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Original Research Article

Neuroprotective Effects of *Centella asiatica* Against $AlCl_3$ and D-Galactose-Induced Astrocyte Activation and Hippocampal Neurodegeneration in Male Albino Wistar Rats

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ABSTRACT

Background: Alzheimer's disease (AD) a neurodegenerative disorder is a leading cause of dementia in the elderly population. The concurrent dosing of rats with aluminium chloride ($AlCl_3$) and D-galactose (D-gal) is regarded an effective approach for developing an animal model to study AD. *Centella asiatica* (CA) demonstrates neuroprotective effects in both in vitro and in vivo studies. This research investigated the protective effects of CA against neurodegeneration of the hippocampus and activation of astrocytes in rats treated with $AlCl_3$ and D-gal.

Materials and methods: Rats received $AlCl_3$ at a dosage of 200 mg/kg body weight daily, D-gal at 60 mg/kg body weight daily, and CA at 100, 200, and 300 mg/kg body weight daily, in conjunction with donepezil at 1 mg/kg body weight daily, for a duration of 70 days. Following treatment, the brain tissue was fixed in 10% formalin for further histological analysis. Nissl staining was applied to examine the survival of CA2 neurons in the hippocampus, whereas Glial Fibrillary Acid Protein (GFAP) was employed to assess active astrocytes in the CA2 hippocampal area.

Results: The findings indicated that $AlCl_3$ and D-gal could substantially harm the hippocampus CA2 pyramidal neurons in rats. Furthermore, it induced the activation of astrocytes in the rat hippocampus. Co-administration of CA at doses of 100mg, 200mg, and 300mg mitigated neurodegeneration and astrocyte activation in the hippocampus of the rats.

Conclusion: The findings indicate that CA may safeguard against morphological changes induced by $AlCl_3$ and D-gal in rats. Molecular investigations are under underway to clarify the potential effects of CA.

KEYWORDS: $AlCl_3$, D-galactose, Neurodegeneration, Astrocytes, *Centella asiatica*, Hippocampus.

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INTRODUCTION

Alzheimer's disease (AD) is an important cause of dementia, including 60–80% of all dementia cases [1]. Currently, over 50 million individuals globally are afflicted with dementia, and with an increase of life expectancy, it has been projected that 139 million people will be having dementia by 2050, exerting a considerable impact on socioeconomic conditions with a strain on healthcare systems [2]. In Malaysia, AD was estimated to affect 0.126% of the population in 2020. It is estimated that it would increase to 0.454% by 2050. The estimated number of individuals in Malaysia now living with AD is roughly 50,000 [3]. The primary forms of AD are early onset (familial) and late onset (sporadic). Early onset, which is rare, arises from genetic defects, while late onset is more common in individuals over 65 [4].

AD is characterized by cognitive impairments in language, spatio-temporal orientation, and executive function, accompanied by behavioral alterations, resulting in a progressive decline in functional autonomy [5]. Histopathological examination reveals two distinct pathognomonic hallmarks of AD which are the intracellular accumulation of abnormally phosphorylated Tau protein leading to the development of neurofibrillary tangles (NFTs) in the cerebral cortex and subcortical grey matter and the presence of extracellular aggregation of Amyloid-beta peptide (Aß) fibrils, which manifest as neuritic plaques [6].

Aluminium chloride ($AlCl_3$) a neurotoxin, is extensively utilized in inducing dementia in various animal models. Aluminium (Al) serves as a cross-linker of amyloid protein, causing oligomerization and consequent neurotoxicity [7]. Al ions contribute to Aß accumulation and the hyperphosphorylation of Tau proteins in the brain, causing injury and subsequent death of neurons [8]. Results of animal studies have demonstrated that prolonged exposure to Al leads to behavioral, pathological, and neurochemical alterations in the brain, impacting spatial memory and learning [9,10,11]. Acetylcholinesterase (AChE) plays an essential role in regulating memory and hippocampal plasticity. it is affected by Al exposure and is a key indicator of neurotoxicity [12]. Excessive D-galactose (D-gal) consumption can result in inflammation, oxidative stress, apoptosis, and increased ageing affecting multiple organs, including the brain [13]. With large amount of D-gal consumption the body's metabolic capacity is saturated, leading to the production of advanced glycation end products (AGEs) that gather and bind to AGE receptors, leading to cellular injury through free radical generation, increased oxidative stress, and cellular inflammation [14]. Investigations indicate that administration of D-gal along with $AlCl_3$ can elicit AD-like symptoms, including cognitive impairment and memory deficits, oxidative damage, and neuroinflammation [15,16,17].

