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Research Article

Neuroprotective role of a protoberberine alkaloid against aluminum-induced neuroinflammation and excitotoxicity

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Abstract

The study was performed to investigate the possible neuroprotective role of palmatine, a protoberberine alkaloid against aluminum-induced aberration in neurotransmitter levels, excitotoxicity, neuronal inflammation, damage, and degeneration. 100 mg/kg of aluminum chloride served as the inducing agent and was administered orally to male Wistar albino rats for 42 consecutive days. Animals were divided into four groups, groups I, II, III, and IV which involve the normal group, the toxic control group receiving aluminum chloride, and two treatment groups administered orally with palmatine at a dose of 10 mg/kg and 20 mg/kg respectively followed by aluminum chloride. Expression of neuronal inflammatory markers like IL-6 and TNF-α were checked by the ELISA method. Deranged neurotransmitter levels of acetylcholine esterase and glutamate in rat brains were measured to determine the extent of excitotoxicity. The neuroprotective role of palmatine was determined based on histopathological studies and by determining BDNF expression by the immunohistochemistry method in rat brains. Palmatine treatment effectively regulated acetylcholinesterase levels and glutamate levels otherwise elevated by aluminum. It lowered excitotoxic damage induced by aluminum and lowered the degree of expression of inflammatory markers IL-6 and TNF- α. Improved expression of BDNF in palmatine-treated groups is indicative of the neuroprotective potential of palmatine in the restoration of neuroplasticity. Histopathology further confirms the neuroprotective potential of palmatine as the treatment significantly prevented neuronal damage degeneration and loss and restored healthy and viable neurons. The findings of the study confirm the neuroprotective potential of palmatine against aluminum-induced neuroinflammation and excitotoxicity.

Keywords: aluminum chloride; BDNF expression; excitotoxicity; neuroprotection; neurotransmitters; neuronal inflammation; palmatine

Abbreviations: AChE-acetyl choline esterase; CA- Cornu Ammonis; DG: Dentate Gyrus.

Introduction

Neurodegenerative disorders are outcomes of aging, oxidative damage induced by free radicals, exposure to neurotoxins, etc. Aluminum, a perilous metal found in copious amounts in the earth's crust poses a serious

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threat to health as it is a neurotoxic and excitotoxic substance that accumulates in the brain and CNS resulting in inflammation, degeneration, and toxicity. Berberine and protoberberine alkaloids are known for their antioxidant and protective effect in various disorders. Palmatine a protoberberine alkaloid is being investigated here for its protective effect against aluminum-induced neuroinflammation and neurodegeneration by opting for an *in silico* and *in vivo* approach.

Neuroinflammation involves an inflammatory process inflicting damage to the brain and associated structures and it involves the production of inflammatory and pro-inflammatory mediators cytokines, chemokines, reactive oxygen species, and secondary messengers. Here, the brain's innate immune system is triggered following an inflammatory challenge caused by injury, infection, exposure to a toxin, neurodegenerative disease, or aging. Aluminum is an abundantly present neurotoxic metal present in the ecosphere and is one of the main culprits contributing to neurodegenerative disorders that entail inflammatory processes and neuronal loss and degeneration, which leads to behavioral and cognitive bereavement and irrevocable brain damage and dysfunction. It is a pro-oxidant that initiates inflammatory and apoptotic changes in neurons (Skalny *et al*., 2021). Aluminum also increases acetylcholine esterase's brain levels, which lowers acetylcholine levels vital for learning and memory. Acetylcholine (ach), an indispensable component neurotransmitter essential for processing memory and learning, is decreased in both concentration and function in patients with Alzheimer's disease. This deficit and other presynaptic cholinergic deficits, including loss of cholinergic neurons and decreased acetylcholinesterase activity (Uddin *et al.,* 2016). Glutamate, elevation resulting from aluminum exposure leads to excitotoxic damage, degeneration, and apoptosis of neurons. Glutamate causes cognitive decline, neuronal degeneration, etc due to overstimulation of NMDA receptors which hinders learning and memory (Nayak *et al.,* 2001; Alghamdi *et al.,* 2018).

Protoberberine alkaloids (PAs) include a range of isoquinoline alkaloids composed of a protoberberine skeleton such as berberine, berberrubine, thalifendine, demethylene berberine, palmatine, jatorrhizine, columbamine, etc and are present in many plants belonging to family Magnoliaceae, Ranunculaceae, Berberidaceae, and Menispermaceae (Zhang *et al.,* 2019). Palmatine is a naturally occurring protoberberine alkaloid present in Chinese medicinal plants such as *Corydalis yanhusuo, Coptis chinesis*, etc. It has shown pharmacological activity against a wide range of conditions like jaundice, dysentery, hypertension (Bhardra *et al.,* 2010), cancer, oxidation, and inflammation and shows anti-bacterial, anti-viral effects and helps manage blood lipids (Long *et al.,* 2019). Palmatine is present in good amounts in *Tinospora* species like *Tinospora cordifolia* and *Tinospora saggitata* and related plants. *Tinospora cordifolia* also known as Guduchi or Amrita balli (Menispermaceae), is used traditionally to treat fever, diabetes, dyspepsia, jaundice, and skin diseases. It has been subjected to extensive phytochemical, pharmacological, and clinical investigation with many interesting findings in the area of immunomodulation, anticancer, hypoglycemic, antiallergic, and anti-inflammatory (Ali *et al*., 2013).

