NeuroLife

Life Neuroscience, 2023; 1(1): 9-17

Original Article

Evaluation of the neuroprotective activity of citral nanoemulsion on Alzheimer's disease-type dementia in a preclinical model: The assessment of cognitive and neurobiochemical responses

Shreya Singh Beniwal[1], Indranath Chatterjee[2], Namra Vinay Gohil[3], Fatemeh Ghalami[4], Dionisio Lorenzo Lorenzo-Villegas[5], Nasrollah Moradikor[4*]

[1]Lady Hardinge Medical College, Connaught Place, New Delhi, India
[2]Department of Computer Engineering, Tongmyong University, Busan, South Korea
[3]Medical College Baroda and SSG Hospital Vadodara, Gujarat, India
[4]Brain Aging and Dementia Group, International Center for Neuroscience Research, Tbilisi, Georgia
[5]University Fernando Pessoa-Canarias, Santa Maria De Guia, Spain

*Correspondence:
moradikor.nasroallah@yahoo.com

Received: April 25, 2023
Accepted: July 10, 2023
Published: July 20, 2023

10.58209/neurolife.2023.09

Checked for Plagiarism: Yes
Peer reviewers approved by:
Dr. Melika Andrew
Language Editor: Dr. Hadi Hamidi
Editor who approved publication:
Prof. Dr. Nanuli Doreulee

Abstract

The current pharmacological treatments for Alzheimer's disease and dementia have their limitations. Therefore, exploring new and safe agents for these conditions is crucial. Medicinal plants and their derivatives have been considered for the treatment of various diseases, including Alzheimer's disease. In this preliminary study, we sought to assess the effects of citral on Alzheimer's disease and dementia by evaluating both biochemical and behavioral responses in an animal model. The rats were divided into different groups: non-Alzheimer rats without surgery (control group), Alzheimer rats administered with 0 (AD), 1 (Citral-1), and 2 mg/kg (Citral-2) of citral, and 1 (N-Citral-1) and 2 mg/kg (N-Citral-2) of citral nanoemulsion. Behavioral responses were observed, and concentrations of brain-derived neurotrophic factor (BDNF), malondialdehyde (MDA), and ferric-reducing ability of plasma (FRAP) were measured. The results revealed that dementia led to increased anxiety and depressive responses and reduced concentrations of BDNF and FRAP compared to the control group. However, the administration of citral in a dose-dependent manner, coated with emulsion, mitigated anxiety and depressive responses and increased concentrations of BDNF and FRAP compared to the control group. Based on these findings, it can be concluded that citral coated with emulsion shows promise in the treatment of dementia by modulating essential biochemical factors. Further clinical studies are warranted to explore its potential for managing anxiety and depression in dementia patients. This research opens up new possibilities for alternative and effective treatments for these neurodegenerative conditions.

KEYWORDS: Antioxidants, Anxiety, BDNF, Citral, Depression, Rat

[DOI: 10.58209/neurolife.2023.09]

1. Introduction

Alzheimer's disease dementia is the most prevalent form of dementia, accounting for 60-70% of all cases. This progressive neurodegenerative disease leads to memory loss, cognitive deficits, and behavioral changes [1]. Dementia is a condition characterized by gradual memory and thinking problems, and it can be caused by various conditions, including Alzheimer's disease [2]. The early symptoms of Alzheimer's disease include behavioral and personality changes and difficulties with language [3]. These behavioral symptoms may resemble those seen in other psychiatric disorders [4]. Anxiety has been found to have a close association with cognitive decline and dementia [5]. Additionally, peripheral serum brain-derived neurotrophic factor (BDNF) levels have been linked to the later stages of the dementia spectrum [6]. Alzheimer's disease also reduces the concentration of antioxidant factors in patients [7]. Hence, it is crucial to find safe and effective agents for treating Alzheimer's disease dementia. Conventional pharmacological treatments often have limited efficacy, necessitating the development of new therapeutic agents. Medicinal plants and their derivatives have been explored for treating various diseases, including Alzheimer's disease dementia, due to their potential effects on biochemical factors [8-10]. These natural compounds hold promise as alternative treatment options.