Astrocytes neurotrophic, and safeguard neurons from oxidative damage. In cases of neurotoxic damage or pathological afflictions, brain vitality is compromised, the astrocytes play a significant function in the survival and function of neurons [18]. Several animal studies have demonstrated that Al toxicity leads to astrogliosis and subsequent neuronal cell death [19,20].

Therefore, protecting the astrocytes and maintaining their vitality hold therapeutic potential in modulating neuronal function and degeneration in Al toxicity [21].

Centella asiatica (CA), is an herb which belongs to the Umbelliferae (Apiaceae) family and has been widely used as a medicinal herb in Ayurvedic and Chinese medicine and is recognized as a cognitive enhancing tonic [22].

The primary active components present in extracts of CA includes a pentacyclic triterpenoid, asiatic acid, flavonoids, madecassoside [23].

The aqueous extract of CA has demonstrated antioxidative properties and enhanced cognitive abilities in human experiments [24,25]. Prior studies, however, did not examine the influence of CA on the morphology and functionality of glial cells. This study evaluated the impact of CA on astrocyte activation and hippocampal neuro degeneration induced by $AlCl_3$ and D-gal in male albino Wistar rats.

MATERIALS AND METHODS

Animals: A total of thirty-six male albino Wistar rats, approximately three months old and weighing between 220-250 grams, were acquired from Perniagaan Usaha Cahaya in Selangor, Malaysia. They were housed two per cage under a climate-controlled environment with 12-hour alternating light and dark cycles at University Putra Malaysia (UPM), Malaysia. The Institutional Animal Care and Use Committee (IACUC) granted ethical permission via ethics certificate UPM/IACUC/AUP-R071/2020. All experiments were performed in compliance with IACUC and UPM procedures.

Chemicals and Plant extract preparation: The chemicals utilized in these studies were of analytical grade standard. The plant materials for the CA plant were obtained from Universiti Teknologi MARA, Malaysia. The leaves were initially desiccated in the shade, after which they were pulverized into a fine powder. Subsequently, 1 kilogram (kg) of the powder was immersed in 3 Liters (L) of distilled water for 48 hours (hrs) to obtain the aqueous extract. The aqueous extract was repeatedly filtered three to four times using Whatman No. 1 filter paper until it became colourless. The extract was further concentrated by a rotary evaporator under low pressure at a temperature of 50 ± 5°C. The resultant product was further lyophilized using a freeze-dryer, resulting in 7% (w/w) of the freeze-dried substance.

Experimental Design: Following a week of acclimatization, the rats were divided into six groups, comprising six rats per group. The control group received normal saline intraperitoneally and distilled water orally, the model group received $AlCl_3$ at a dosage of 200 mg/kg orally and D-gal at a dosage of 60 mg/kg intraperitoneally daily, the CA 100 group (model + CA at 100 mg/kg orally daily), the CA 200 group (model + CA at 200 mg/kg orally daily), the CA 300 group (model + CA at 300 mg/kg orally daily), and the donepezil group (model + 1mg of donepezil /kg/i.p/daily).The rats had seventy days of treatment, after which they were decapitated, and their brain tissues were extracted for analysis.

Sample Collection and Preparation: The removed rats brain tissues were placed in an ice-cold saline. The brain tissues which were intended for histological analysis were rinsed well using normal saline and subsequently immersed in 10% formalin solution for one week. The brain tissues were further processed to observe the histological alterations.

Histopathological Changes of the hippocampus: Using a Rotary Microtome tissue sections of 5 ìm thickness were obtained, following which they were stained with Nissl stain to observe the neurodegenerative cells in the cornu ammonis 2 (CA2) sub regions of hippocampus. Then using a microscope (Olympus, BX43), observation. of histopathological alterations and quantification of neurons in the CA2 sub region was performed. Images were captured at a magnification of 40X utilizing an image analyzer (Nikon H500L). The number of viable neurons (identified as neurons with distinct nucleus) were counted while the neurons with very dark stain, shrunken cell body and irregular nuclei were not considered for quantification.

Immunohistochemistry for Astrocytes activation in the hippocampal CA2 subregion: Prior to the initiation of immunohistochemistry processing, the tissue sections were treated in 0.01 M citrate buffer at pH 6.0 for 10 minutes at 100 °C to facilitate antigen retrieval. Subsequently, 3% H2O2 was added to phosphate-buffered saline (PBS) to inactivate the endogenous peroxidase. The sections were subsequently blocked in standard serum, thus inhibiting non-specific binding and then they were treated with primary polyclonal antibodies for astrocytes in 5% normal serum at a dilution of 1:500 for 1 hour at room temperature. After 1 hour, the tissue segment underwent four rinses with PBS, followed by a 30-minute incubation with a biotinylated secondary antibody, and was subsequently treated with an avidin-biotinylated HRP complex in PBS for an additional 30 minutes. Sections were subsequently counterstained with haematoxylin to enhance nuclear visibility. IHC images were captured using a Nikon Eclipse 80i microscope (SEO Enterprises, Inc, Lakeland, FL, USA) equipped with a Nikon DS

Ri1 12-megapixel camera (Nikon, Tokyo, Japan) at a magnification of 40X. The measurement of activated astrocytes was conducted using Image J software by examining the thicker processes and heightened expression of the intermediate filament protein Glial Fibrillary Acid Protein (GFAP), which are indicative of active astrocytes.

