Title: Short-term Efficacy of Commercial Curcumin Nanomicelle on Cognition, Oxidative Stress and Choline Esterase in AD's Disease

Running Title: Curcumin Nanomicelle in Alzheimer's Disease

Authors: Samira Kazemi[1], Narges Hesni Langroudi[1], Peyman Hasani Abharian[2], Kamran Tavakol[3], Mohammad Taghi Joghataei[4,5], Shima Tavakol[4,6]*, Sepide Arbabi Bidgoli[1,]*

1. *Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Islamic Azad University, Tehran, Iran.*
2. *Institute for Cognitive Science Studies, Department of Cognitive Rehabilitation, Brain and Cognition Clinic, Tehran, Iran.*
3. *College of Medicine, Howard University, Washington DC, USA.*
4. *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.*
5. *Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
6. *Department of Research and Development, Tavakol BioMimetic Technologies Co, Tehran, Iran.*

***Corresponding Author**: Shima Tavakol, Sepide Arbabi Bidgoli, Cellular and Molecular Research Center, Iran University of Medical Sciences, Department of Research and Development, Tavakol BioMimetic Technologies Co, Tehran, Iran.; Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Islamic Azad University, Tehran, Iran. Email: Shima.tavakol@yahoo.com; arbabi@iaups.ac.ir

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease accompanied by cognitive dysfunction. Preclinical changes can precede the onset of clinical symptoms by a decade, highlighting the need for preventative and therapeutic strategies to mitigate or delay disease progression This pilot clinical trial, for the first time, investigated the effects of commercially available curcumin nanomicelles on oxidative stress pathways and serum cholinesterase levels in patients with AD. Fifteen volunteers with mild to severe AD and fifteen age-matched healthy controls were enrolled. Participants with AD received 80 mg of thermodynamically stable 10 nm curcumin nanomicelles on alternate days for two months. cognitive function, as assessed by the Mini-Mental State Examination (MMSE) , did not exhibit significant changes in AD patients following curcumin nanomicelle administration (19.8 versus 20.6). Serum levels of oxidative stress biomarkers, including catalase, superoxide dismutase (SOD) inhibition, malondialdehyde (MDA) concentration, and cholinesterase activity, were evaluated before and after intervention. The results showed no significant differences between the cognition improvement, catalase activity, SOD inhibition, MOD concentration, and cholinesterase activity between AD patients and healthy controls, or before and after curcumin nanomicelle administration. It might be concluded that although curcumin nanocarriers did not enhance antioxidant biomolecules, they did not provoke lipid peroxidation mechanisms. Therefore, the study suggests that optimization of nanocarrier parameters, including concentration, particle size larger than 10 nm, and blood-brain barrier targeting, warrants further investigation in a long-term study to explore their potential as a supplemental therapy for AD.

Keywords: Alzheimer’s disease; Cognitive function; Nano- corcumin; Oxidative stress; Clinical trial

Highlight

- Commercial curcumin nanomicelles did not change the score of MMSE questionnaire
- Commercial curcumin nanomicelles did not provoke lipid peroxidation mechanisms
- Commercial curcumin nanomicelles did not enhance the oxidative stress biomarkers, and cholinesterase activity in AD
- There was no difference between the level of oxidative stress biomarkers, and cholinesterase activity in healthy and AD
- Optimal concentration and particle size larger than 10 nm maybe improve the outcomes

1. Introduction

Dementia encompasses a constellation of clinical symptoms, including impairments in memory, problem-solving, cognitive abilities, communication, eating, and mobility. Alzheimer's disease (AD) constitutes a significant proportion of dementia cases, accounting for an estimated 60-780% of all dementia diagnoses diagnosis (Silva et al., 2019). Epidemiological projections estimate that 6.2 million individuals will be affected by AD in the US by 2021 (Association, 2010). Furthermore, AD is considered the sixth leading cause of mortality in the US. In Iran, the mortality rate attributed to AD was 25.34 per 100,000 individuals in 2019 (Balooch Hasankhani, Naziri, & Roudbari). The annual cost of care for AD patients in the US was estimated at 15.3 million hours and $256.7 billion in 2020, increasing to $355 billion in 2021 (Wiley, 2021). It is noteworthy that the prevalence of AD in India is significantly lower (4.4 times) than in the US, potentially attributable to the frequent consumption of spices, such as turmeric, in the Indian diet (Ege, 2021). In Iran, an estimated 2-3% of the population aged 67-78 years' experience AD, with healthcare costs for these individuals exceeding those of the general population by a factor of three (Navipour, Neamatshahi, Barabadi, Neamatshahi, & Keykhosravi, 2019). In other words, specifically, the cost of AD healthcare in Iran ranges from 434 to 2480 USD, depending on the stage of the disease (Aajami, Kebriaeezadeh, & Nikfar, 2019). It is noteworthy that the Age-Standardized Incidence Rate (ASIR) for AD is decreasing in men, while the Age-Standardized Mortality Rate (ASMR) is increasing in women in Iran (Balooch Hasankhani et al.).