Aluminum triggers glutamate-mediated excitotoxicity that causes severe neuronal damage and neuronal loss and it also elevates acetylcholine esterase levels which give rise to the symptoms such as neurobehavioral alterations, cognitive and memory loss, etc. Excitotoxicity and oxidative stress mediated by ROS generation activate nuclear factor κB (NF-κB), neuronal inflammatory mediators, and cytokines like TNF-α, IL-6, and IL-1β which further worsens the condition. Aluminum exposure over a period of time. This would contribute to serious brain damage and neurotoxicity (Cao *et al*., 2019).

BDNF expression is crucial for neuronal preservation, viability, neuroplasticity, and for regulation of levels of neurotransmitters. BDNF expression gradually declines with the progression of neurodegeneration. and neuronal inflammation (Giacobbo *et al.,* 2019). Aluminum, a potent inducer of free radical-mediated oxidative stress, triggers proinflammatory cytokines and inflammatory mediators. Neuronal inflammation over a period of time suppresses BDNF expression which is a vital part of the brain's defense mechanism and for the purpose of neuronal survival signaling and neuroplasticity.

Materials and Methods

Isolation of palmatine from leaves of Tinospora cordifolia

Palmatine, the protoberberine alkaloid was isolated from the methanolic extract of leaves of TC plant. The extract was partitioned to $CHCl_3$ and aqueous fractions followed by evaporation of $CHCl_3$ solution to a viscous brownish mass (10.2 gm). The residue was then loaded on a column packed with silica gel (grade 120/60 mess size) and was run. The components were eluted with $CHCl_3$ and then gradually enriched with methanol to get 7 fractions. Yellow-colored fraction (fraction 5) was further eluted with $CHCl_3$-MeOH (10: 1) mixture and subjected to silica gel column chromatography to give a single compound (2.6 gm). The isolate was then purified by recrystallization with methanol. It was then subjected to TLC and the Spectroscopic analysis (infrared (IR), and nuclear magnetic resonance (NMR) spectroscopy) confirmed the presence of palmatine (purity: 98.37%) in the said plant (Hsieh *et al.,* 2004).

Neuroprotective study

Induction of aluminium chloride-mediated oxidative stress (neurotoxicity) in animals and evaluation of the neuroprotective activity of Palmatine in male Wistar rats

Chemicals and reagents

Aluminium chloride (Thermofiescher), Palmatine.

Experimental animals

A number of 40 male Wistar albino rats weighing around 200-250 g were procured from Krupanidhi College of Pharmacy, Bangalore, India. The animals were housed and acclimatized in a well-ventilated animal house under appropriate laboratory conditions a few days prior to the experiment maintained at 25±4 °C and 50-60% relative humidity, a 12-hour light-dark cycle with food and water ad libitum as per the guidelines of (CPCSEA) Committee for the Purpose of Control and Supervision on Experiments on Animals. The Institutional Ethical Committee approved the experiment protocol by the number KCP/IAEC/PCOL/61/2020

Male Wistar albino rats (n=10) were grouped as follows:

Group I: Normal group administered with distilled water,

Group II: $AlCl_3$ group (induced neurotoxicity) (100 mg/kg *p.o* $AlCl_3$ *p.o*.),

Group III: Palmatine (10 mg/kg *p.o*) +$AlCl_3$ (100 mg/kg *p.o*),

Group IV: Palmatine (20 mg/kg *p.o*) +AlCl3 (100 mg/kg *p.o*) (Auti *et al.,* 2019).

The dose of palmatine was selected based on the previous literature (Ma *et al.,* 2016). The treatment was performed for 42 days and at the end of treatment, rats were sacrificed and the brain areas the study mainly focused on i.e., hippocampus and cortex were separated and dissected out and washed with ice-cold isotonic saline maintained at −80 ◦C. This was then homogenized with 10 parts of ice-cold phosphate buffer (0.1M) maintained at pH 7.4 and was centrifuged at 4000 rpm for 20 mins. The supernatant was collected to perform biochemical estimations.

Estimation of brain levels of Acetylcholinesterase (AChE) (Ellman *et al*., 1961)

AChE is an enzyme involved in the breakdown of the neurotransmitter acetylcholine, the levels of which get elevated in the brain with the administration of cholinotoxic aluminum. The levels of AChE were estimated by Ellman's method with modifications. Standard acetylcholinesterase, acetyl thiocholine iodide (ATCI) and DTNB (5, 5-dithiobis (2- nitro benzoic acid) were used for the purpose. Admixture of all these with the brain supernatant added with the substrate (10 µl of ATCI, 14 mM solution) initiates the reaction. The colored adducts 5- thio-2- nitrobenzoate anion so formed is measured at 410 nm after 10 mins. Physostigmine

(dissolved in ethanol) (AchE inhibitor), served as a positive control. Percentage inhibition of acetylcholinesterase was determined using below formula.

$$\text{Percentage inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 10$$

Brain levels of glutamate

Rat brain homogenate was prepared by homogenizing the brain with 2 parts by weight of perchloric acid, centrifuged for 10 min at 3,000 rpm. About 3 ml of brain supernatant was adjusted to pH 9 with 1 ml phosphate solution and rested for 10 min in an ice bath followed by filtering it through fluted filter paper The absorbance was measured at 340 nm. A blank reading at 340 nm was measured. *(*Paul Babu *et al.,* 2014).

Estimation of inflammatory markers

ELISA method was followed for the determination of IL-6 and TNF-α levels in the hippocampus and cortex and was performed as per the instructions of the manufacturer (Millipore). The results are expressed in ng of cytokine per ml of brain tissue homogenate. (Barichello *et al.,* 2010) (Godbout *et al.,* 2003) (Matsusaka *et al*., 1993).