Citral is the main product of lemongrass which is mainly found in oils extracted from different plant species such as Lemon myrtle and Listea citrata, etc. It has a strong lemon smell and essence and is applied as an additive in diets [11]. It has antibacterial activities [12] through the production of reactive oxygen species in microorganisms [13]. Citral also exhibited antioxidant activity in DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging tests [14]. It also increased the expression of BDNF [15]. Citral is an unstable and hydrophobic compound under normal storage conditions, so it can easily be degraded and lose its pharmaceutical properties. Nanoemulsion technology is an excellent method to hydrophilize, microencapsulate, and protect the compound [16]. Nanoemulsion protects citral from degradation and also helps to increase its delivery [17]. In sum, citral has antioxidant activity and can have roles in increasing BDNF which can be profitable for the treatment of Alzheimer's disease dementia. However, degradation limitation can be decreased by coating with nanoemulsions. Since no study was found to evaluate the effects of citral on Alzheimer's disease dementia, this preliminary study aimed to evaluate the effects of citral on Alzheimer's disease dementia by evaluating the biochemical and behavioral responses in an animal model.

2. Materials and Methods

2.1. Materials

Citral (97% Cis+Trans) was prepared by Sigma Aldrich Company (Madrid, Spain). Citral nanoemulsion was produced based on previous studies [18].

2.2. Animals

This study followed the recommendations and ethical guidelines provided by the Ethics Committee of the International Center for Neuroscience Research. To begin, seventy-two male rats weighing 183 ± 13 g were acquired and divided into six groups, each consisting of 12 rats. The groups were categorized as follows: first, the non-Alzheimer rats without surgery formed the control group. Then, the Alzheimer rats were further subdivided into groups receiving different doses of citral and citral nanoemulsion. The doses administered were 0 mg/kg for the AD group, 1 mg/kg for the Citral-1 group, and 2 mg/kg for the Citral-2 group. Additionally, the Alzheimer rats were also given 1 mg/kg and 2 mg/kg of citral nanoemulsion, forming the N-Citral-1 and N-Citral-2 groups, respectively.

[DOI: 10.58209/neurolife.2023.09]

Before proceeding with the study, a dose-finding phase was conducted. The rats were exposed to varying doses of citral and its nanoemulsion, ranging from 1 to 5 mg/kg, over two weeks. The researchers closely monitored the rats for any signs of toxicity, such as diarrhea, vomiting, or mortality. Fortunately, no adverse effects were observed at any of the tested doses, allowing the researchers to confidently select the lowest effective doses for the study. Throughout the entire duration of the experiment, the rats were provided with unrestricted access to food and water, and their living conditions were consistently monitored and controlled to ensure accurate results. Daily, citral and its nanoemulsion were administered orally via gavage to the respective groups for a total of 28 days.

2.3. Surgery and drug administration

We anesthetized the rats by intraperitoneal administration of 90 mg/kg ketamine HCl and 10 mg/kg xylazine. The rats were then fixed in a stereotaxic apparatus (Narishige, Tokyo, Japan), and oligomers Aβ1-42 (1μg/μL in each site) were infused into the hippocampal CA1 area bilaterally at a rate of 1μL/5min with the help of a 10-μL Hamilton syringe connected to an infusion pump as reported by others [19]. Following infusion, the cannula was left in place for additional three minutes to allow the complete diffusion of the drug.

2.4. Behavioral tests

Open-field test (OFT) was conducted as reported by other studies with the help of a dark area (72×72×45 cm) for 20 min [20]. An elevated plus maze (EPM) was utilized to assess anxiety with the help of apparatuses consisting of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm, surrounded by 40-cm high wooden walls), raised 50 cm above the floor [20]. The force swimming test (FST) was evaluated as described by previous studies [21]. Immobility, swimming, and climbing were evaluated.

2.5. The assessment of BDNF and antioxidant-associated factors

The animals were decapitated, the prefrontal cortex was isolated and frozen at -80°C. Tissue was homogenized in cold lysis buffer, and BDNF was assessed by ELISA kits (Hangzhou Eastbiopharm Co., LTP). Malondialdehyde (MDA) and ferric-reducing ability of plasma (FRAP) were also assessed by ELISA kits (Hangzhou Eastbiopharm Co., LTP) as recommended by producer companies.

2.6. Data analysis

The obtained data were analyzed for normality and since the data were normal, the parametric test of ANOVA was used. Duncan's test was used to compare between groups. A $P<0.05$ was considered significant. The data were analyzed by Graph Pad Prism (Version of 6.07).