AD is a progressive neurodegenerative disorder characterized by selective neuronal loss and cognitive decline. Notably, degenerative physiological changes can commence several decades prior to the manifestation of clinical symptoms (Wiley, 2021). Consequently, preventative strategies aimed at mitigating or delaying the onset of AD symptoms are of significant interest. The molecular hallmark of AD is the accumulation of tau protein tangles within neurons and amyloid-beta plaques in the extracellular space of the brain, leading to neuronal death (Wiley, 2021). While reactive oxygen and nitrogen species are essential for normal cellular function at basal levels, aberrant changes in their levels can contribute to cellular and neural damage, as observed in AD. For instance, reactive lipid species (RLS) generated by lipid peroxidation and peroxynitrite (ONOO-) accumulate in the brains of AD

patients, contributing to neuronal damage. Malondialdehyde (MDA), a product of polyunsaturated fatty acid peroxidation, serves as another marker of oxidative stress in AD patients (Tavakol et al., 2022).

Cells employ a range of biomolecules, including catalase and superoxide dismutase (SOD), to counteract the detrimental effects of oxidative stress. Mitochondrial superoxide dismutase (MnSOD) and catalase are responsible for scavenging superoxide radicals and H2O2 (Lee, Giordano, & Zhang, 2012). Consequently, SOD levels are crucial for mitigating oxidative stress in neurons and potentially slowing the progression of Alzheimer's disease (AD). Overexpression of SOD in transgenic mice of AD's model improves spatial memory and catalase and decreases oxidized and amyloid proteins, (Dumont et al., 2009). Beyond oxidative stress homeostasis, the levels of cholinergic proteins such as acetylcholine (ACh), is crucial to transmitting synaptic signals favorably. Degeneration of cholinergic neurons in the basal forebrain and increased acetylcholinesterase (AChE) activity around amyloid plaques contribute to amyloid toxicity and AD progression (Melo, Agostinho, & Oliveira, 2003).

Despite extensive research efforts in neuropharmacology and pharmaceutics, effective treatment for neurodegenerative diseases and disorders remains a significant challenge (Hamidpour et al., 2024; Tavakol et al., 2014; Tavakol et al., 2022; Tavakol et al., 2017; Tavakol, Zahmatkeshan, & Rahvar, 2023). While therapeutic agents are crucial, preventative strategies that delay disease progression are also highly desirable. Curcumin, a polyphenolic compound derived from turmeric root, possesses anti-inflammatory, antioxidant, and anti-amyloid properties (Tavakol, Zare, Hoveizi, Tavakol, & Rezayat, 2019). Both curcumin and its nanoformulation have been explored as potential alternatives to anti-amyloid antibodies for labeling amyloid-beta (Aβ) plaques in imaging studies, offering advantages over conventional dyes such as Congo red and thioflavin-S (Maiti et al., 2016). However, curcumin's clinical application is hindered by several limitations, including rapid metabolism, poor solubility and bioavailability, sensitivity to light, metal ions, alkaline conditions and heat, physicochemical instability, and rapid degradation (Flora, Gupta, & Tiwari, 2013; Karthikeyan, Senthil, & Min, 2020).