Histopathology

After sacrifice, rat brains washed in saline were fixed in 10% formaldehyde and transverse sections of the hippocampus and cortex areas were sliced out from brain tissue using a microtome and were fixed in paraffin blocks. Hematoxylin and eosin (H&E), Congo red dye stained the thin sections and were then examined under a digital microscope at a magnification of 100X, Brain areas evaluated include the cortical, hippocampal, and pyramidal regions (Figures 6-20).

Estimation of BDNF

Expression of the brain-derived neurotrophic factor was estimated on rat brains to determine the extent of the protective effect brought about by palmatine treatment. The immunohistochemistry method was utilized to determine BDNF expression. Immunohistochemical staining was performed on the hippocampus and cortex regions of the brain. Tissue blocks (paraffin wax embedded) were sliced using microtome into fine transverse sections of 5-6 μm thickness. They were placed on slides coated with Poly-L-Lysine and incubated overnight at 37 ◦C for 1 h. The brain sections were then deparaffinized followed by rehydration and incubated with citrate buffer (pH 6). The slides were then incubated in 3% hydrogen peroxide for about 20 min to inhibit the activation of endogenous peroxidase. BDNF antibody (polyclonal) (Immunotag, Inc., USA) was applied as the primary antibody and secondary antibody was mouse anti-rabbit-IgG-HRP (Diagnostic Bio system) was used. The stained sections were visualized after its reaction with the diaminobenzidine (DAB) reagent. This was then counterstained with hematoxylin. The brain sections were washed with Tris buffer saline (TBS) followed by dehydration of the same in alcohol followed by xylene and were mounted using DPX. The sections were visualized under the microscope carried out at 100X to determine the extent of interaction between the antigen and antibody. Morphometric analysis was carried to observe the extent of immunoreactivity (Serra *et al.,* 2017) (Figures 21-35).

Statistical methods

Statistical significance of all the results were tested by comparing treatment groups with the respective positive control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD.

Results

Extraction and isolation of palmatine from Tinospora cordifolia leaves

The Soxhlet extraction of *Tinospora cordifolia* leaves (hydroalcoholic extract) yielded about 127.6 g of crude extract and from which 2.6 g of a bright yellow colored alkaloid compound palmatine was isolated with 98.37 percent purity.

TLC: A solvent system composed of 2% cetylpyridinium chloride and ethyl acetate (9:1) served for the purpose of TLC. By means of TLC, compared the isolated palmatine with standard palmatine which gave an Rf value of 0.49 (Figure 1).

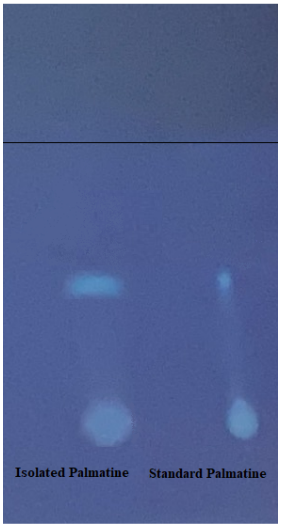

Figure 1. TLC of isolated palmatine compared to standard palmatine

Spectral analysis of isolated palmatine

IR study of palmatine unveiled mid-IR from 4000 ~ 200 cm^{-1}, and far-infrared region (50 ~ 1000 cm^{-1}) indicating a set of spectra for both standard and isolated palmatine both of which were overlapping each other. IR data reveals that, 1067, 1194, 1208, 1240 cm^{-1} exhibits strong C-O stretching bond. 1434, 1445, 1461, 1510, 1567 cm^{-1} representing benzene rings, 2920, 2946, 2967, 2981 cm^{-1} indicative of C-H (alkane) stretching bond. 3322 cm^{-1} is characteristic of medium stretching N-H bond. (Figure 2)

LC-MS results reveal an m/z value of 352.72 for the isolated palmatine which is almost identical to that of the palmatine standard. As per the standard literature, the molecular weight of palmatine is 352.4 g/mol (Figure 3).

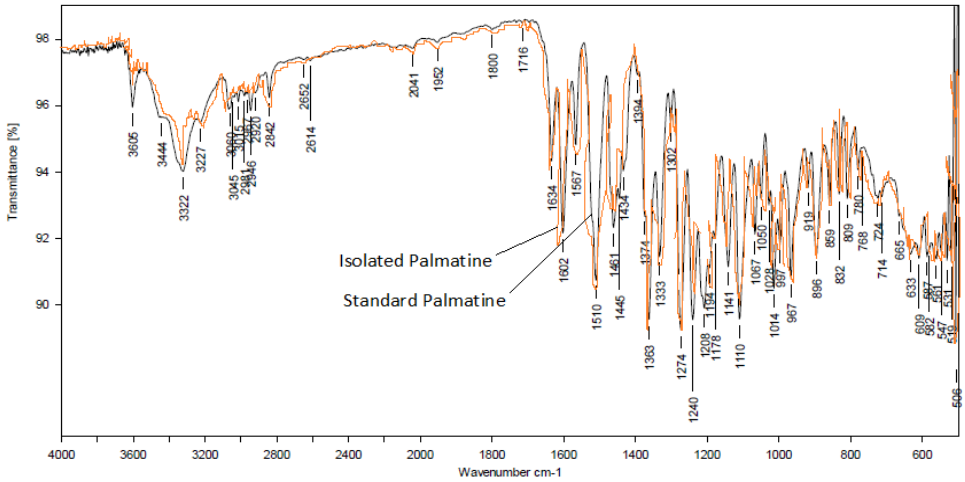

Figure 2. IR spectrum of palmatine
IR with orange lines indicates isolated Palmatine
IR with black lines indicates Standard Palmatine

