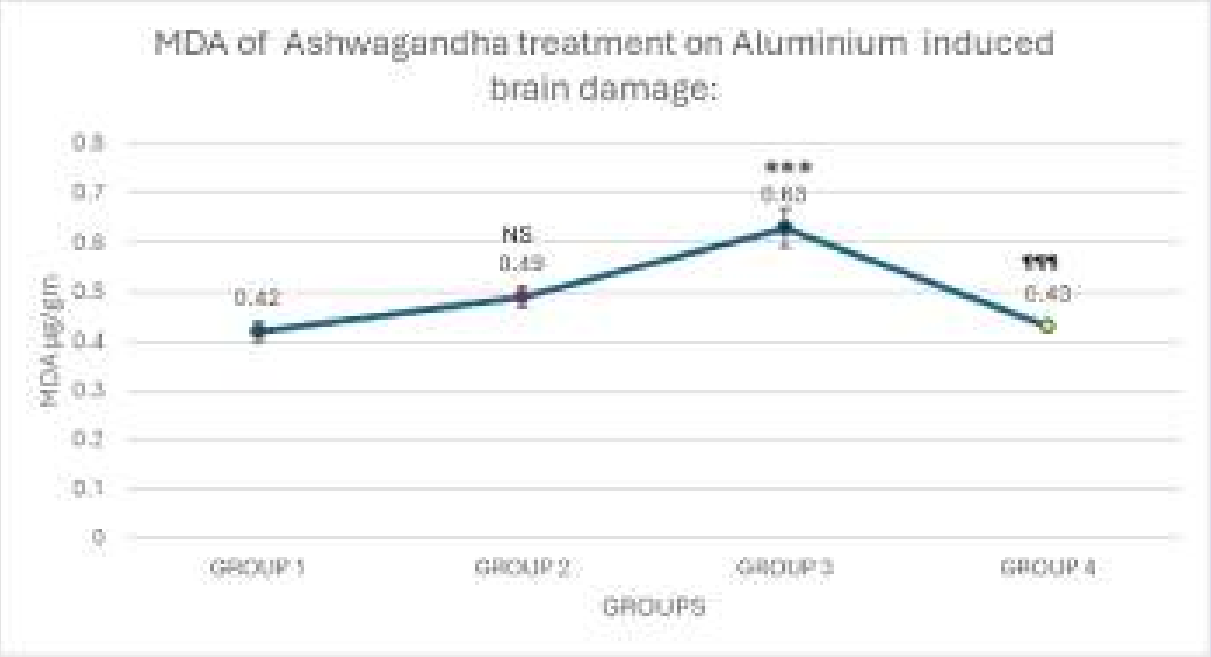
Figure 3. Figure represents the Group 4:AL +AS group- dentate gyrus (150 x 150 sq µm² area), the Cornu ammonis (300 sq µm length area - CA 1 to CA4) parts of the hippocampus, and Frontal Cortex (250 x 250 sq µm² area FC) respectively

Table 1. Neuronal count in different Regions of the Hippocampus and Dentate gyrus. 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

Groups	DG Mean ± SD	CA4 Mean±SD	CA3 Mean±SD	CA2 Mean±SD	CA1 Mean±SD	FC Mean±SD
Gr.I	30.19±1.2	29.12±1.3	18.12±1.7	16.13±1.01	15.32±0.18	27.5±1.3
Gr.II	29.17 ± 2.0^{NS}	26 ± 1.6^{NS}	17.33 ± 1.5^{NS}	15.50 ± 1.0^{NS}	14.50 ± 1.0^{NS}	26.50 ± 1.2^{NS}
Gr.III	18.83 ± 1.6***	5.67 ± 1.6***	7.67 ± 1.3***	4.83 ± 1.1***	4.17 ± 0.7***	5.17 ± 0.75***
Gr.IV	24.50 ± 2.5¶¶¶	8.67 ± 1.2¶¶¶	10.67 ±1.6¶¶¶	8.83 ± 0.9¶¶¶	7.17 ± 0.9¶¶¶	20.67 ± 2.4¶¶¶



Graph 1. Effect of Ashwagandha treatment on brain tissue level on 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.IV



Graph 2. Effect of Ashwagandha treatment on brain tissue level on 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

Japan), and images were captured using the connected Olympus DP 27 digital camera.