While a comprehensive search of www.ClinicalTrials.gov reveals a substantial number of registered studies (2759) pertaining to AD, only five specifically investigate the effects of curcumin on AD. Notably, four additional clinical trials evaluating curcumin's impact on AD have been reported in the

literature but are not currently registered on ClinicalTrials.gov (Brondino et al., 2014). To the best of our knowledge, to date, only one clinical trial has been identified that evaluates the efficacy of nano-curcumin in AD patients, utilizing solid lipid nanoparticles (NCT01001637). Despite promising preclinical findings in animal models (Chen et al., 2018), a long-term, 6-month study involving curcumin consumption in AD patients did not demonstrate significant improvements in clinical symptoms (Baum et al., 2008). This lack of efficacy is attributed, in part, to curcumin's poor water solubility, physicochemical instability, and high Log P value of curcumin, resulting in limited bioavailability.

To overcome these limitations and enhance curcumin's water solubility and bioavailability, encapsulation into nanocarriers, such as nanomicelles, has emerged as a promising strategy. Several studies have investigated the efficacy of nano-curcumin in AD. For instance, PEGylated curcumin nanoparticles of polylactic acid (PLA) have been shown to inhibit the production and depolarization of Aβ plaque fibers in PC12 cells (Yang, Sun, Huang, Zhu, & Li, 2021). Moreover, encapsulation of curcumin in poly-lactide-co-glycolide (PLGA)-selenium nanoparticles has demonstrated a reduction in Aβ load and memory deficits in a mouse model of AD (Huo, Zhang, Jin, Li, & Zhang, 2019). Furthermore, encapsulation of curcumin into PLGA nanoparticles exhibits an anti-amyloid effect through the Wnt/β-catenin pathway in rat models of AD (Tiwari et al., 2014).

This study employed commercially available curcumin nanomicelles, characterized and subsequently administered to Alzheimer's disease (AD) patients. The investigation focused on assessing the impact of curcumin nanomicelles on cholinesterase activity. Given curcumin's established role in mitigating oxidative stress within cells, the study further examined the effects of curcumin nanomicelles on enzymes implicated in oxidative stress pathways, including superoxide dismutase (SOD), catalase, and malondialdehyde (MDA). d.

2. Materials and Methods

2.1. General Experimental Procedures

Nano curcumin (Sina curcumin) from Exir Nano Sina Company (Iran, Tehran), catalase, Malondialdehyde (MDA) and Superoxide dismutase (SOD) kit were purchased from ZellBio GmbH (Germany), Choline esterase assay kit was purchased from Pars Azmon kit (Karaj, Tehran, Iran). Dynamic light scattering instrument was from Zeta sizer, Malvern, UK.

2.2 Nanocurcumin characterization: Particle size and zeta potential, entrapment efficacy and drug release studies in water, simulated gastric fluid, and simulated intestinal fluid

Commercial nano curcumin (Sina curcumin) was a gift from Exir Nano Sina Company (Iran, Tehran). Hatamipour et al. characterized the nanomicelles of Sina Curcumin as follows. In brief, the hydrodynamic particle size was determined using dynamic light scattering (Zeta sizer, Malvern, UK) at a 633 nm wavelength under normal and accelerated conditions (Hatamipour, Sahebkar, Alavizadeh, Dorri, & Jaafari, 2019).

The entrapment efficacy of the nanomicelles was determined using the ultrafiltration method. The molecular weight of the centrifugal filter was 12 KD. The diluted nanomicelles with 5% dextrose were added to the centrifugal filter and centrifuged at 4000 × g for 30 min. HPLC determined the amount of free curcuminoid that crossed the filter. The percent entrapment efficacy formula was as follows:

Entrapment efficacy %= (total drug concentration- supernatant drug concentration/total drug concentration) *100

The release profile of curcuminoids from nanomicelles was evaluated in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The pH of SGF and SIF was adjusted to 2.2 and 6.8, respectively. The release profile was studied for 72 h, and the number of released curcuminoids was evaluated using HPLC. All characterization experiments were repeated three times. The mean and SD were reported and analyzed (Hatamipour et al., 2019).

2.3. Clinical Trial Study

2.3.1. Participants

The Ethics Committee approved the controlled clinical trial study of Islamic Azad University (IR.IAU.PS.REC.1396.98). The protocol was explained to all participants, and all participants enrolled in written informed consent. Fifteen subjects with mild to severe probable AD's disease were enrolled at the Neurology Clinic of Firoozgar Hospital (Tehran, Iran). In addition, fifteen healthy subjects were age matched and considered the control group.