Statistical Analysis: The values were presented as mean ± standard deviation (SD) with a sample size of n=3. Subsequent to the one-way ANOVA, Tukey's post hoc test was utilized to examine the data. Statistical significance was established for p values below 0.05 in the comparisons.

RESULTS

CA prevented neurodegeneration of the hippocampal CA2 pyramidal neurons in $AlCl_3$ and D- gal induced neurodegeneration: The Nissl's staining was used to evaluate the neuroprotective effects of CA on neurons in CA2 hippocampal region of rat brains. Substantial changes were noticed in the quantity of viable neurons within the CA2 hippocampal region among various groups of rats. The hippocampus of rats from the model group demonstrated a significantly reduced number of viable neurons compared to the control group (*p<0.05). The rats from CA100, CA 200, CA 300 and Donepezil group exhibited a significantly greater count of viable neurons when compared to model group (*p<0.05 (Figure 1)

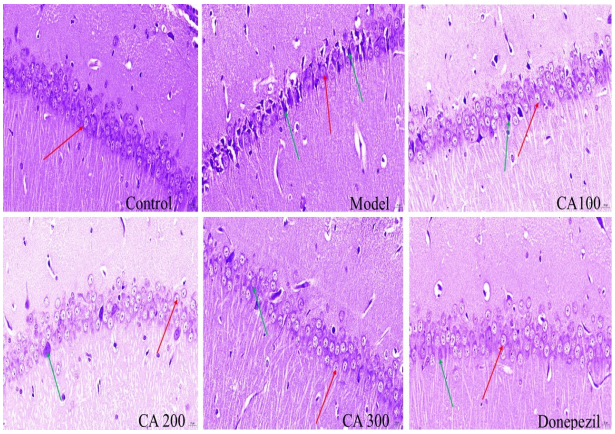

Fig. 1: Neuroprotective effects of CA on the CA2 area of hippocampal neurons, as demonstrated through Nissl staining, in rats treated with $AlCl_3$ and D-gal. Images of Nissl-stained hippocampal tissue illustrating variations in the quantity of viable cells (shown by red arrows) and degenerated cells (indicated by green arrows)

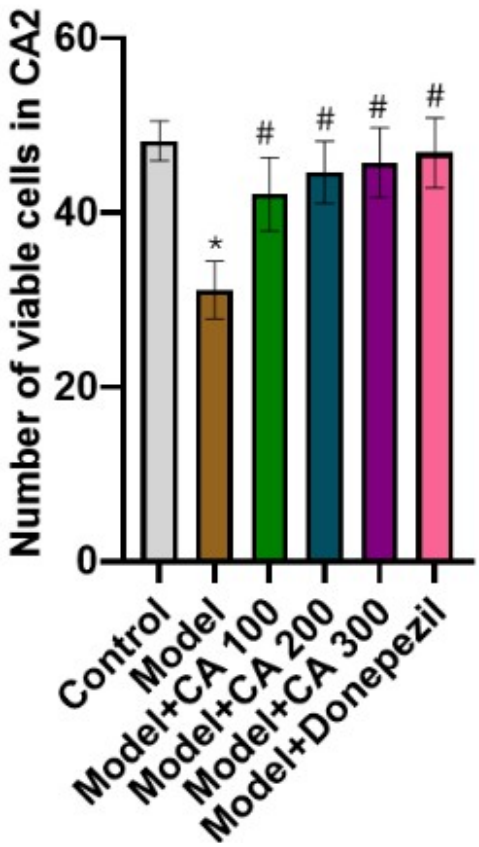

Fig. 2: Indicates functional neurons in the CA2 region of the hippocampus subjected to $AlCl_3$ and D-gal exposure. Data are presented as mean ± S.D, n = 6, *p < 0.05 compared to control and AlCl3 + D-gal; #p < 0.05 compared to $AlCl_3$ + D-gal + CA treatment at 100 mg, 200 mg, and 300 mg, as well as donepezil groups.