3. Results

3.1. Anxiety-like behaviors

Figure 1 shows the effects of citral and its nanoemulsion on anxiety-like behavior in rats with dementia. The results showed that the induction of diseases significantly decreased the number of visits, center time, and total distance compared with control rats ($P=0.001$). The results showed that oral administration of citral increased the number of visits, center time, and total distance compared with AD in rats in a dose-dependent manner ($P=0.001$). However, the effects were higher citral coated with nanoemulsion. It can be stated that the number of visits, center time, and total distance were significantly higher in rats treated with 2 mg/kg and 1 mg/kg citral nanoemulsion and 2 mg/kg and 1 mg/kg citral, respectively.

[DOI: 10.58209/neurolife.2023.09]

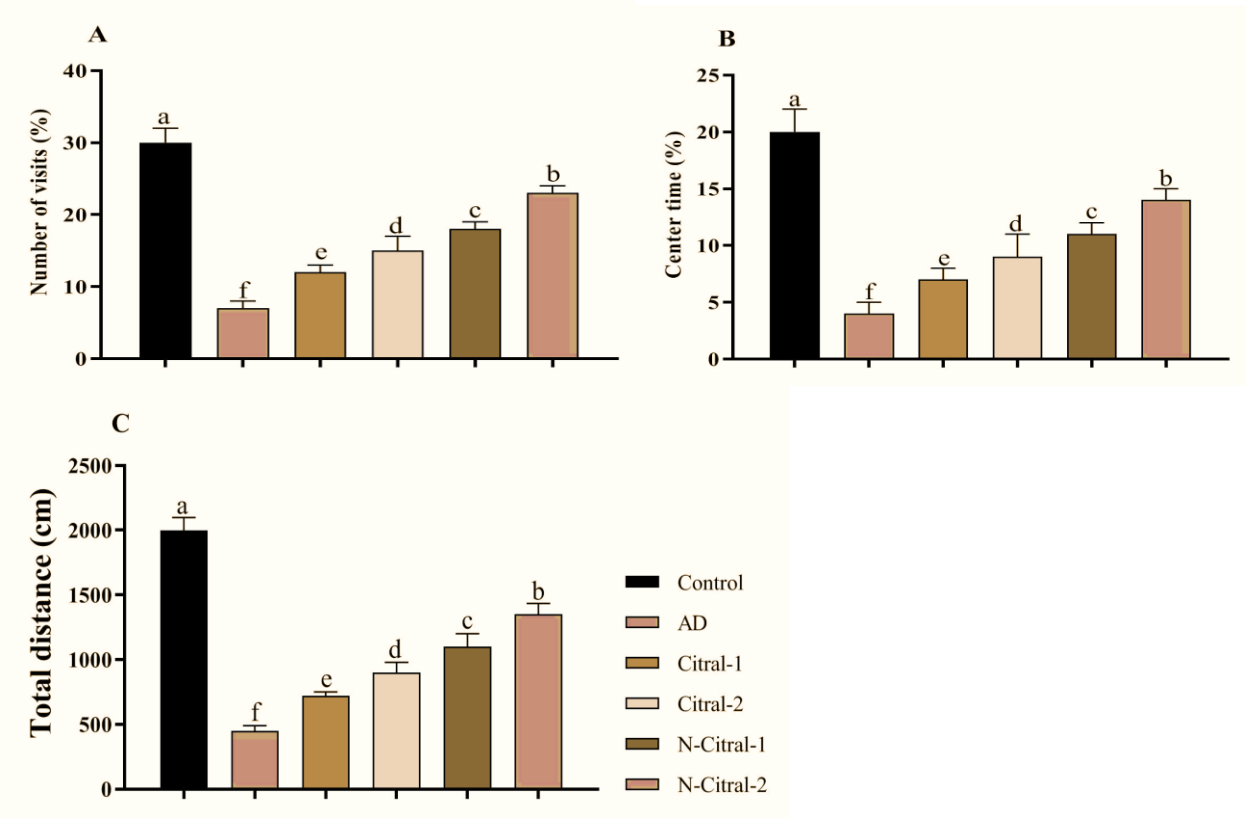

Fig. 1. The effects of citral and its nanoemulsion on anxiety-like behaviors in rats with dementia. Different words (a-f) on figures show significant differences between groups at p<0.05.