Statistical analysis

Statistical analysis was done using SPSS statistical software version 17. Normality and variance homogeneity tests were carried out for all values prior to statistical analysis.The data was presented as mean ± SD. Parametric unpaired t- test was used to evaluate the differences in parameters between the groups. P<0.05 was taken as statistically significant

RESULT

Effect of Ashwagandha treatment on Aluminium induced brain damage

Biochemical Parameters

In the present study administration of aluminum in rats showed a significant(P<0.0001) decrease in the brain tissue level of GSH when compared to normal control group. There was also a significant (P<0.05) increase in the tissue level of MDA in aluminum treated group compared to normal control. Treatment with Ashwagandha showed a significant increase in brain GSH level (Gr.IV) (Graph 1) and decrease in MDA level (GR.IV)(Graph 2) compared to aluminum intoxicated rats(Gr.III)

Histological Parameters

Photomicrographic sections of the brain in Ashwagandha-treated (Gr.II) (Figure 1) rats showed a Normal Neuronal Count. (Table 1) as seen in control, significant changes were not observed. But, exposure to Aluminum(Gr.3) (Figure 2) has caused significant reduction in neuronal count in contrast to group Gr.1 (Table 1) and (figure 1).Neuronal count in the experiment group pretreated with ashwagandha (Gr.4) (Figure 3) showed a visible increase in neuronal count in different regions of the Hippocampus,Dentate Gyrus and Frontal Cortex of rat brain when compared to Aluminum intoxicated group(Gr.3) (Table 1)

DISCUSSION

Aluminum toxicity in rat brains can lead to behavioral changes like decreased motor activity and impaired learning, along with biochemical and structural changes. Studies show it causes oxidative stress, disrupts cholinergic systems, and increases aluminum accumulation in specific brain regions like the hippocampus[15,16,17]. Numerous studies have established the neurotoxic nature of Aluminium chloride[18]. Several clinical investigations suggested that Aluminium could be a factor in the development of neurological illness, including Alzheimer's disease, Parkinson's Disease, and Multiple Sclerosis. Some of the studies have shown that long-term Aluminum exposure causes accumulation in cortex and limbic structures and fall in the pyramidal cells leading to neurites and loss of synapses[19]. In the present study administration of aluminum in rats showed a significant decrease in the brain tissue level of GSH when compared to normal control group. There was also a significant increase in the tissue level of MDA in aluminum treated group compared to normal control. Our results were in accordance with previous study proved that Aluminum can induce oxidative stress in the brain by increasing the production of reactive oxygen species and damaging antioxidant defenses[20]. It is known that one of the most important components of Ashwagandha–withanolide A combats neurodegenerative processes in Alzheimer's disease and Parkinson's disease. In the present study, significantly decreased level of GHS and increased level of MDA which are the potent biomarker of oxidative stress in the Aluminium exposed rats, signifies rise in free radicals which affects the neuronal count. Accordingly, the results of current study have shown a clear decline in neuronal count in different areas of brain under the exposure of Aluminium Chloride along with lack of architecture in brain histology. Herbal plants have been the primary source of medicines for humans since ancient times, and currently around eighty per cent of global population depends on traditional medical system. Ashwagandha is one among these herbal plants seems to have significant antioxidant activity, which is also one of its signalling pathways. No studies have been explored the antioxidant role of ashwagandha against aluminium induced oxidative stress focusing distinct brain tissue. In our research, the treatment with ashwagandha in Aluminium toxicated rats showed increase in brain level of GSH and decrease in MDA level indicating the protective role of ashwagandha extract against oxidative stress induced by Aluminium. Ashwagandha induced increase GSH level indicates the antioxidant action of Wthania Somnifera and can act as protective agent against cellular damage induced by free radicals. Few studies have reported that GSH forms S-nitrosoglutathione with NO thus anchors as an endogenous reservoir for NO and the increased GSH synthesis induced by ashwagandha extract can modulate the rise in the level of NO induced by aluminium[21]. Present study has also shown that ashwaghandha plant extract has also reduced the MDA, which contribute a major proportion of ROS in cells. The presence of powerful antioxidants such as flavonoids and withanolides in large quantities may account for the ashwagandha root extract's antioxidant properties. This study adds more evidence to the neuroprotective action of WS by an appreciable increase in neuronal count in all areas of brain including dentate gyrus,cornis ammonis,hippocampus and frontal cortex .In addition to that, histological examination of brain has shown satisfactory improvement in tissue morphology in rats pre-treated with Ashwagandha.Certain studies have demonstrated the therapeutic action of WS extract can improves cognition[22] and improves Alzheimer pathology in mice[23]. This traditional mode of medicine has been used for centuries to treat anxiety and mental disorders.

CONCLUSION

The results of this study revealed that oral aluminium chloride administration induced marked oxidative stress in the exposed animals, as reflected by significant abnormalities in the measured biochemical parameters. These biochemical findings were further supported by notable histopathological alterations in the examined organs. Treatment with Ashwagandha (*Withania somnifera*) exerted a protective antioxidant effect, effectively mitigating these adverse changes.

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