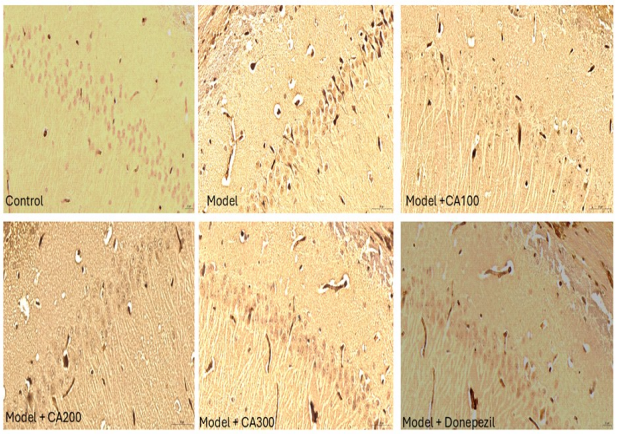

Fig. 3: Impact of CA on the CA2 region of the hippocampus, as evidenced by GFAP staining, in rats administered $AlCl_3$ and D-gal. Images of hippocampus tissue demonstrate activated astrocytes with a bushier morphology and an increased number of processes.

CA reduced the activation of astrocytes in $AlCl_3$ and D -gal induced astrocytes activation in CA 2 hippocampus: The CA2 region of the hippocampus was also assessed for astrocyte activation using GFAP staining. The comparison between the control group and model

group revealed a considerable rise (*p<0.05) in the number of activated astrocytes in the model group. When comparing the model group to the CA100. CA200, CA300 and Donepezil group treatment reduced (*p<0.05) the number of activated astrocytes. (Figure 2)

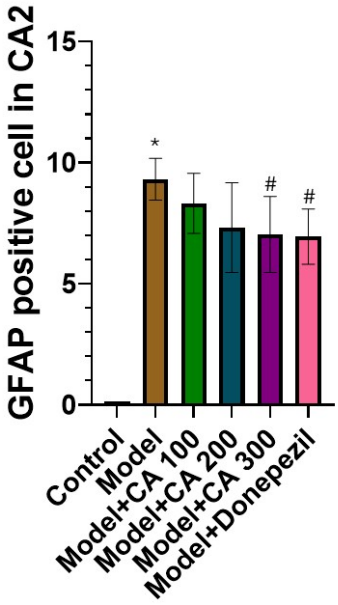

Fig. 4: Demonstrates GFAP positive cells in the CA2 region of the hippocampus following exposure to $AlCl_3$ and D-gal. Data are expressed as mean ± S.D, n = 6. Statistical significance is indicated by *p < 0.05 when compared to control and $AlCl_3$ + D-gal; #p < 0.05 when compared to $AlCl_3$ + D-gal + CA treatment at 100 mg, 200 mg, and 300 mg, as well as donepezil groups.

DISCUSSION

This study shows that CA can prevent neurodegeneration in the hippocampal CA2 pyramidal neurons in an $AlCl_3$ and D-gal induced neurodegeneration model. CA2 pyramidal neurons are characterized by large cell bodies and dendrites which branch along the transverse axis of the hippocampus [26]. Unlike CA3 pyramidal neurons, CA2 neurons do not have thorny excrescences on their apical dendrites [27]. CA2 neurons play a critical role in forming social memories. Although small, the CA2 region contributes significantly to functions like social memory and anxiety regulation [28]. Among adults, CA2 is a highly interconnected area, receiving input from over ten different hippocampal subregions. This region is particularly impacted in conditions such as schizophrenia and neurodegenerative diseases [29]. Early research on human hippocampal tissue has shown that CA2 undergoes distinctive changes in various pathologies and psychiatric disorders [30].

Neurodegenerative illnesses, such as AD, are characterised by the progressive degeneration of neurons and susceptible regions of the central nervous system. The pathways behind neurodegeneration are believed to be complex, encompassing various aspects which include mitochondrial dysfunction, oxidative stress, defective protein breakdown and aggregation. Furthermore, it also involves genetic, environmental, and intrinsic variables [31,32]. This study has demonstrated that rats when subjected to $AlCl_3$ and D-gal induced notable morphological alterations in the CA2 region of the hippocampus of brain. These modifications encompassed a heightened number of pyknotic cells, reconfigurations in the organization of pyramidal cells, and disturbances in the nuclei.

Histological examination of the hippocampus in the animal model revealed that $AlCl_3$ in conjunction with D-gal caused gradual pathological alterations, including nuclear disintegration, karyorrhexis, intense cytoplasmic staining, and disruption of CA2 pyramidal cells. CA mitigates neurodegeneration by suppressing hyperphosphorylated tau (P-tau) biosynthesis proteins, averting apoptosis and maintaining cellular integrity [33].

