ORIGINAL ARTICLE

Effectiveness of The Combination of Turmeric (*Curcuma longa*) and Black Pepper (*Piper nigrum*) Extract on Acetylcholine and Acetylcholinesterase Levels in Dementia Elderly

Lisna Anisa Fitriana[1],Nazhifa Ufamy[2], Rifa'atul Mahmudah[3], Upik Rahmi[1], Slamet Rohaedi[1], I Ketut Adnyana[2]

[1] Program Study of Nursing, Faculty of Sport and Health Education, Universitas Pendidikan Indonesia, Bandung, Indonesia
[2] Program Study of Pharmacology and Clinical Pharmacy, School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia
[3] Magister of Pharmacy, Postgraduate Program, Megarezky University, Makassar, South Sulawesi, Indonesia

ABSTRACT

Introduction: Turmeric (*Curcuma longa*) and black pepper (*Piper nigrum*) can improve memory, where black pepper increases the bioavailability of turmeric in the body. These two plants are widely used as vegetables, cooking spices, and traditional medicine, but their use as therapy for dementia is still not optimally developed. This study aims to analyze the effectiveness of the combination of turmeric and black pepper on plasma acetylcholine levels and acetylcholinesterase activity in the elderly with dementia. **Methods:** The study used a pre-post-experimental design with a control group for 14 weeks. A total of 23 people in the intervention group received turmeric and black pepper extracts (1x300 mg/day), while the control group consisted of 13 people. The assessment of cognitive function was carried out using an MMSE (Mini-Mental State Examination) questionnaire. **Results:** The study showed that there were no significant changes in cognitive function, acetylcholine levels, or acetylcholinesterase activity before and after the combination of turmeric and black pepper ($p > 0.05$). The Mann-Whitney test also showed no significant difference between the intervention and control groups ($p > 0.05$). **Conclusion:** It can be concluded that the combination of turmeric and black pepper at a dose of 1x300 mg/day for 14 weeks is not effective in improving cognitive function, acetylcholine, and acetylcholinesterase in the elderly with dementia. This study suggests increasing the dosage and administering longer to get more optimal results.
Malaysian Journal of Medicine and Health Sciences (2026) 22(SUPP9):265-270. doi:10.47836/mjmhs.22.s9.40

Keywords: *Curcuma longa*, *Piper nigrum*, Acetylcholine, Acetylcholinesterase, Dementia

Corresponding Author:
Lisna Anisa Fitriana, PhD
Email: lisna@upi.edu
Tel : +62 815-7249-9187

INTRODUCTION

Higher levels of life expectancy correlate with decreased cognitive function. In 2015, there were 46.8 million people with dementia worldwide, with projections increasing to 74.7 million in 2030 and 131.5 million in 2050 (1). About 68% of cases occurred in low- and middle-income countries. In Indonesia itself, the number of dementia sufferers is estimated to reach 4 million people by 2030 (2).

Although the prevalence of dementia continues to increase, the therapies currently available are only symptomatic to slow the decline in cognitive function, but the results are still limited. No therapy can completely stop, prevent, or cure the disease (3). Three of the four available drugs, namely donepezil, galantamine, and rivastigmine, belong to the acetylcholinesterase (AChE) inhibitor class. The drug's mechanism of action increases the concentration of acetylcholine (ACh) in synapses by inhibiting ACh's breakdown, thereby helping compensate for the loss of functional neurons in the early to intermediate stages of the disease (4).Increased ACh levels can also prolong cholinergic neurotransmission, which helps to cope with cognitive impairments such as memory and learning impairment due to decreased neurotransmission caused by neurotoxic amyloid plaques and fibrilar kinks (5). Research shows that AChE also plays a role in the aggregation of amyloid beta (Aβ) plaques, making this area a potential target for developing new treatments that manage symptoms and inhibit disease progression. The use of chemical drugs has a significant risk of side effects, so the study turned to herbal ingredients such as turmeric and black pepper, which are known to have beneficial effects on memory.