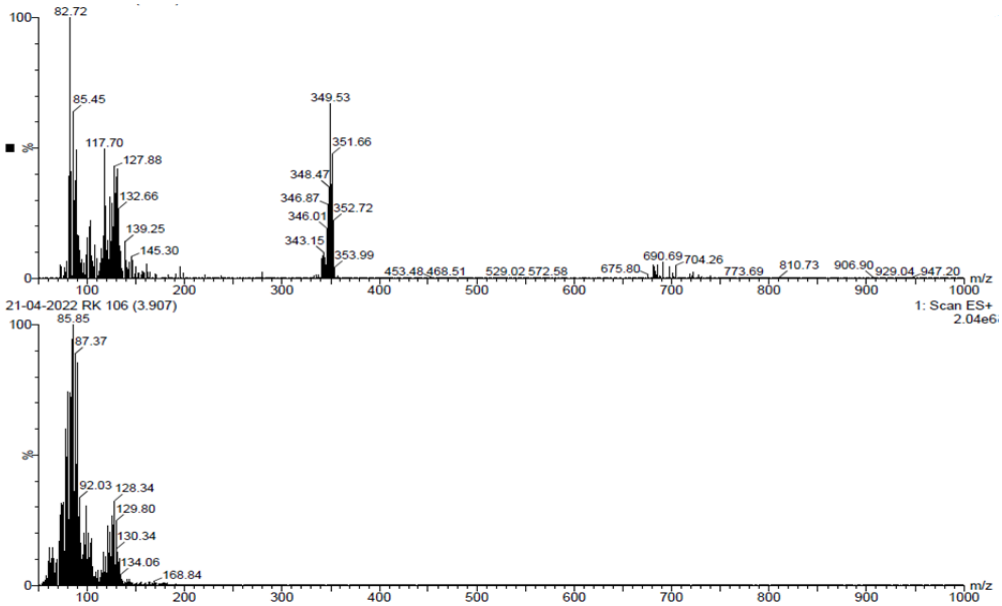

Figure 3. LC-MS graph for isolated palmatine

Neuroprotective study (in vivo studies)

Analysis of brain levels of AChE

The brain levels of acetylcholine esterase elevated with aluminum exposure in induced group. Palmatine treatment helped regulate the brain AChE levels to a good extent in a dose-dependent manner (Table 1).

Analysis of brain levels of glutamate

Aluminum exposed group showcased an exacerbated level of glutamate in the brain which was found to decrease in a dose-dependent manner with palmatine treatment which was close to the normal values (Table 2).

Table 1. Analysis of brain levels of AChE

Sl no:	Treatment group	Brain levels of AChE (µmol/min/g)
1	Normal group	5.61± 0.09
2	$AlCl_3$ group	15.98 ± 0.1[a]
3	Palmatine 10 mg/kg	10.37 ± 0.29[b]
4	Palmatine 20 mg/kg	9.3± 0.34[c]

Statistical significance of AChE levels was assessed by comparing treatment groups with the respective positive control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group. [b,c] $p<0.01$ & when compared to the positive control group.

Table 2. Analysis of brain levels of glutamate

Sl.No	Treatment group	Brain levels of Glutamate (nmol/min/g)
1	Normal group	0.12 ±0.01
2	$AlCl_3$ group	0.29±0.02[a]
3	Palmatine 10 mg/kg	0.19±0.02[b]
4	Palmatine 20 mg/kg	0.14±0.01[c]

Statistical significance of glutamate levels was determined by comparing treatment groups with the respective positive control group by employing One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group. [b,c] $p<0.01$ & when compared to the positive control group

Determination of brain levels of inflammatory markers

Analysis of brain levels of IL-6

Aluminum administration resulted in elevated levels of IL-6 in the brains of the aluminum-induced group compared to the normal group suggestive of the inflammatory changes brought about by aluminum which was lowered by palmatine treatment (Figure 4).

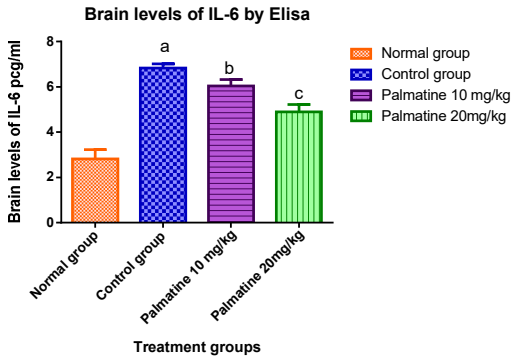

Figure 4 Analysis of brain levels of IL-6

Statistical significance of IL-6 results was determined by comparing treatment groups with the respective positive control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group. [b,c] $p<0.01$ & when compared to the positive control group.

Analysis of brain levels of TNF-α

Aluminum exposure intensified the inflammatory reactions in the brain elevating the brain levels of TNF-α in the induced group. Palmatine administration reduced the inflammatory changes brought by aluminum as it lowered the levels of TNF-α in rat brains (Figure 5).