2.3.2. Inclusion and exclusion criteria

Inclusion criteria for the intervention group were the male-female age above 65 years old, diagnosed with AD using Mini-Mental State Exam (MMSE) questionnaire and neurologist confirmation, and liver problems, and not being treated by aspirin and anticoagulants. However, inclusion criteria for the control group included in the range of intervention groups and not having AD.

Exclusion criteria for the intervention group included death, discontinuation of treatment for more than a week, instability of patients with curcumin, and allergic reaction to nano curcumin.

2.3.3. Intervention

Patients took medications prescribed by their physician during the study and used nano curcumin as a supplement. AD patients took a Sina Curcumin (80 mg) capsule for eight weeks on even days (three times a week).

2.4. MMSE questionnaire for cognition evaluation consumption and smoke

Age, weigh, and height of patients were studied in both groups. MMSE questionnaire is a relatively easy and quick questionnaire that evaluates cognition. It was performed before and after curcumin administration in AD's disease patients for two months. In brief, the MMSE measures orientation to time and place, registration, attention and calculation, recall, repetition, language (reading and writing), and copying. MMSE scores were evaluated and compared before and after curcumin consumption in AD's patients.

2.5. Blood collection

At the time of enrollment and after the termination of the study, 5 ml of blood was collected from patients to prepare serum. The blood was centrifuged, and serum was isolated and kept at -80 °C until analysis.

2.6. Oxidative stress analysis

2.6.1. Catalase activity

Catalase is an antioxidant heme enzyme in the peroxisome of human tissues. It degrades hydrogen peroxide to water and hydrogen. Therefore, it protects tissues from the disruptive effects of reactive hydroxyl radicals (Goyal & Basak, 2010). Catalase activity was measured using a ZellBio GmbH (Germany) assay Kit. It was performed based on a manual kit. In brief, serum was diluted with assay buffer at a ratio of 1:5. Then, standards were diluted with assay buffer (1:20). Then, 25 µl of serum was mixed with 25 µl of hydrogen peroxide reagent in duplicate wells and incubated at room temperature for 30 min. Then, 25 µl of the substrate and 25 µl of HRP reagent were added to each well and incubated at room temperature for 15 min. The absorbance was read using a microplate reader at 560 nm wavelength. The activity (U/ml) was calculated based on the formula extracted from the curve standard. Analysis of catalase activity was performed in duplicate, and the values provided are the normalized mean ± SD.

2.6.2. Superoxide dismutase (SOD) inhibition

Superoxide is a harmful byproduct of oxygen metabolism in cells. Dismutation of superoxide is catalyzed by SOD to hydrogen peroxide and molecular oxygen (Younus, 2018). A SOD colorimetric assay kit was purchased from ZellBio GmbH (Germany). In brief, standards were prepared based on a manual kit. Ten microliters of serum, 50 µl of the substrate working solution, and 25 µl of xanthine oxidase were added to each well and incubated at room temperature for 20 min. The absorbance was read using a microplate reader at 450 nm wavelength. The concentration was calculated based on the following formula:

SOD inhibition= (mean OD of sample/mean OD of zero standard) *100

Analysis of SOD inhibition was performed in duplicate, and the values provided are the normalized mean ± SD.

2.6.3. Malondialdehyde (MDA) concentration

MDA is a final byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid. Therefore, it is considered an index of oxidative stress (Maurya et al., 2021). An MDA/thiobarbituric acid reactive substances (TBARS) colorimetric assay kit was purchased from ZellBio GmbH company (Germany). Analysis was performed based on a manual kit. In brief, 50 µl of serum, 50 µl of sample diluent, and 50 µl of TBA substrate were added to each well and incubated at 37 °C for one h with shaking. The absorbance was read using a microplate reader at 535 nm wavelength. The concentration (µM) was calculated based on the formula extracted from the curve standard. Analysis of MDA concentration was performed in duplicate, and the values provided are the normalized mean ± SD.

2.6.4. Choline esterase activity

Choline esterase is an enzyme involved in the degradation of choline-based esters, which are critical in neurotransmission (KONDAPALLI & SRUTHI, 2020).. The choline esterase activity (U/l) was determined based on the Butyryl thiocholine technique using the Pars Azmon kit (Karaj, Tehran, Iran). In brief, two reagents, A and B, and serum were added to the wells, and after incubation, the absorbance was read at 405 nm using a microplate reader. Analysis of choline esterase activity was performed in duplicate, and the values provided are the normalized mean ± SD.