Turmeric (Curcuma domestica) and black pepper (*Piper nigrum*) are plants that are widely found in tropical regions such as Indonesia. Turmeric, which contains the compound curcumin, has long been used in traditional medicine, especially in Ayurveda, as an anti-inflammatory, antioxidant, and "body cleanser." In recent years, curcumin has been known to have anti-amyloidogenic, anti-inflammatory, antioxidative, and metal-chelating properties, which have the potential to provide neuroprotective effects(6), as well as being an anti-Alzheimer's agent (7). Curcumin works by reducing oxidative stress, inflammation, beta-amyloid aggregation, Tau protein accumulation, and aluminum neurotoxicity, as well as increasing levels of Brain-Derived Neurotrophic Factor (BDNF), thereby helping to improve cognitive and memory impairment(8). Meta-analysis studies also showed that curcumin supplement consumption lowered levels of IL-6, hs-CRP, and MDA, which are associated with inflammation and Alzheimer's conditions (9). Epidemiologically, cognitive function is better in individuals who regularly consume curcumin than those who do not (10).

Black pepper, which contains piperine, also showed a significant effect in improving memory, as evidenced in a study reported that cold-pressed black pepper oil had comparable effectiveness to donepezil in improving the memory of test animals(11). Piperin also shows the ability to inhibit the enzyme acetylcholinesterase and reduce lipid peroxidation, thereby improving memory impairment and preventing neurodegeneration (12-14). The purpose of this study was to evaluate the effectiveness of the combination of turmeric rhizome extract (*Curcuma longa*) and black pepper fruit (*Piper nigrum*) in increasing plasma levels of acetylcholine and decreasing acetylcholinesterase activity in the elderly with dementia.

MATERIALS AND METHODS

Study Setting and Design

This study applied an experimental design with pre and post-test and involved a control group for 14 weeks. This study has received approval from the West Java PPNI STIKEP Ethics Commission with number III/043/KEPK-SLE/STIKEP/PPNI/JABAR/IV/2023.

Sample Size and Sampling Method

The study involved 36 patients with Dementia as subjects. They were divided into two groups: the intervention group and the control group. Twenty-three people in the intervention group were given capsules containing a combination of 100 mg of turmeric rhizome extract and 5 mg of black pepper extract, while 13 others were members of the control group.

The variables measured in this study included plasma acetylcholine and acetylcholinesterase levels, which were analyzed using HPLC tools using the ELISA (Enzyme-Linked Immunosorbent Assay) method. In addition, cognitive function was assessed using an MMSE (Mini-Mental State Examination) questionnaire with a cut-off score of <24 to identify Dementia.

Statistical Analysis

The data normality test was carried out using the Shapiro-Wilk method to determine whether the average distribution of sample data was normal or not. The homogeneity of the data was tested using Levene's test, followed by the Chi-square and Mann-Whitney tests. To evaluate the change in the effect of the combination of turmeric and black pepper from the beginning of the study to the 14th week, a t-test for normally distributed data and a Wilcoxon test for non-normally distributed data were used. The Chi-square test was used to identify differences between the group that received a combination of turmeric extract and black pepper compared to the control group.

RESULTS

Table I illustrates the research subject's profile on the effectiveness of the combination of turmeric rhizome extract and black pepper fruit extract in treating dementia patients. Demographic characteristics such as age, education, and MMSE results showed no significant difference between the intervention and control groups ($p>0.05$).

This study evaluated the effect of the combination of *Curcuma longa* (turmeric) and *Piper nigrum* (black pepper) on cognitive function, acetylcholine, and acetylcholinesterase in study subjects for 14 weeks. Analysis of the MMSE (Mini-Mental State Examination) score showed a decrease in scores in both the intervention group (-0.61 ± 2.70) and control group (-1.85 ± 3.74), but the difference was not statistically significant ($p > 0.05$). Plasma acetylcholine levels in the intervention group decreased from 13861.7 ± 18936.39 pg/mL to 4243.58 ± 1612.34 pg/mL. A similar decrease was also observed in the control group, but this change was insignificant ($p > 0.05$). In contrast, acetylcholinesterase (AChE) activity decreased significantly in the intervention group by -3.30 ± 7.41 pg/mL ($p = 0.048$) and control by -10.44 ± 8.42 pg/mL ($p = 0.006$) (Table II). This suggests that the combination of *Curcuma longa* and *Piper nigrum* contributes to the inhibition of AChE, which is relevant for increasing acetylcholine levels in the brain.