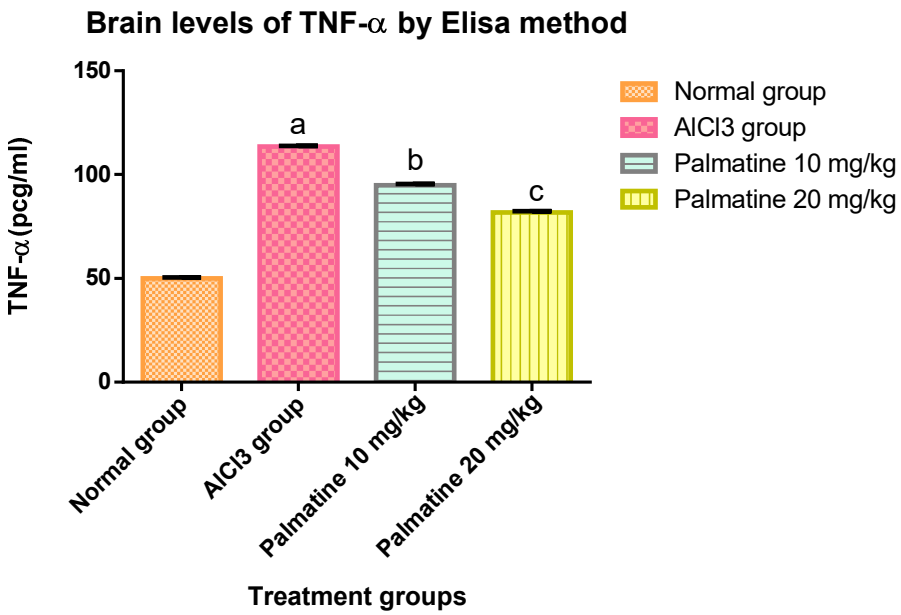

Figure 5. Analysis of brain levels of TNF-α

Statistical significance of TNF-α results was tested by comparing treatment groups with the respective positive control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group. [b, c] $p<0.01$ & when compared to the positive control group.

The results are indicative of the protective role of palmatine treatment against aluminum-induced neuro-inflammation by modulating the levels of IL-6 and TNF—α in the brain. Also indicates the potential of palmatine in regulating exacerbated levels of acetylcholine esterase and glutamate in rat brains.

Histopathology

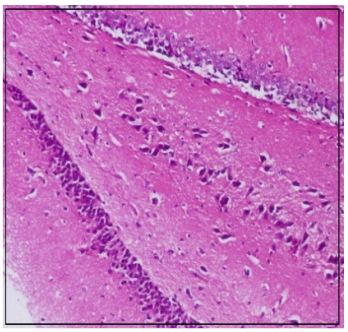

Figure 6. Cortical region showing glial cells & neuronal cells normal morphology (X100)

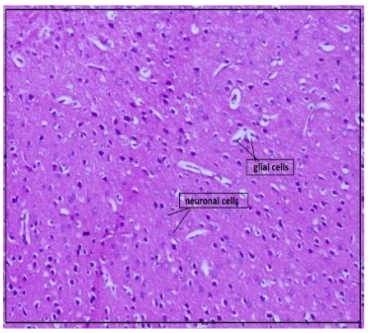

Figure 7. Hippocampus region showing normal morphology (X100)

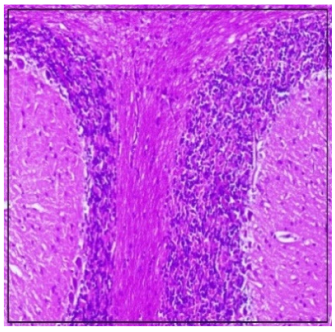

Figure 8. Pyramidal region showing normal morphology (X100)

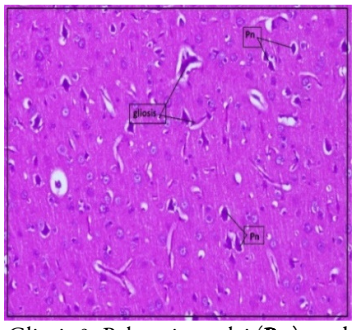

Figure 9. Cortical region showing Gliosis & Pyknotic nuclei (**Pn**) moderate: 3+ (X100)

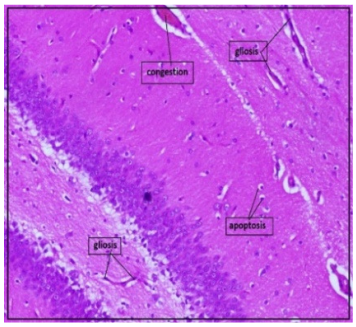

Figure 10. Hippocampus showing blood vessel Congestion apoptosis & Gliosis were moderate – 3+ (X100)

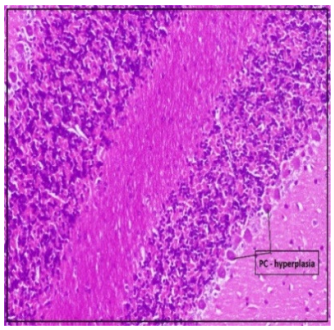

Figure 11. Pyramidal region showing Purkinje cells hyperplasia was observed (PC) – (X100)

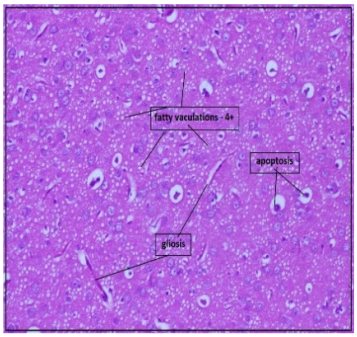

Figure 12. Cortical region showing fatty vacuolations – severe: 4+. Gliosis & apoptosis were moderate: 3+ (X100)

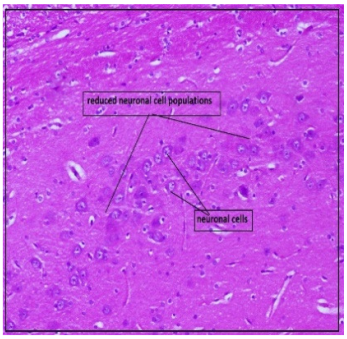

Figure 13. Hippocampus showing reduced neuronal cell populations (X100)

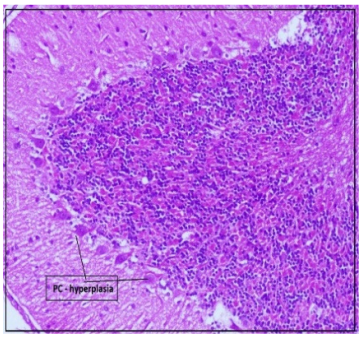

Figure 14. Pyramidal region showing Purkinje cells hyperplasia was observed (PC) – (X100)