CA provides further protective benefits by elevating protein phosphatase 2 (PP2A) levels, reducing glycogen synthase kinase-3 beta (GSK-3ß) levels, enhancing mRNA expression of Bcl-2, and averting structural anomalies in the CA2 region of the hippocampus [34]. CA also enhances cognitive improvement and facilitates non-spatial learning and memory. CA also possesses promise as a cholinesterase inhibitor to assist in improving memory function. Recent data indicate that CA improves learning and memory function in rats by increasing the expression of AMPAR subunits GluA1 and GluA2, as well as the N-methyl-D-aspartate receptor (NMDAR) component GluN2B, while decreasing the expression of the NMDAR subunit GluN2A in the hippocampus and also in entorhinal cortex [35]. The findings of the present study indicate that CA has cytoprotective properties

and contributes to the preservation of the normal cytoarchitectural pattern of the CA2 sub-region of the hippocampus. Neuro degeneration in the hippocampus CA2 pyramidal region is mitigated by CA administration at escalating dosages, with the maximum dose of 300mg nearly equivalent to the conventional medication donepezil.

Neuroinflammation is recognized as a central feature in AD pathology and a key target for therapeutic approaches. In AD, the primary contributors to inflammation include microglia, astrocytes, and some neurons, which play essential roles in brain homeostasis and function [36]. Reactive gliosis, a process where astrocytes and microglia become activated in response to various toxins, contributes to neuroinflammation and neurodegeneration [37]. GFAP, which is an astrocyte-specific intermediate filament protein is crucial for maintaining central nervous system homeostasis, is upregulated during reactive gliosis following exposure to $AlCl_3$ and D-gal [38]. In this study, the hippocampal CA2 pyramidal region in $AlCl_3$+D-gal treated animals exhibited signs of reactive gliosis. The reduced GFAP expression with CA treatment suggested an anti-inflammatory effect.

Reactive gliosis is seen to be alleviated by CA administration in increasing doses with the highest administered dose of 300mg, nearly comparable to the standard drug donepezil. This demonstrates that other than neuroprotective and antioxidant properties, CA has anti-inflammatory properties that can help reduce neurodegeneration in AD patients. CA is believed to inhibit phospholipase A2, an enzyme that promotes inflammation [39]. It may also decrease nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) expression, potentially aiding in mitochondrial function. Mitochondrial-dependent oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) are linked to anti-inflammatory responses [40,41].

CONCLUSION

AD has affected millions across the world and stays as a leading cause of dementia and no treatment to slow the process of neurodegeneration.

This study neuroprotective effect of the CA on $AlCl_3$+D-gal induced neurodegeneration on rat models showed that CA at a dosage of 300 mg/kg body weight reduced loss of neurons in the CA2 hippocampal region and prevents neuroinflammation by reducing the GFAP expression. This study firmly concluded that CA has a great potential to prevent neurodegeneration, however more pathways need to be studied to confirm the exact mechanism of action.

Author Contributions

Thirupathirao. Vishnumukkala: Designed and performed the experiments. Analysed the data and wrote the manuscript.
Ravindra Kumar Boddeti: Assisted in manuscript preparation and reviewed manuscript.
Prarthana Kalerammana Gopalakrishna: Analysed the data and assisted in manuscript preparation.
Barani Karikalan: Prepared the figures and assisted in manuscript preparation.
Saravanan Jagadeesan: Performed the experiments and reviewed the manuscript.
Mohamad Taufik Hidayat B. Baharuldin: Design of the study, guidance in manuscript preparation
Nurul Huda Mohd Nor: Design of the study, guidance in manuscript preparation
Mohamad Aris Mohd Moklas: Designed and conceptualised the study, reviewed the results, and wrote the manuscript for final submission.

Conflicts of Interests: None

REFERENCES

[1]. Silva MVF, Loures C de MG, Alves LCV, de Souza LC, Borges KBG, Carvalho M das G. Alzheimer's disease: risk factors and potentially protective measures. J Biomed Sci. 2019;26(1):33. https://doi.org/10.1186/s12929-019-0524-y PMid:31072403 PMCid:PMC6507104

[2]. Collaborators GD, Szoeke NE, Vollset C, Abbasi SE, Abd-Allah N. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study. Lancet Neurol. 2016; 18:88-106.