3.2. Plus-maze behaviors

Figure 2 depicts the effects of citral and its nanoemulsion on plus-maze behavior in rats with dementia. Our findings showed that induction of diseases significantly decreased the open arm time, and open arm distance compared with control rats (P=0.001). The results showed that oral administration of citral increased the open arm time and open arm distance compared with AD in rats in a dose-dependent manner (P=0.001). However, the effects of citral coated with nanoemulsion were higher. It can be stated that the open arm time and open arm distance were significantly higher in rats treated with 2 mg/kg and 1 mg/kg citral nanoemulsion and 2 mg/kg and 1 mg/kg citral, respectively.

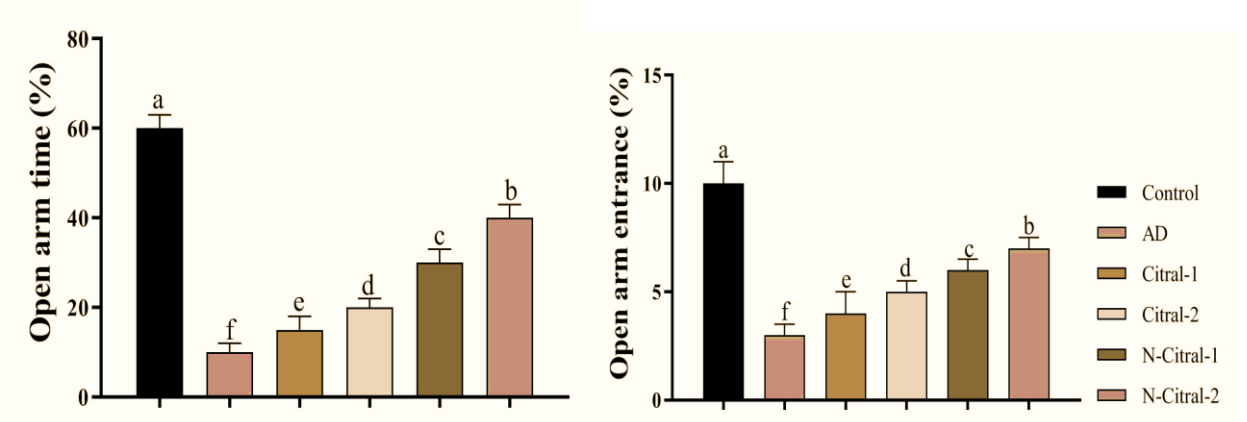

Fig. 2. The effects of citral and its nanoemulsion on plus-maze behavior in rats with dementia. Different words (a-f) on figures show significant differences between groups at p<0.05.

[DOI: 10.58209/neurolife.2023.09]

3.3. Depression-like behavior

Figure 3 illustrates the effects of citral and its nanoemulsion on depression-like behavior in rats with dementia. The results showed that Alzheimer decreased swimming duration while increasing immobility duration (P=0.001). The treatment with citral in higher concentrations and coating with nanoemulsion significantly increased swimming duration while decreasing immobility duration (P=0.001).

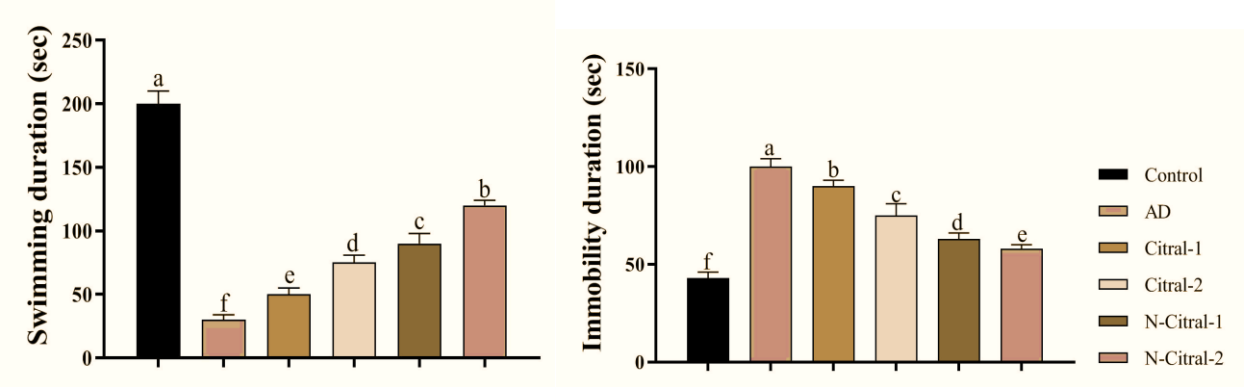

Fig. 3. The effects of citral and its nanoemulsion on depression-like behavior in rats with dementia. Different words (a-f) on figures show significant differences between groups at p<0.05.