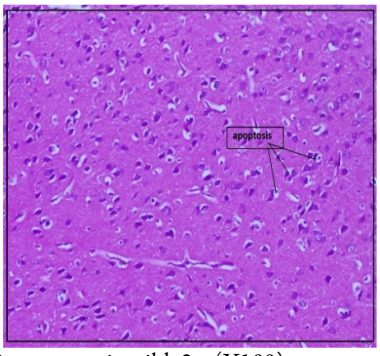

Figure 15. Cortical region showing apoptosis mild: 2+ (X100)

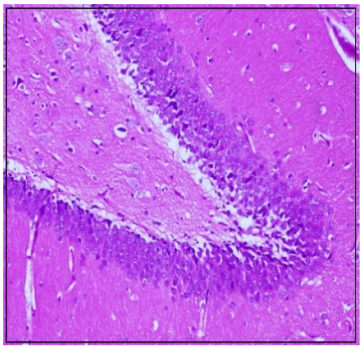

Figure 16. Hippocampus showing near normal morphology (X100)

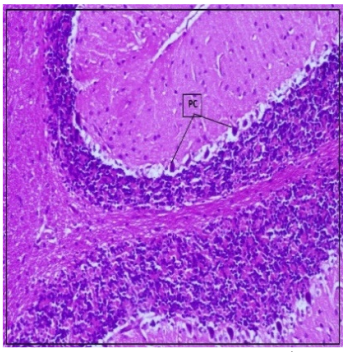

Figure 17. Pyramidal region showing near normal Purkinje cells (X100)

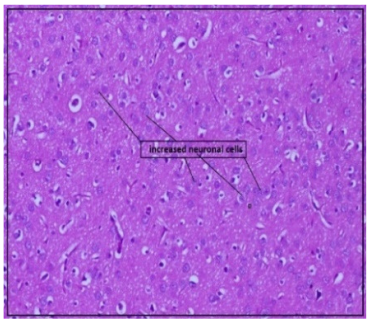

Figure 18. Cortical region showing near normal neuronal cells (X100)

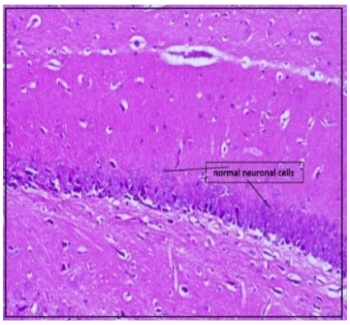

Figure 19. Hippocampus showing near normal morphology with normal neuronal cells (X100)

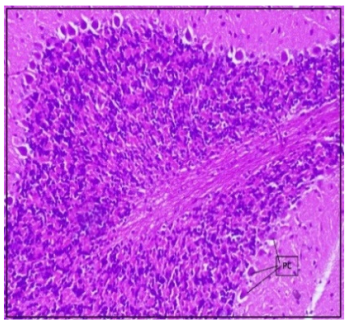

Figure 20. Pyramidal region showing near normal Purkinje cells (PC) – (X100)

Estimation of BDNF by immunohistochemistry

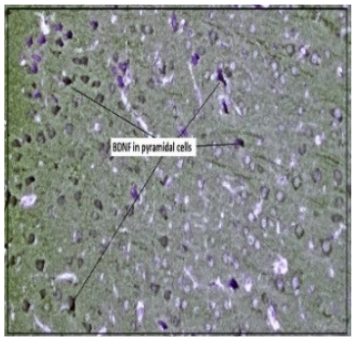

Figure 21. Cortical region showing BDNF in pyramidal neuronal cells – Dark brown colour (X100)

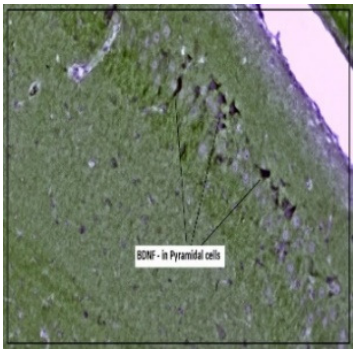

Figure 22. Hippocampus – **CA1 region** showing BDNF in pyramidal neuronal cells (X100)

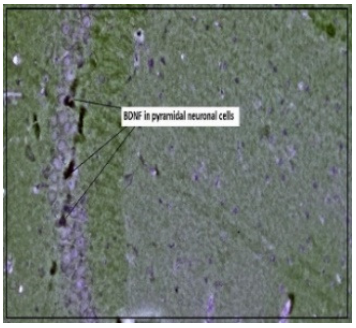

Figure 23. Hippocampus – **CA3 region** showing BDNF in pyramidal neuronal cells (X100)

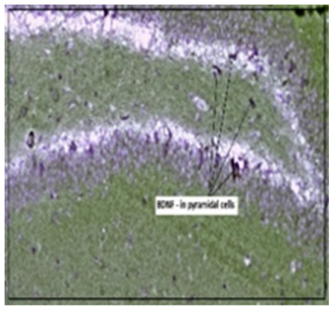

Figure 24. Hippocampus – **DG region** showing BDNF in pyramidal neuronal cells(X100)

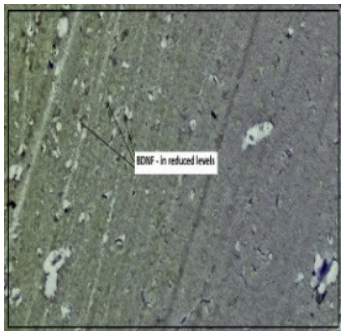

Figure 25. Cortical region showing BDNF in pyramidal neuronal cells
(Note: BDNFi n reduced levels) Dark brown color (X100)