[3]. Ali, M. F., Ja'afar, N. I. S., Krishnan, T. G., Zulkifle, M. A. M., Khaidzir, N. K., Jamil, T. R., Aziz, A. F. A. Dementia awareness among elderly at risk for developing mild cognitive impairment: a cross-sectional study at a university-based primary care clinic. BMC Geriatrics. 2023; 23(1):496. https://doi.org/10.1186/s12877-023-04230-4 PMid:37592221 PMCid:PMC10436505

[4]. Vishnumukkala T, Gopalakrishna PK, Jagadeesan S, Chiroma SM, Nor NHM, Baharuldin MTH, et al. Herbal medicine: A promising approach for the treatment of Alzheimer's disease. Int J Ayurveda Pharma Res. 2024;142-50. https://doi.org/10.47070/ijapr.v12i1.3103

[5]. García-Morales V, González-Acedo A, Melguizo-Rodríguez L, Pardo-Moreno T, Costela-Ruiz VJ, Montiel-Troya M, et al. Current understanding of the physiopathology, diagnosis and therapeutic approach to Alzheimer's disease. Biomedicines. 2021;9(12):1910. https://doi.org/10.3390/biomedicines9121910 PMid:34944723 PMCid:PMC8698840

[6]. Thirupathirao. Vishnumukkala, Prarthana Kalerammana Gopalakrishna, Barani Karikalan, Saravanan Jagadeesan, Mohamad Taufik Hidayat B. Baharuldin, Warren Thomas, Mohamad Aris Mohd Moklas. Protective Effect of Centella asiatica on AlCl3 and D-Galactose Induced Hepatotoxicity in Rats through the Alleviation of Oxidative Stress as Demonstrated by Histological Changes in Liver. Int J Anat Res 2023;11(4):8740-8747. https://doi.org/10.16965/ijar.2023.204

[7]. Khan KA, Kumar N, Nayak PG, Nampoothiri M, Shenoy RR, Krishnadas N, et al. Impact of caffeic acid on aluminium chloride-induced dementia in rats: Caffeic acid and dementia. J Pharm Pharmacol. 2013;65(12):1745-52. https://doi.org/10.1111/jphp.12126 PMid:24236984

[8]. Xue J-S, Li J-Q, Wang C-C, Ma X-H, Dai H, Xu C-B, et al. Dauricine alleviates cognitive impairment in Alzheimer's disease mice induced by D-galactose and AlCl3 via the Ca2+/CaM pathway. Toxicol Appl Pharmacol. 2023;474(116613):116613. https://doi.org/10.1016/j.taap.2023.116613 PMid:37414289

[9]. Aimone JB, Deng W, Gage FH. Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. Neuron. 2011;70(4):589-96. https://doi.org/10.1016/j.neuron.2011.05.010 PMid:21609818 PMCid:PMC3240575

[10]. Prakash D, Gopinath K, Sudhandiran G. Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. Neuromolecular Med. 2013;15(1):192-208. https://doi.org/10.1007/s12017-012-8210-1 PMid:23315010

[11]. Junior AFS, Aguiar MSS, Junior OSC, Luana De Nazaré SS, Franco EC, Lima RR, et al. Hippocampal neuronal loss, decreased GFAP immunoreactivity and cognitive impairment following experimental intoxication of rats with aluminum citrate. Brain research. 2013;1491;23-33. https://doi.org/10.1016/j.brainres.2012.10.063 PMid:23131585

[12]. Kaizer RR, Corrêa MC, Spanevello RM, Morsch VM, Mazzanti CM, Gonçalves JF, et al. Acetylcholinesterase activation and enhanced lipid peroxidation after long-term exposure to low levels of aluminum on different mouse brain regions. J Inorg Biochem. 2005;99(9):1865-70. https://doi.org/10.1016/j.jinorgbio.2005.06.015 PMid:16055195

[13]. Shwe T, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. Role of D-galactose-induced brain aging and its potential used for therapeutic interventions. Exp Gerontol. 2018; 101:13-36. https://doi.org/10.1016/j.exger.2017.10.029 PMid:29129736

[14]. Vishnumukkala T, Gopalakrishna PK, Karikalan B, Thomas W, Jagadeesan S, Musa Chiroma S, et al. Centella asiatica ameliorates AlCl3 and D-galactose induced nephrotoxicity in rats via modulation of oxidative stress. Bioinformation. 2024;20(5):508-14. https://doi.org/10.6026/973206300200508 PMid:39132239 PMCid:PMC11309103

[15]. Xue J-S, Li J-Q, Wang C-C, Ma X-H, Dai H, Xu C-B, et al. Dauricine alleviates cognitive impairment in Alzheimer's disease mice induced by D-galactose and AlCl3 via the Ca2+/CaM pathway. Toxicol Appl Pharmacol. 2023;474(116613):116613. https://doi.org/10.1016/j.taap.2023.116613 PMid:37414289