Statistical analysis

SPSS software (v23) was applied to analyze the data related to oxidative stress and choline esterase. A paired t-test was used to compare the before and after the intervention and the control and patients. $P<0.05$ was considered statistically significant.

3. Result

3.1. Nanoparticle characterization

Hatamipour et al. characterized the nanomicelles of Sina Curcumin. They reported that the particle size of nanomicelles was 9.5± 0.1 nm and that the particle size did not change during the 24-month incubation period under normal and 6-month accelerated conditions. The percentage of entrapment efficacy was 100± 0% (Hatamipour et al., 2019).

The drug release study in SGF showed an 18% release of curcumin from the micelles by 72 h, while the curcumin release from the nanomicelles was 11% when they were kept in SIF. Moreover, curcumin release from the nanomicelles was 2% by six h in both SGF and SIF (Fig. 1 a-c) (Hatamipour et al., 2019).

3.2. Cognitive function score

The demographic data was shown in Table 1. The MMSE questionnaire was administered to AD's patients to evaluate the effect of curcumin nanomicelles on cognitive impairment. Scores showed that curcumin naomicelles did not significantly change MMSE scores in AD patients (P= 0.730). There was no change in orientation to time (P= 1.00) and place (P= 0.644), registration (P= 0.541), recall (P= 0.756), nomination (P= 1), repetition (P= 1), conception (P= 0.517), reading (P= 1), writing (P= 1), copying (P= 0.726) or attention (P= 0.796) (Table 2).

3.3. Oxidative stress response before and after intervention

3.3.1. Catalase activity

Catalase activity as an oxidative stress index was evaluated in patients before and after the intervention. The results indicated no significant difference in catalase activity of AD patients when they took nanocurcumin (P=0.268). On the other hand, there was no significant difference between the catalase activity in the control and intervention groups by eight weeks posttreatment with nanocurcumin in AD patients (P= 0.745) (Fig. 2a).

3.3.2. SOD inhibition

SOD inhibition as an oxidative stress index showed a nonsignificant difference in SOD inhibition before and after nanocurcumin prescription (*P*= 0.787). Furthermore, there was no significant difference between the SOD inhibition in the control and intervention groups by eight weeks posttreatment with nano curcumin in AD patients (*P*= 0.621) (Fig. 2b).

3.3.3. MDA concentration

As a final byproduct of lipid peroxidation indicated, the results derived from MDA concentration showed no significant difference between the amount of MDA in AD patients when they took nanocurcumin in the short term (*P*= 0.924). However, there was no significant difference between the amount of MDA in the control and intervention groups by eight weeks posttreatment with nanocurcumin in AD patients (*P*= 0.957) (Fig. 2c).

3.3.4. Choline esterase activity

Choline esterase is an enzyme involved in choline degradation, and butyrylcholine esters were evaluated. The data showed no significant difference between choline esterase activity in AD patients before and after the prescription of nanocurcumin (*P*= 0.549). Moreover, there was no significant difference between cholinesterase activity in the healthy control and AD patient groups (*P*= 0.088) (Fig. 3).

4. Discussion

AD is a progressive neurodegenerative disorder typically diagnosed when degenerative physiological changes in the brain have already been underway for approximately three years (Beason-Held et al., 2013). To effectively design and select an ideal therapeutic agent, a thorough understanding of the underlying cellular mechanisms beyond the disease pathology is essential. The brain, consuming approximately 20% of the body's total oxygen, is particularly susceptible to oxidative damage. Catalase and SOD play crucial roles in scavenging oxidative stress byproducts.

Our findings revealed no significant changes in cognitive scores following the administration of curcumin nanomicelles to AD patients. Furthermore, no significant differences were observed in cholinesterase, SOD, or catalase levels after curcumin nanomicelle administration in AD patients. These data suggest that 10 nm curcumin nanomicelles, at a concentration of 80 mg every two days, did not significantly alter the activity of SOD and catalase over a two-month period. Interestingly, the concentration of MDA, a byproduct of lipid peroxidation, remained unchanged. It indicates that curcumin nanomicelles did not induce or exacerbate lipid peroxidation, suggesting their potential safety in terms of extra lipid peroxidation.