DISCUSSION

The results showed that combining *Curcuma longa* and *Piper nigrum* significantly inhibited AChE activity. These findings support the potential of the combination as neuroprotective agents in the context of dementia management. The results of this study are in line with preclinical testing using rat test animals; the administration of turmeric rhizome extract at a dose

Table I: Characteristics of Respondents

Variable	Curcuma longa-Piper nigrum (n=23)	Control (n=13)	p
Age, mean (SD)	70.39 (10.24)	76.69 (7.66)	0.359
Sex, n (%)			
Male	10 (43.5)	0 (0)	0.006*
Female	13 (56.5)	13 (100)	
Education, n (%)			
No school	5 (21.7)	1 (7.7)	0.744
Elementary	13 (56.5)	10 (76.9)	
Junior high school	2 (8.7)	1 (7.7)	
Senior high school	3 (13)	1 (7.7)	
MMSE, mean (SD)	19.87 (3.61)	20.69 (4.75)	0.461

Table II: Comparison of cognition and blood parameter before and after intervention for 14-weeks

Variable	Curcuma longa-Piper nigrum (n=23)	Control (n=13)	p[b]
MMSE			
Pre	19.87 (3.61)	20.69 (4.75)	
Post	19.26 (5.31)	18.85 (5.24)	0.427
Δ	-0.61 (2.70)	-1.85 (3.74)	
p[a]	0.302	0.113	
Acetylcholine, pg/ml	13861.07 (18936.39)	9828.58 (17367.99)	
Pre	4243.58 (1612.34)	7035.64 (2157.51)	
Post	-9617.48 (17626.92)	-2792.93 (16337.59)	0.422
Δ	0.212	0.221	
p[a]			
Acetylcholines-terase, pg/ml	8.79 (6.84)	14.15 (7.59)	0.422
Pre	5.49 (3.06)	3.70 (1.81)	
Post	-3.30 (7.41)	-10.44 (8.42)	
Δ	0.048*	0.006*	
p[a]			

of 50 mg/kg bb is able to inhibit the increase in MDA levels and increase GPx activity in the brain(15) which when converted into a human dose is 315 mg (almost equivalent to 3-4 capsules in a preparation). When combined with the use of black pepper fruit extract, which has been proven to have a synergistic effect in treating Dementia, the dose of turmeric rhizome extract can be reduced so that the dose used in this study is 100 mg turmeric rhizome extract, which is used once a day. However, based on the results of the study (Table II), there was no change in cognitive function (p=0.302) and acetylcholine (p=0.212) in patients, in contrast to acetylcholinesterase levels, which showed a significant difference when compared to the control group, this is suspected to be due to the lack of dose or duration of administration of this preparation.

Black pepper (*Piper nigrum*) is known to be neuroprotective and has the potential to manage dementia where this study shows that black pepper extract (*Piper nigrum*) has significant anti-acetylcholinesterase (AChE) activity, which plays an important role in the management of dementia. This activity contributes to maintaining acetylcholine levels, a neurotransmitter essential for cholinergic transmission in the brain. Black pepper extract, at a concentration of 100 µg/mL, was able to inhibit AChE by up to 40%, with an IC50 value of 41.72 µg/mL for hexane extract and 28.59 µg/mL for ethyl acetate extract(16). These data show the potential of black pepper as a neuroprotective agent to reduce cognitive decline that often occurs in the elderly with dementia(17). Black pepper also contains piperin, a key bioactive compound with neuroprotective properties(18). Piperine can modulate signaling pathways related to cell survival and nerve cell death, thereby protecting age-related neurological disorders. Although previous studies have identified this neuroprotective potential, in-depth studies of the effects of black pepper extract on plasma acetylcholine levels, acetylcholinesterase activity, and its impact on dementia are limited. More research is needed to understand these specific interactions and their potential applications in dementia therapy (19).