[16]. Aihaiti M, Shi H, Liu Y, Hou C, Song X, Li M, et al. Nervonic acid reduces the cognitive and neurological disturbances induced by combined doses of D-galactose/AlCl3 in mice. Food Sci Nutr. 2023;11(10):5989-98. https://doi.org/10.1002/fsn3.3533 PMid:37823115 PMCid:PMC10563680

[17]. Haider S, Liaquat L, Ahmad S, Batool Z, Siddiqui RA, Tabassum S, et al. Naringenin protects AlCl3/D-galactose induced neurotoxicity in rat model of AD via attenuation of acetylcholinesterase levels and inhibition of oxidative stress. PLoS One. 2020;15(1):e0227631. https://doi.org/10.1371/journal.pone.0227631 PMid:31945778 PMCid:PMC6964982

[18]. Prarthana Kalerammana Gopalakrishna S, Venkatesh R, Naik S, Sura VT. The Role of Astrocytes in Alzheimer's disease. Int Res J Biological Sci. 2023;12(3):17-20.

[19]. De Bastiani MA, Bellaver B, Brum WS, Souza DG, Ferreira PCL, Rocha AS, et al. Hippocampal GFAP-positive astrocyte responses to amyloid and tau pathologies. Brain Behav Immun. 2023; 110:175-84. https://doi.org/10.1016/j.bbi.2023.03.001 PMid:36878332

[20]. Laabbar W, Abbaoui A, Elgot A, Mokni M, Amri M, Masmoudi-Kouki O, et al. Aluminum induced oxidative stress, astrogliosis and cell death in rat astrocytes, is prevented by curcumin. J Chem Neuroanat. 2021;112(101915):101915. https://doi.org/10.1016/j.jchemneu.2020.101915 PMid:33370573

[21]. Jeong H-K, Ji K-M, Min K-J, Choi I, Choi D-J, Jou I, et al. Astrogliosis is a possible player in preventing delayed neuronal death. Mol Cells. 2014; 37(4): 345-55.
https://doi.org/10.14348/molcells.2014.0046
PMid:24802057 PMCid:PMC4012084

[22]. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, et al. Therapeutic Potential of Centella asiatica and Its Triterpenes: A Review. Front Pharmacol. 2020; 11:568032.
https://doi.org/10.3389/fphar.2020.568032
PMid:33013406 PMCid:PMC7498642

[23]. Jagadeesan S, Gopalakrishna PK, Sura S, Karikalan B, Dandala KCR, Ravindranadh G, et al. Prevention of neuronal damage in brains of chronic stress-induced Male Wistar rats administering Centella asiatica (L) urban. J Anat Soc India. 2024;73(3):204-13.
https://doi.org/10.4103/jasi.jasi_80_24

[24]. Xu M-F, Xiong Y-Y, Liu J-K, Qian J-J, Zhu L, Gao J. Asiatic acid, a pentacyclic triterpene in Centella asiatica, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacol Sin. 2012;33(5):578-87.
https://doi.org/10.1038/aps.2012.3
PMid:22447225 PMCid:PMC4010358

[25]. Rather A, Justin-Thenmozhi M, Manivasagam A, Saravanababu T, Guillemin C, Essa GJ. Asiatic acid attenuated aluminum chloride-induced tau pathology, oxidative stress and apoptosis via AKT/GSK-3â signaling pathway in wistar rats. Neurotoxicity research. 2019; 35:955-68.
https://doi.org/10.1007/s12640-019-9999-2
PMid:30671870

[26]. Insausti, R., Muñoz-López, M., & Insausti, A. M. The CA2 hippocampal subfield in humans: A review. Hippocampus.2023;33(6), 712-729.
https://doi.org/10.1002/hipo.23547
PMid:37204159

[27]. Ding, L., Chen, H., Diamantaki, M., Coletta, S., Preston-Ferrer, P., & Burgalossi, A. Structural Correlates of CA2 and CA3 Pyramidal Cell Activity in Freely Moving Mice. The Journal of neuroscience: the official journal of the Society for Neuroscience.2020;40(30):5797-5806.
https://doi.org/10.1523/JNEUROSCI.0099-20.2020
PMid:32554511 PMCid:PMC7380973

[28]. Lehr, A. B., Kumar, A., Tetzlaff, C., Hafting, T., Fyhn, M., & Stöber, T. M. CA2 beyond social memory: Evidence for a fundamental role in hippocampal information processing. Neuroscience and biobehavioral reviews.2021;126, 398-412.
https://doi.org/10.1016/j.neubiorev.2021.03.020
PMid:33775693

[29]. Piskorowski, R. A., & Chevaleyre, V. Hippocampal area CA2: interneuron disfunction during pathological states. Frontiers in neural circuits.2023;17:1181032.
https://doi.org/10.3389/fncir.2023.1181032
PMid:37180763 PMCid:PMC10174260