Curcumin exhibits not only antioxidant properties but also a high affinity for amyloid-beta (Aβ) plaques, effectively inhibiting plaque aggregation (McClure et al., 2017; Mei, Zhu, Zhou, Li, & Chen, 2020; Yanagisawa et al., 2011). However, the efficacy of these actions hinges on the ability of curcumin to cross the blood-brain barrier (BBB) and maintain its concentration within the target brain regions. Research has demonstrated that NP size, targeting functionalization, and payload drug concentration within the brain are critical factors for efficient drug delivery in AD. As mentioned earlier, in the present study, 10 nm nanocarriers were employed. While studies have indicated that optimal particle size for BBB passage is approximately 40 nm or less than 50 nm, nanocarriers smaller than 10 nm may be filtered by the kidneys, hindering their optimal retention within the brain (Danaei et al., 2018).

The optimal concentration of curcumin within the brain is another critical consideration. Studies have demonstrated that curcumin concentrations of 500 nM and 100 μM in the brain can promote and inhibit neurogenesis, respectively, in animal models of AD (Begum et al., 2008). Therefore, both excessively high and low concentrations of curcumin may prove ineffective. Encapsulation of curcumin within nanocarriers has been shown to significantly enhance its payload in the brain, resulting in notable improvements in cognitive function (90%) and acetylcholine esterase inhibition (52%) (Kakkar, Mishra, Chuttani, & Kaur, 2013). Nanoencapsulation of drugs enhances both the bioavailability and retention time of curcumin in the brain (Tsai, Chien, Lin, & Tsai, 2011). Certain nanocarrier systems, such as curcumin lipid NPs, demonstrate enhanced affinity for Aβ plaques compared to free curcumin, leading to a decline in Aβ aggregation (Maiti, Paladugu, & Dunbar, 2018). To achieve successful delivery of nano-curcumin to the brain, targeted delivery strategies are recommended. Lactoferrin, T807

molecules, insulin, transferrin and integrin receptors, Tet-1 peptide, B6 peptide, odorranalectin, and N-trimethyl chitosan are all potential targeting agents for functionalizing nanocarriers to facilitate BBB crossing (Ege, 2021). For example, functionalization of pegylated PLGA NPs with the B6 peptide (Orlando, Gonzales, Royer, Deck, & Vander Jagt, 2012) or transferrin-Tet-1 has been shown to significantly enhance brain uptake of curcumin compared to their non-targeting counterparts (Ramalingam & Ko, 2015).

Although there are several successful preclinical studies related to the improvement of cognition using curcumin NPs, clinical findings have been controversy regarding the effectiveness of curcumin in AD's patients and healthy subjects, in part owing to structural changes in the BBB or involved signaling pathways in AD's patients, indicating the necessity of targeted delivery to the brain (Goozee et al., 2016; Voulgaropoulou, Van Amelsvoort, Prickaerts, & Vingerhoets, 2019). In a clinical trial study, 1500 mg curcumin (Biocurcumax™) and a placebo were administered to 160 cases without cognitive impairment. Findings by six months indicated that the intervention group did not show a decline in cognition, while the placebo group showed a decline in cognitive function (Rainey-Smith et al., 2016) In other words, although curcumin did not improve cognition, it stabilized cognition for six months as a protective agent (Reddy et al., 2018). Evaluation of cognitive function, working memory and sustained attention, in healthy older people who were administered solid lipids of curcumin (Longvida®, 80 mg/day) showed that on the one hand, four weeks after the consumption of Longvida®, working memory, sustained attention, and mood improved. However, these findings were not significant three h post-treatment (Cox, Pipingas, & Scholey, 2015). Another study on healthy people showed that the administration of curcumin (Theracurmin®, 90 mg/twice a day) for 18 months decreases amyloid and tau accumulation in the brain while improving short-term memory, attention, visual and verbal (Small et al., 2018).

A clinical trial involving individuals with AD demonstrated that administration of curcumin (2-4 g/day) for six months did not result in significant changes in cognitive function, tau levels, or amyloid-beta (Aβ) concentrations in plasma and cerebrospinal fluid (CSF) in patients with mild to moderate AD (Ringman et al., 2012). Similarly, Baum et al. (Baum et al., 2008) reported that administering 1 or 4 g

of curcumin to individuals with progressive cognitive impairment did not significantly alter MMSE scores after six months, despite an observed enhancement in vitamin E levels compared to the placebo group. Furthermore, serum Aβ1-40 levels did not exhibit significant increases. This study represents the first pilot clinical trial to evaluate the efficacy of curcumin nanomicelles on cognitive function and oxidative stress. The findings align with previous research investigating the efficacy of curcumin in AD patients, indicating that no significant differences in MMSE scores. were observed.