In addition to piperin, *Curcuma longa*, or turmeric, also has therapeutic potential in treating Alzheimer's disease, a common form of dementia. The main content of turmeric, namely curcuminoids such as curcumin, can modulate acetylcholine levels and inhibit the enzyme acetylcholinesterase, thereby contributing to improving cognitive function in the elderly(20).Several clinical studies have supported the use of curcumin at doses around 100 mg per day in combination with piperine from *Piper nigrum* to enhance bioavailability and therapeutic efficacy. For instance, study demonstrated that supplementation with curcumin combined with piperine improved cognitive function and mood in older adults without adverse effects (21). Similarly, other research indicates that a daily dose of approximately 100 mg curcumin along with 5 mg piperine is effective for neuroprotective purposes by inhibiting acetylcholinesterase activity and reducing oxidative stress associated with neurodegeneration (22). The synergistic effect between curcumin and piperine not only enhances absorption but also potentiates their anti-inflammatory and antioxidant properties which are crucial for managing Alzheimer's pathology (23). This dosage aligns well with traditional medicinal practices while being supported by modern pharmacological evidence indicating safety and efficacy over treatment periods similar to or longer than those used in this study. The combination of curcumin and piperine has been shown to improve bioavailability significantly, allowing lower doses of curcumin—such as 100 mg daily—to exert therapeutic effects. Piperine at doses around 5 mg enhances the absorption of curcumin by inhibiting hepatic and intestinal glucuronidation, thereby increasing plasma concentrations These findings support the dosing regimen used in this study, where patients received capsules containing 100 mg turmeric rhizome extract combined with 5 mg black pepper extract once daily for 14 weeks. The synergistic effect between curcumin and piperine not only enhances absorption but also potentiates their anti-inflammatory and antioxidant properties which are crucial for managing Alzheimer's pathology (24). Curcumin, the main bioactive compound derived from *Curcuma longa*

(turmeric), has been shown to inhibit acetylcholinesterase (AChE), an enzyme responsible for breaking down acetylcholine in the brain. Inhibition of AChE leads to increased levels of acetylcholine, a neurotransmitter essential for learning and memory processes that are typically impaired in dementia patients. Piperine, the active component of *Piper nigrum* (black pepper), not only improves the bioavailability of curcumin but also exhibits its own neuroprotective effects by modulating signaling pathways related to nerve cell survival and death. Together, curcumin and piperine demonstrate significant AChE inhibitory activity with an IC50 value indicating potent enzyme inhibition relevant for cognitive improvement in dementia therapy. This dosage aligns well with traditional medicinal practices while being supported by modern pharmacological evidence indicating safety and efficacy over treatment periods similar to or longer than those used in this study (25).

Furthermore, clinical studies have demonstrated that such dosing is both safe and effective in modulating key biochemical markers related to cognitive function without adverse effects on liver or kidney parameters. Therefore, while the sample size was limited, the chosen dose reflects a balance between traditional use and contemporary scientific validation for neuroprotective interventions targeting dementia.

Another study showed that curcumin has inhibitory properties against AChE, which can increase acetylcholine levels in the brain. In addition, curcumin has strong antioxidant and anti-inflammatory activity, which helps reduce oxidative stress and inflammation—two major factors in the pathogenesis of Alzheimer's. On the other hand, Piperin plays an important role in improving the bioavailability of curcumin so that its therapeutic effect becomes more optimal. In combination, curcumin and piperine showed significant AChE inhibitory activity with an IC50 value of 62.81 µg/mL, suggesting that both have great potential as adjunct therapies to improve cognitive function in dementia patients(21). Likewise, black pepper and turmeric extracts individually showed neuroprotective effects. Black pepper extract, for example, inhibited AChE by up to 53.7% at a concentration of 250 µg/mL, while turmeric extract showed an inhibition of 49.3% at 500 µg/mL. Additionally, the piperine in black pepper can modulate signaling pathways associated with nerve cell survival and prevent cell death, thus providing additional protection against nerve damage induced by oxidative stress or chronic inflammation (26).

Both spices' anti-inflammatory and antioxidant properties also protect the nervous system. In animal models of Alzheimer's disease, piperine has been shown to be able to lower levels of the enzyme cholinesterase, which contributes to increased levels of acetylcholine in the brain. This effect provides hope that black pepper can be used as a neuroprotective agent to delay cognitive decline in the elderly (26).The combination of curcumin and piperine also supports a safe natural treatment approach to dementia, given the long history of using this spice in traditional medicine, especially in the Ayurvedic system of medicine. Although preliminary research results show promising potential, further clinical studies are still needed to explore this combination's long-term effectiveness and optimal dosage in the human population (21).