[30]. Piskorowski, R. A., Nasrallah, K., Diamantopoulou, A., Mukai, J., Hassan, S. I., Siegelbaum, S. A., Gogos, J. A., & Chevaleyre, V. Age-Dependent Specific Changes in Area CA2 of the Hippocampus and Social Memory Deficit in a Mouse Model of the 22q11.2 Deletion Syndrome. Neuron. 2016;89(1), 163-176.
https://doi.org/10.1016/j.neuron.2015.11.036
PMid:26748091 PMCid:PMC4706988

[31]. Wilson, D. M., 3rd, Cookson, M. R., Van Den Bosch, L., Zetterberg, H., Holtzman, D. M., & Dewachter, I. Hallmarks of neurodegenerative diseases. Cell.2023; 186(4), 693-714.
https://doi.org/10.1016/j.cell.2022.12.032
PMid:36803602

[32]. Sheppard, O., & Coleman, M. Alzheimer's Disease: Etiology, Neuropathology and Pathogenesis. In X. Huang (Ed.), Alzheimer's Disease: Drug Discovery. Exon Publications.2020;1-22.
https://doi.org/10.36255/exonpublications.alzheimersdisease.2020.ch1
PMCid:PMC6889002

[33]. Zhang, H., Wang, X., Xu, P. et al. Tolfenamic acid inhibits GSK-3â and PP2A mediated tau hyperphosphorylation in Alzheimer's disease models. J Physiol Sci.2020;70:29.
https://doi.org/10.1186/s12576-020-00757-y
PMid:32517647 PMCid:PMC10717460

[34]. Farhani, N. I. B. R., Chiroma, S. M., Mohamad, T. A. S. T., & Mohd Moklas, M. A. Centella asiatica L. Urban protects against cognitive dysfunction in alluminum chloride-induced neurotoxicity in rats via inhibition of acetylcholinesterase level. Egyptian Journal of Basic and Applied Sciences.2022;10(1):33-44.
https://doi.org/10.1080/2314808X.2022.2139114

[35]. Wong, J. H., Muthuraju, S., et. al. Differential expression of entorhinal cortex and hippocampal subfields á-amino-3-hydroxy-5-methyl- 4 isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors enhanced learning and memory of rats following administration of Centella asiatica. Biomedicine & pharmacotherapy. Biomedecine & pharmaco-therapie2019;110:168-180.
https://doi.org/10.1016/j.biopha.2018.11.044
PMid:30469081

[36]. Di Benedetto, G., Burgaletto, C., Bellanca, C. M., Munafò, A., Bernardini, R., & Cantarella, G. Role of Microglia and Astrocytes in Alzheimer's Disease: From Neuroinflammation to Ca2+ Homeostasis Dysregulation. Cells.2022;11(17):2728.
https://doi.org/10.3390/cells11172728
PMid:36078138 PMCid:PMC9454513

[37].Zhang, W., Xiao, D., Mao, Q. et al. Role of neuroinflammation in neurodegeneration development. Sig Transduct Target Ther.2023;8:267.
https://doi.org/10.1038/s41392-023-01486-5
PMid:37433768 PMCid:PMC10336149

[38]. Yang, K.; Liu, Y.; Zhang, M. The Diverse Roles of Reactive Astrocytes in the Pathogenesis of Amyotrophic Lateral Sclerosis. Brain Sci. 2024;14:158. https://doi.org/10.3390/brainsci14020158 PMid:38391732 PMCid:PMC10886687

[39]. Wong, J. H., Barron, A. M., & Abdullah, J. M. Mitoprotective Effects of Centella asiatica (L.) Urb.: Anti-Inflammatory and Neuroprotective Opportunities in Neurodegenerative Disease. Frontiers in pharmacology.2021;12:687935. https://doi.org/10.3389/fphar.2021.687935 PMid:34267660 PMCid:PMC8275827

[40]. Sun, S., Gu, Y., Wang, J., Chen, C., Han, S., & Che, H. Effects of Fatty Acid Oxidation and Its Regulation on Dendritic Cell-Mediated Immune Responses in Allergies: An Immunometabolism Perspective. Journal of immunology research, 2021:7483865. https://doi.org/10.1155/2021/7483865 PMid:34423053 PMCid:PMC8376428

[41]. Wculek, S. K., Heras-Murillo, I., Mastrangelo, A., Mañanes, D., Galán, M., Miguel, V., Curtabbi, A., Barbas, C., Chandel, N. S., Enríquez, J. A., Lamas, S., & Sancho, D. Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis. Immunity, 2023;56(3), 516-530.e9. https://doi.org/10.1016/j.immuni.2023.01.011 PMid:36738738

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