5. Conclusion

The aging global population is projected to lead to a significant increase in neurodegenerative diseases, including AD. Projections indicate that the number of individuals affected by dementia and AD will reach 152 million and 106.8 million cases, respectively, by 2050 (Nichols, Feigin, & Collaborators, 2022). Therefore, the identification of preventative and therapeutic agents capable of inhibiting or slowing AD progression remains a critical research priority. Curcumin, with its established antioxidant, anti-inflammatory, and anti-amyloid-beta (Aβ) plaque properties, holds promise as a potential therapeutic agent. However, its poor solubility and bioavailability pose significant challenges. Encapsulation of curcumin within nanocarriers has emerged as a promising strategy to address these limitations. Our study suggests that further optimization of nanocarrier parameters, including particle size and duration of administration, is crucial for achieving clinical efficacy. Specifically, the development of curcumin nanoparticles exceeding 10 nm in size, functionalized with blood-brain barrier (BBB)-targeting biomolecules such as transferrin and Tat-1, warrants further investigation. Additionally, identifying the optimal concentration of curcumin NPs that induce neurogenesis, exhibit antioxidant and anti-Aβ plaque activity, and possess anticholinesterase potential is essential. Although there seems to be a long way to go to optimize curcumin NPs to treat AD in the clinic, it will not be far off. The findings of our study serve as a beacon for future research, highlighting the need to optimize particle size, targeting agents, concentration, and duration of administration of curcumin nanocarriers.

Competing Interests

There are no conflicts to declare.

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N/A

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Authors' contribution

S.K and N. H. L contributed in material preparation, data collection, and analysis, K. T and S. T wrote the paper and provided technical assistance, P. H. A analyzed MMSE questionnaire, M. T.J analyzed data, S. A. B supervised the overall study and advised on study design and data interpretation All authors read and approved the final manuscript."

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Table 1. Demographic data in the control and AD patients' groups

	N=15	Minimum	Maximum	Mean± Sd
Age	AD	65.00	80.00	72.8667± 5.06905
	Control	65.00	92.00	70.666± 8.85330
Weight	AD	47.00	90.00	65.3333±12.99267
	Control	52.00	85.00	70.666± 9.05276
Height	AD	154.00	185.00	165.2000± 8.95385
	Control	157.00	185.00	168.26± 7.62952

Table 2. MMSE scores before and after curcumin nanomicelle consumption in AD patients

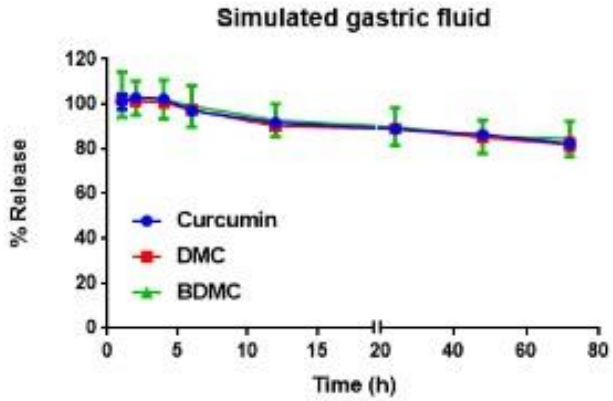
	Mean Before (n=15)	Mean After (n=15)
Orientation to time	3	3
Orientation to place	3.8	4
Registration	2.6	2.8
recall	0.4	0.5
Nomination	2	2
repetition	0.8	0.8
Conception	2.7	2.8
Reading	0.8	0.8
Writing	0.7	0.7
copying	0.4	0.5
Attention	2.3	2.5
Total score	19.8	20.6