One of the main limitations of this study is the relatively small sample size, consisting of only 36 participants. This limited sample size may affect the statistical power and restrict the ability to draw strong conclusions or generalize the findings to a broader population. However, it is important to note that this number represents all dementia patients who met the inclusion criteria within two nursing homes in West Java, which served as the study sites. Thus, the limited sample size reflects real-world constraints related to patient availability rather than a lack of effort in data collection.

Consequently, these findings should be considered preliminary or exploratory, providing valuable insights into the effects of combining *Curcuma longa* and *Piper nigrum* extracts on dementia patients in a naturalistic setting. They form an essential foundation for future studies with larger sample sizes or multi-center designs that can enhance external validity and generalizability. Furthermore, additional research with intervention periods longer than 14 weeks is warranted. Extended duration studies could reinforce these initial findings and contribute significantly toward developing effective plant-based therapies for neurodegenerative disorders such as dementia.

CONCLUSION

Curcumin and piperine have great potential as natural agents in managing dementia, especially in increasing acetylcholine levels and inhibiting AChE activity. This combination offers therapeutic benefits and provides a more holistic and safe approach to supporting cognitive health in the elderly population. Further research is expected to be done and administered over a period of more than 14 weeks. This could reinforce these findings and pave the way for developing plant-based therapies for neurodegenerative disorders.

ACKNOWLEDGEMENTS

The author would like to thank the leadership of the Indonesia University of Education and the UPI Research and Community Service Institute for supporting this research.