3.4. Antioxidant-associated factors

Figure 4 shows the effects of citral and its nanoemulsion on antioxidant-associated factors in rats with dementia. The results showed that Alzheimer's disease decreased BDNF and FRAP while increasing MDA (P=0.001). The treatment with citral in higher concentrations and coating with nanoemulsion significantly increased BDNF and FRAP while decreasing MDA concentration (P=0.001).

4. Discussion

This study was conducted to evaluate the neuroprotective activity of citral and its nanoemulsion on dementia type of Alzheimer's disease in rats by assessing behavioral and neurobiochemical responses. We found that citral in a dose-dependent manner improved anxiety and depression in the rats with Alzheimer's disease and improved biochemical parameters parallel with behavioral responses. The induction of Alzheimer's disease decreased behavioral responses in the animals. A close relationship was reported between Alzheimer's disease and anxiety and depression which are in agreement with other studies [22, 23]. Possible mechanisms for the effects of Alzheimer's disease on anxiety and depression are attributed to an increase in amyloid-β [23].

The oxidation promotes the progression of the disease, anxiety, and depression [24, 25]. The results showed that Alzheimer's disease decreased FRAP and increased MDA which confirms the effects of the disease on the oxidation system. Another mechanism of dementia is via BDNF. It was reported that BDNF concentration is reduced in Alzheimer's disease [26, 27]. The results showed that the disease reduced BDNF concentration which confirms the effects of Alzheimer's disease on BDNF. The mentioned biochemical factors have a close relationship with behavioral responses and can affect responses. The effects of antioxidant properties in decreasing anxiety and depression have been confirmed [28, 29]. The results showed that citral decreased anxiety signs. The results are in agreement with other studies on the effects of plant derivations on anxiety [30-32]. Depression and anxiety are known as clinical illnesses associated with the central nervous system. As mentioned, BDNF and antioxidant factors are involved in behavioral responses. Based on the findings, citral in a dose-dependent manner improved behavioral responses and decreased anxiety and depression in rats. It means that citral works as an anti-depression and anti-anxiety.

[DOI: 10.58209/neurolife.2023.09]