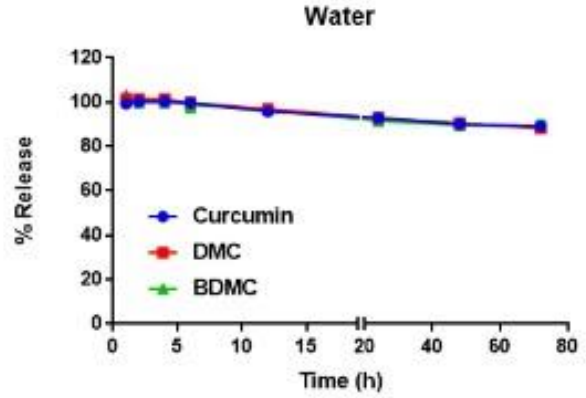
Legend

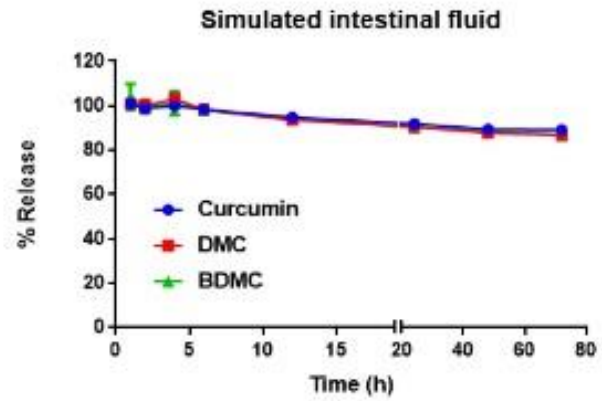
Fig.1 Release profiles of demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and curcumin in simulated gastric fluid (SGF). b) Water. c) Simulated intestinal fluid (SIF) at 0, 1, 2, 4, 6, 12, 24, 48 and 72 h. The figure was reused from (Hatamipour et al., 2019), and permission was received.

Fig. 2 a) Catalase activity in the serum of AD's patients before and after nanocurcumin administration indicating the non-significant difference before and after of the nanocurcumin prescription. In addition, there was no significant difference between the healthy control and patients. b) SOD inhibition in the serum of AD's patients before and after nanocurcumin administration indicating the non-significant difference before and after of the nanocurcumin prescription. In addition, there was no significant difference between the healthy control and patients c) MDA concentration in the serum of AD's patients before and after nanocurcumin administration. Nanocurcumin did not induce a significant difference in SOD, indicating the non-significant difference before and after of the nanocurcumin prescription. In addition, there was no significant difference between the healthy control and patients

Fig.3 Cholinesterase activity in the serum of AD's patients before and after nanocurcumin administration. Nanocurcumin did not induce a significant difference in Cholinesterase activity. In addition, there was no significant difference between the healthy control and patients







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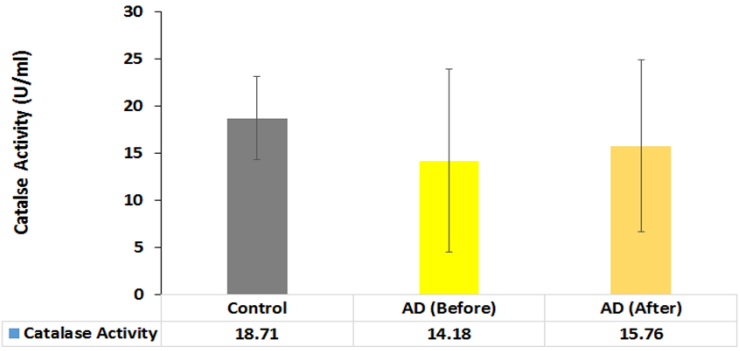

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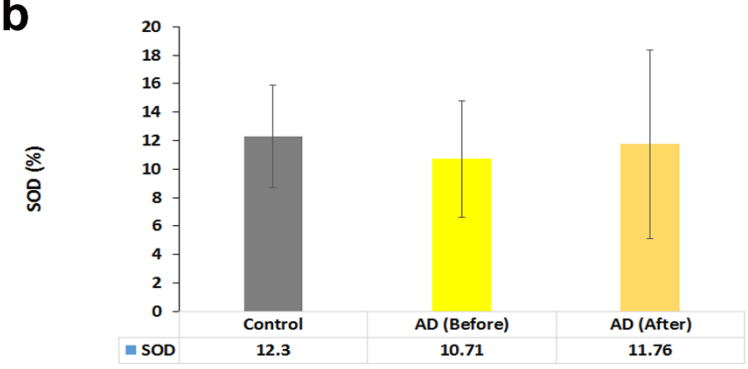

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