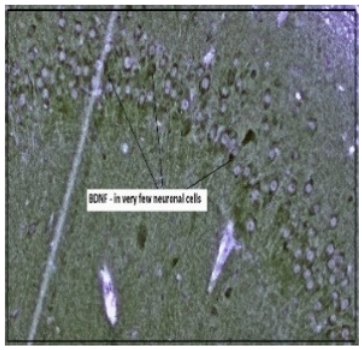

Figure 26. Hippocampus showing – CA1 region showing BDNF in few pyramidal neuronal cells (X100)

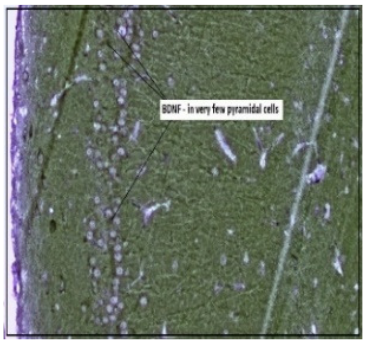

Figure 27. Hippocampus showing – **CA3 region** showing BDNF in very few pyramidal neuronal cells (X100)

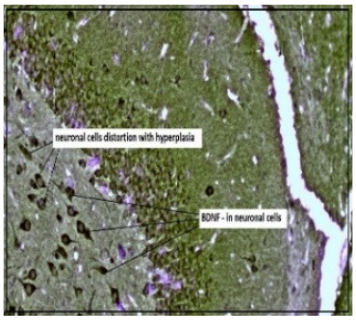

Figure 28. Hippocampus showing – **DG region** showing BDNF in pyramidal neuronal cells (X100) (Neuronal cells – distorted with hyperplasia)

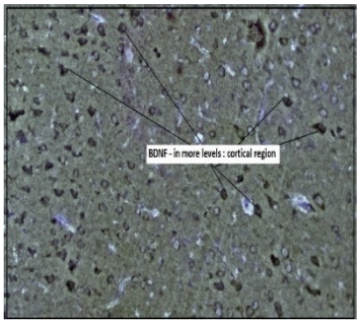

Figure 29. Cortical regions showing BDNF in pyramidal neuronal cells
(More BDNF levels noticed) Dark brown color (X100)

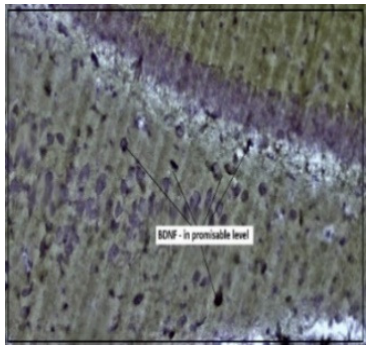

Figure 30. Hippocampus – **CA1 region** showing BDNF in pyramidal neuronal cells at a promising level (X100)

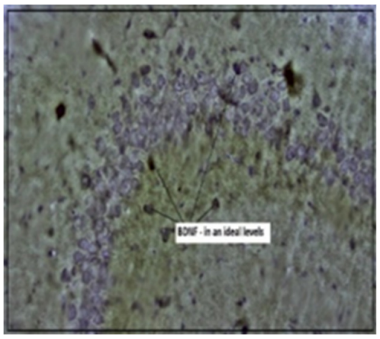

Figure 31. Hippocampus – **CA3 region** showing BDNF in pyramidal neuronal cells at an ideal level (X100)

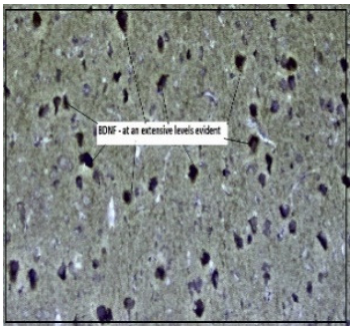

Figure 32. Cortical region showing BDNF in pyramidal neuronal cells (Extensive BDNF levels noticed) Dark brown colour (X100)

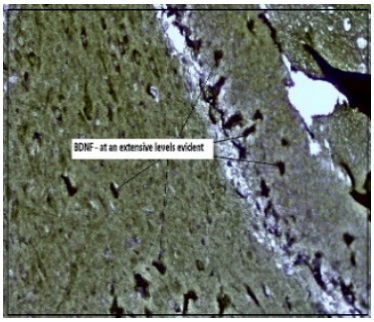

Figure 33. Hippocampus – **CA1 region** showing BDNF in pyramidal (Extensive BDNF levels noticed) neuronal cells (X100)

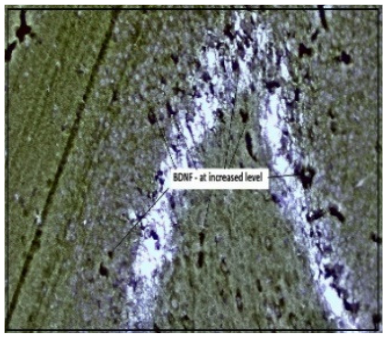

Figure 34. Hippocampus – CA3 region showing BDNF in pyramidal neuronal cells at an increased level (X100)

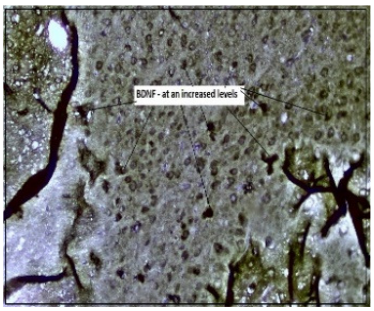

Figure 35. Hippocampus – **DG region** showing BDNF in pyramidal neuronal cells (X100)

Discussion

The neuroprotective potential of palmatine isolated from *Tinospora cordifolia* was determined by administering palmatine to male Wistar albino rats induced with aluminum chloride-mediated neurotoxicity. This study with palmatine unveils the interrelation between oxidative stress, neuronal inflammation, neurotransmitter imbalance, excitotoxicity neurodegeneration, and neuronal loss, alteration in synaptic plasticity, BDNF expression, etc brought about by aluminum exposure and how the treatment helps to combat the damage induced.