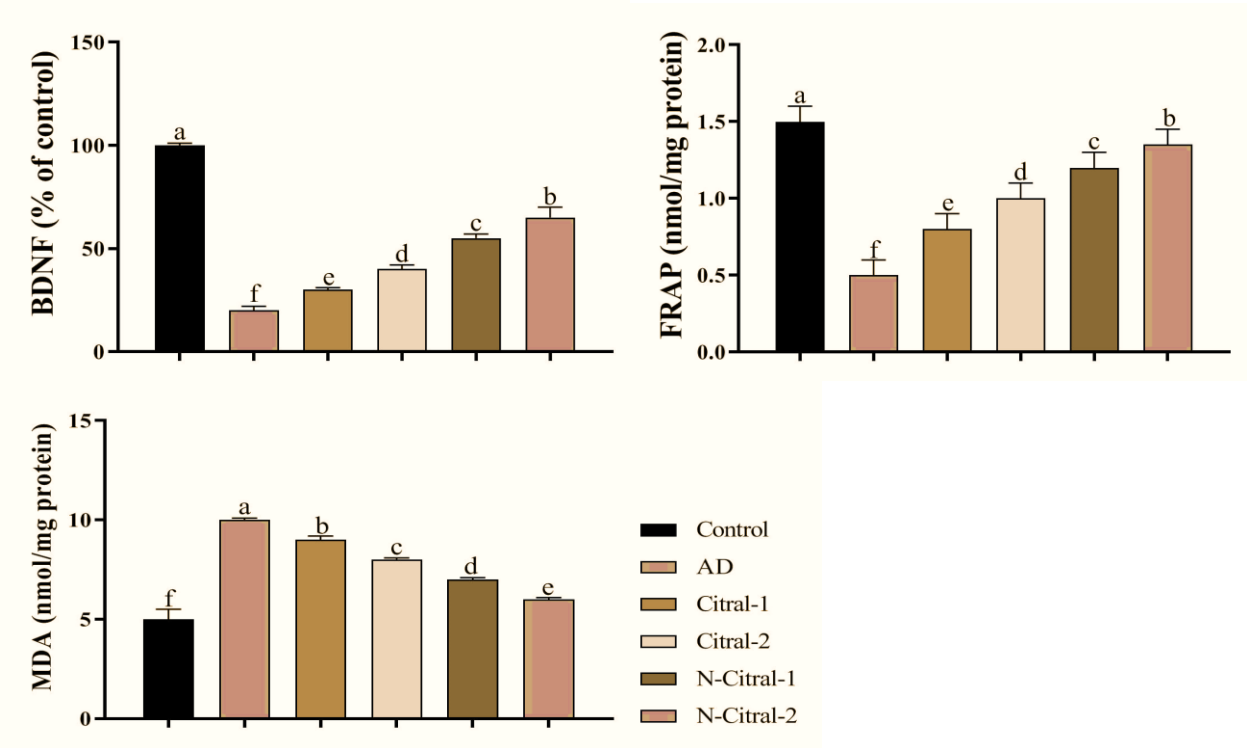

Fig. 4. The effects of citral and its nanoemulsion on BDNF and antioxidant-associated factors in rats with dementia. Different words (a-f) on figures show significant differences between groups at $p<0.05$.

The mechanism of citral is via increasing BDNF and improving antioxidant-oxidant status. The results showed that nano-emulsion coating improved responses compared with a non-coated form which could be attributed to emulsion coating. We believe that emulsion coating protects citral from degradation and causes active compounds to work with the most efficiency. Seemingly, nanoemulsion not only decreases the degradation of citral but also increases the delivery of citral in the targeted organ. Our findings showed that citral prevented the decrease of BDNF in the treated rats. The results are in agreement with other studies on the effects of citral on the expression of BDNF [15]. Seemingly, citral prevents damage in the brain region involved in the synthesis and the expression of BDNF and keeps the expression of BDNF. The results are in agreement with other studies on the antioxidant properties of citral [33, 34]. It can be stated that citral exhibits its antioxidant properties in a dose-dependent manner which helps to decrease symptoms of dementia.

5. Conclusions

This study demonstrated that citral, when administered in a dose-dependent manner and coated with emulsion, effectively reduced anxiety and depression in rats with Alzheimer's disease dementia. The beneficial effects were attributed to improvements in antioxidant status and BDNF levels. The results suggest that citral, when formulated into an emulsion, holds promise as a potential treatment for Alzheimer's disease. However, further clinical studies are necessary to validate its efficacy and safety in human subjects. Considering the complex nature of Alzheimer's disease, citral-based treatment could potentially be utilized in combination with other drugs for more comprehensive therapeutic approaches. The findings of this research contribute valuable insights into the potential use of citral-loaded emulsion as an adjunct therapy for Alzheimer's disease. Continued investigation into this promising compound could pave the way for novel and effective treatment strategies to alleviate the burden of this devastating neurodegenerative disorder.

[DOI: 10.58209/neurolife.2023.09]

Author Contributions: All authors contributed toward data analysis, drafting, and revising the paper and agreed to be responsible for all the aspects of this work. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare that there are no competing interests in this work.

References:

1. Mielke MM. Sex and gender differences in Alzheimer's disease dementia. The Psychiatric times. 2018;35(11):14
2. Kokkinou M, Beishon LC, Smailagic N, Noel-Storr AH, Hyde C, Ukoumunne O, et al. Plasma and cerebrospinal fluid ABeta42 for the differential diagnosis of Alzheimer's disease dementia in participants diagnosed with any dementia subtype in a specialist care setting. Cochrane Database of Systematic Reviews. 2021;(2). https://doi.org/10.1002/14651858.cd010945
3. Rosness TA, Engedal K, Chemali Z. Frontotemporal dementia: an updated clinician's guide. Journal of Geriatric Psychiatry and Neurology. 2016;29(5):271-80. https://doi.org/10.1177/0891988716654986
4. Bang J, Spina S, Miller BL. Frontotemporal dementia. The Lancet. 2015;386(10004):1672-82. https://doi.org/10.1016/s0140-6736(15)00461-4
5. Becker E, Rios CLO, Lahmann C, Ruecker G, Bauer J, Boeker M. Anxiety as a risk factor of Alzheimer's disease and vascular dementia. The British Journal of Psychiatry. 2018;213(5):654-60. https://doi.org/10.1192/bjp.2018.173
6. Ng TKS, Ho CSH, Tam WWS, Kua EH, Ho RC-M. Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): a systematic review and meta-analysis. International journal of molecular sciences. 2019;20(2):257. https://doi.org/10.3390/ijms20020257
7. Amato A, Terzo S, Mulè F. Natural compounds as beneficial antioxidant agents in neurodegenerative disorders: A focus on Alzheimer's disease. Antioxidants. 2019;8(12):608. https://doi.org/10.3390/antiox8120608
8. Uddin M, Al Mamun A, Kabir M, Jakaria M, Mathew B, Barreto GE, et al. Nootropic and anti-Alzheimer's actions of medicinal plants: molecular insight into therapeutic potential to alleviate Alzheimer's neuropathology. Molecular neurobiology. 2019;56(7):4925-44. https://doi.org/10.1007/s12035-018-1420-2
9. Vyas S, Kothari S, Kachhwaha S. Nootropic medicinal plants: Therapeutic alternatives for Alzheimer's disease. Journal of Herbal Medicine. 2019;17:100291. https://doi.org/10.1016/j.hermed.2019.100291
10. Gul R, Jan H, Lalay G, Andleeb A, Usman H, Zainab R, et al. Medicinal Plants and Biogenic Metal Oxide Nanoparticles: A Paradigm Shift to Treat Alzheimer's Disease. Coatings. 2021;11(6):717. https://doi.org/10.3390/coatings11060717
11. Idrees M, Hakkim FL, Naikoo GA, Ul Hassan I. Recent advances in extraction, characterization, and potential use of citral. Natural Bio-active Compounds. 2019:225-36. https://doi.org/10.1007/978-981-13-7438-8_9
12. Cao Y, Zhou D, Zhang X, Xiao X, Yu Y, Li X. Synergistic effect of citral and carvacrol and their combination with mild heat against Cronobacter sakazakii CICC 21544 in reconstituted infant formula. LWT. 2021;138:110617. https://doi.org/10.1016/j.lwt.2020.110617
13. Zheng S, Jing G, Wang X, Ouyang Q, Jia L, Tao N. Citral exerts its antifungal activity against Penicillium digitatum by affecting the mitochondrial morphology and function. Food chemistry. 2015;178:76-81. https://doi.org/10.1016/j.foodchem.2015.01.077

[DOI: 10.58209/neurolife.2023.09]

14. Habib S, Gupta P, Bhat SS, Gupta J. In silico, in-vitro and in vivo screening of biological activities of citral. International Journal for Vitamin and Nutrition Research. 2020. https://doi.org/10.1024/0300-9831/a000625
15. Charret TS, Pereira MT, Pascoal VD, Lopes-Cendes I, Cristina Rheder Fagundes Pascoal A. Citral Effects on the Expression Profile of Brain-Derived Neurotrophic Factor and Inflammatory Cytokines in Status Epilepticus-Induced Rats Using the Lithium–Pilocarpine Model. Journal of Medicinal Food. 2021;24(9):916-24. https://doi.org/10.1089/jmf.2020.0073
16. Lu W-C, Huang D-W, Wang C-C, Yeh C-H, Tsai J-C, Huang Y-T, et al. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. Journal of food and drug analysis. 2018;26(1):82-9. https://doi.org/10.1016/j.jfda.2016.12.018
17. Luciano WA, Pimentel TC, Bezerril FF, Barão CE, Marcolino VA, Carvalho RdSF, et al. Effect of citral nanoemulsion on the inactivation of Listeria monocytogenes and sensory properties of fresh-cut melon and papaya during storage. International Journal of Food Microbiology. 2023;384:109959. https://doi.org/10.1016/j.ijfoodmicro.2022.109959
18. Prakash A, Baskaran R, Vadivel V. Citral nanoemulsion incorporated edible coating to extend the shelf life of fresh cut pineapples. Lwt. 2020;118:108851. https://doi.org/10.1016/j.lwt.2019.108851
19. Hajipour S, Sarkaki A, Farbood Y, Eidi A, Mortazavi P, Valizadeh Z. Effect of gallic acid on dementia type of Alzheimer disease in rats: electrophysiological and histological studies. Basic and clinical neuroscience. 2016;7(2):97. https://doi.org/10.15412/j.bcn.03070203
20. Moradi-Kor N, Dadkhah M, Ghanbari A, Rashidipour H, Bandegi AR, Barati M, et al. Protective effects of spirulina platensis, voluntary exercise and environmental interventions against adolescent stress-induced anxiety and depressive-like symptoms, oxidative stress and alterations of BDNF and 5HT-3 receptors of the prefrontal cortex in female rats. Neuropsychiatric Disease and Treatment. 2020;16:1777. https://doi.org/10.2147/ndt.s247599
21. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology. 1988;94(2):147-60. https://doi.org/10.1007/bf00176837
22. García-Alberca JM, Lara JP, Berthier ML. Anxiety and depression in caregivers are associated with patient and caregiver characteristics in Alzheimer's disease. The International Journal of Psychiatry in Medicine. 2011;41(1):57-69. https://doi.org/10.2190/pm.41.1.f
23. Pietrzak RH, Lim YY, Neumeister A, Ames D, Ellis KA, Harrington K, et al. Amyloid-β, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. JAMA psychiatry. 2015;72(3):284-91. https://doi.org/10.1001/jamapsychiatry.2014.2476
24. Salim S, Chugh G, Asghar M. Inflammation in anxiety. Advances in protein chemistry and structural biology. 2012;88:1-25. https://doi.org/10.1016/b978-0-12-398314-5.00001-5
25. Lopresti AL, Maker GL, Hood SD, Drummond PD. A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2014;48:102-11. https://doi.org/10.1016/j.pnpbp.2013.09.017
26. Amidfar M, de Oliveira J, Kucharska E, Budni J, Kim Y-K. The role of CREB and BDNF in neurobiology and treatment of Alzheimer's disease. Life sciences. 2020;257:118020. https://doi.org/10.1016/j.lfs.2020.118020
27. Gao L, Zhang Y, Sterling K, Song W. Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. Translational Neurodegeneration. 2022;11(1):1-34. https://doi.org/10.1186/s40035-022-00279-0
28. Habtemariam S. Antioxidant and anti-inflammatory mechanisms of neuroprotection by ursolic acid: Addressing brain injury, cerebral ischemia, cognition deficit, anxiety, and depression. Oxidative medicine and cellular longevity. 2019;2019. https://doi.org/10.1155/2019/8512048

[DOI: 10.58209/neurolife.2023.09]

29. Manosso LM, Camargo A, Dafre AL, Rodrigues ALS. Vitamin E for the management of major depressive disorder: possible role of the anti-inflammatory and antioxidant systems. Nutritional Neuroscience. 2022;25(6):1310-24. https://doi.org/10.1080/1028415x.2020.1853417
30. Hosein Farzaei M, Bahramsoltani R, Rahimi R, Abbasabadi F, Abdollahi M. A systematic review of plant-derived natural compounds for anxiety disorders. Current topics in medicinal chemistry. 2016;16(17):1924-42. https://doi.org/10.2174/1568026616666160204121039
31. Fajemiroye JO, da Silva DM, de Oliveira DR, Costa EA. Treatment of anxiety and depression: medicinal plants in retrospect. Fundamental & clinical pharmacology. 2016;30(3):198-215. https://doi.org/10.1111/fcp.12186
32. Kenda M, Kočevar Glavač N, Nagy M, Sollner Dolenc M. Medicinal plants used for anxiety, depression, or stress treatment: An update. Molecules. 2022;27(18):6021. https://doi.org/10.3390/molecules27186021
33. Ali RA, Abdel-Tawab HS, Mostafa DEF. Nigella sativa Mitigative Antioxidant Properties against Citral Induced Oxidative Stress and Biochemical changes during Development of Chick Embryo.
34. Liu C, Li Q, Shan Y, Du C, Chen S, Yin W, et al. Antioxidant-Mediated Modification of Citral and Its Control Effect on Mildewy Bamboo. Polymers. 2022;14(21):4652. https://doi.org/10.3390/polym14214652

[DOI: 10.58209/neurolife.2023.09]