Palmatine treatment ameliorated the neuroinflammatory status and regulated the levels of glutathione, neurotransmitters namely AChE and Glutamate and lowered excitotoxicity, gliosis pyknosis, and neuronal apoptosis brought about by aluminum, thereby alleviated the viability of neurons. Findings of the ELISA test for inflammatory markers confirms that palmatine effectively regulates aluminum chloride-induced spikes in cytokines like TNF-alpha and IL-6 and curtailed the levels of excitotoxic glutamate and acetylcholine esterase levels in rat brains. Palmatine helped to maintain the number of intact neurons to a good extend, and improved BDNF expression thereby improving synaptic plasticity. The findings confirm the neuroprotective potential of the protoberberine alkaloid palmatine. Another study involving ischemia-reperfusion injury in the brain wherein the treatment with palmatine effectively lowered inflammatory response in the brain after cerebral I/R injury wherein the treatment effectively lowered IL-1β, IL-6, and TNF-α in the brain after middle cerebral artery occlusion which was further confirmed by RT-PCR results. These results are supportive of the potential of palmatine against neuronal inflammation (Tang *et al.,* 2021).

The histopathological evaluations were performed to evaluate the ameliorative properties of palmatine treatment at doses 10 mg/kg and 20 mg/kg respectively against aluminum chloride (AlCl3)-induced AD in animals via the suppression of oxidative stress and neuroinflammation. In the aluminum chloride (AlCl3)-induced group, the brain showed evidence to implicate potential damage in all the 3 regions of the rat brain,

including oxidative stress-induced damage, Gliosis, apoptosis, increased congestion and increased congestion and pyknotic nuclear condensation, etc. The brain of the induced group animals showed severe hyperplastic vacuolar changes in the Cortical & hippocampus regions, pyramidal region exhibited vacuolar changes with Purkinje cells hyperplasia. In treated groups there was a marked reduction in aluminum chloride (AlCl3)-induced severity of neurodegeneration with the restoration of the brain histological architecture to near normal morphology.

Brain-derived neurotrophic factor (BDNF) is the most widely expressed and well-characterized member of the neurotrophin family in the mammalian brain. Modest changes in BDNF levels affect the development and regulation of neural circuits and brain function. In the mature nervous system, BDNF promotes the elaboration and refinement of neuronal circuit structure, modulates synaptic plasticity, and, consequently, regulates cognitive brain function (including learning and memory). Although BDNF does not seem essential for the survival of most CNS neurons, it modulates dendritic complexity and spine density, which markedly affects behavior and suggests that it acts more as a differentiation and plasticity factor in the CNS.

Alterations in BDNF levels are associated with neurodegenerative disorders (including Alzheimer's disease, Huntington's disease, and epilepsy), neuropsychiatric disorders (including depression, anxiety disorders, bipolar disorders, schizophrenia, and addiction), and obesity. The hallmark of BDNF deficiency is synaptic degeneration, and increased levels of BDNF can promote synaptic repair in preclinical models. Moreover, BDNF could potentially be used to treat diseases in which alterations in its levels are not directly involved in the pathogenesis (for instance, in Parkinson's disease, amyotrophic lateral sclerosis, stroke, and spinal cord injury). BDNF is a highly charged protein that does not readily cross the blood-brain barrier (BBB), so effective CNS delivery is a challenge

Evaluation of BDNF levels in rat brains by means of IHC unveil that palmatine prevented the steep fall in dBDNF expression observed in aluminum chloride (AlCl3)-induced group by protecting brain from damage inflicted by oxidative stress and neuroinflammation. Aluminium chloride ($AlCl_3$)-induced groups exhibited a decreased level of expression of BDNF compared to the treatment group. In both Cortical & hippocampus - severe hyperplastic vacuolar changes, and pyramidal cell vacuolar changes were observed. But in the palmatine-treated groups at the dose of 10 mg/kg b. wt. & 20 mg /kg b. wt., a marked increase in BDNF levels in a dose-dependent manner was observed. The findings from the treatment through immunohistochemical staining and histopathological examinations are conclusive of the protective role of palmatine in aluminum chloride-induced neurotoxicity.

Conclusions

The findings of the study related to the neuroprotective potential of protoberberine alkaloid palmatine against aluminum-induced neuroinflammation and excitotoxicity reveal that the phytocompound palmatine effectively regulates the levels of AChE, glutamate, inflammatory markers IL-6, TNF-α, prevented neuronal degeneration and apoptosis to a good extend and improved neuronal viability synaptic plasticity and BDNF expression which is otherwise disturbed by the deleterious effects of aluminum in rat brain.

Authors' Contributions

The experimental work and manuscript writing was performed by R.B under the guidance of co-authors R.S.V and K.D. who also supported to shape the research. Plant identification extraction and isolation was performed by R.B. under K.D. and animal experiments were performed by R.B. under R.S.V. who also helped with proofreading and refining the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The institutional Ethical committee of Krupanidhi College of Pharmacy, Bangalore approved the experiment protocol by the number KCP/IAEC/PCOL/61/2021.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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