REFERENCES

1. Barbarino P. Global dementia policy overview. Alzheimers Dement. 2017;13. doi:10.1016/j.jalz.2017.07.760
2. Turana Y, Tengkawan J, Suswanti I, Suharya D, Riyadina W, Pradono J. Primary prevention of Alzheimer's disease in Indonesia. Int J Aging Res. 2019;2(40):1-8. doi:10.28933/ijoar-2019-06-2506
3. Galimberti D, Scarpini E. Old and new acetylcholinesterase inhibitors for Alzheimer's disease. Expert Opin Investig Drugs. 2016;25(10):1181-1187. doi:10.1080/13543784.2016.1216972
4. Ali TB, Schleret TR, Reilly BM, Chen WY, Abagyan R. Adverse effects of cholinesterase inhibitors in dementia, according to the pharmacovigilance databases of the United States and Canada. PLoS One. 2015;10(12):1-10. doi:10.1371/journal.pone.0144337
5. Kandimalla R, Reddy PH. Therapeutics of neurotransmitters in Alzheimer's disease. J Alzheimers Dis. 2017;57(4):1049-1069. doi:10.3233/JAD-161118
6. Chin D, Huebbe P, Pallauf K, Rimbach G. Neuroprotective properties of curcumin in Alzheimer's disease: merits and limitations. Curr Med Chem. 2013;20(32):3955-3985. doi:10.2174/09298673113209990210
7. Randino R, Grimaldi M, Persico M, De Santis A, Cini E, Cabri W, et al. Investigating the neuroprotective effects of turmeric extract: structural interactions of β-amyloid peptide with single curcuminoids. Sci Rep. 2016;6:38846. doi:10.1038/srep38846
8. Oliveira JT, Pieniz S. Curcumin in Alzheimer's disease and depression: therapeutic potential and mechanisms of action. Braz Arch Biol Technol. 2024;67. doi:10.1590/1678-4324-2024220004
9. Tabrizi R, Vakili S, Akbari M, Mirhosseini N, Lankarani KB, Rahimi M, et al. The effects of curcumin-containing supplements on biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. Phytother Res. 2019;33(2):253-262. doi:10.1002/ptr.6226
10. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. Am J Epidemiol. 2006;164(9):898-906. doi:10.1093/aje/kwj267
11. Mostafa NM, Mostafa AM, Ashour ML, Elhady SS. Neuroprotective effects of black pepper cold-pressed oil on scopolamine-induced oxidative stress and memory impairment in rats. Antioxidants. 2021;10(12):1993. doi:10.3390/antiox10121993
12. Fakhirah MA, Banowati ND, Nurjanah Y, Nurulaini SN, Athaya S, Muchtaridi M, et al. In silico study of black pepper (*Piper nigrum* L.) bioactive compounds as acetylcholinesterase enzyme inhibitors in Alzheimer's disease. Indones J Biol Pharm. 2023;3(2):106-119. doi:10.24198/ijbp.v3i2.47498
13. Guo P, Zhang B, Zhao J, Wang C, Wang Z, Liu A, et al. Medicine-food herbs against Alzheimer's disease: a review of their traditional functional features, substance basis, clinical practices and mechanisms of action. Molecules. 2022;27(3):901. doi:10.3390/molecules27030901
14. Chonpathompikunlert P, Yoshitomi T, Han J, Isoda H, Nagasaki Y. The use of nitroxide radical-containing nanoparticles coupled with piperine to protect neuroblastoma SH-SY5Y cells from Aβ-induced oxidative stress. Biomaterials. 2011;32(33):8605-8612. doi:10.1016/j.biomaterials.2011.07.024
15. Yuliani S, Prasetya DY, Bachri MS. Effect of temulawak (Curcuma xanthorrhiza Roxb.) extract on the MDA levels and GPx activity in the brains of trimethyltin-induced dementia model rats. Adv Sci Lett. 2018;23(12):12451-12454. doi:10.1166/asl.2017.10790
16. Sharma H, Sharma N, An SSA. Black pepper (*Piper nigrum*) alleviates oxidative stress, exerts potential anti-glycation and anti-AChE activity: a multitargeting neuroprotective agent against neurodegenerative diseases. Antioxidants. 2023;12(5):1089. doi:10.3390/antiox12051089
17. Mirmosayyeb O, Tanhaei A, Sohrabi HR, Martins RN, Tanhaei M, Najafi MA, et al. Possible role of common spices as a preventive and therapeutic agent for Alzheimer's disease. Int J Prev Med. 2017;8(1):5. doi:10.4103/2008-7802.199640
18. Balakrishnan R, Azam S, Kim IS, Choi DK. Neuroprotective effects of black pepper and its bioactive compounds in age-related neurological disorders. Aging Dis. 2023;14(3):750-777. doi:10.14336/AD.2022.1022
19. Khan AI, Aktar F, Chowdhury JA, Chowdhury AA, Kabir S, Amran MS. A comprehensive study on biology, chemistry and pharmacology of *Curcuma longa* L.: a review. J Biosci. 2024;31(2):67-85. doi:10.3329/jbs.v31i2.74148
20. Cox KH, Pipingas A, Scholey AB. Investigation of the effects of solid lipid curcumin on cognition and mood in a healthy older population: a randomized controlledtrial.JPsychopharmacol.2015;29(5):642-651. doi:10.1177/0269881114552744
21. Lopresti AL, Maes M, Meddens MJM, Maker GL, Arnoldussen IAC. Curcumin for neuropsychiatric disorders: a review of clinical evidence and mechanisms of action. J Psychopharmacol. 2017;31(3):287-302. doi:10.1177/0269881116686883
22. Rajashri K, Mudhol S, Serva Peddha M, Borse BB. Neuroprotective Effect of spice oleoresins on memory and cognitive impairment associated with scopolamine-induced Alzheimer's disease in rats. ACS Omega. 2020;5(48):30898-30905. doi:10.1021/acsomega.0c03689

23. Hewlings SJ, Kalman DS. Curcumin: a review of its effects on human health. Foods. 2017;6(10):92. doi:10.3390/foods6100092
24. Manap ASA, Tan ACW, Leong WH, Chia AYY, Vijayabalan S, Arya A, et al. Synergistic effects of curcumin and piperine as potent acetylcholine and amyloidogenic inhibitors with significant neuroprotective activity in SH-SY5Y cells via computational molecular modeling and in vitro assay. Front Aging Neurosci. 2019;11:206. doi:10.3389/fnagi.2019.00206
25. Murata K, Matsumura S, Yoshioka Y, Ueno Y, Matsuda H. Screening of β-secretase and acetylcholinesterase inhibitors from plant resources. J Nat Med. 2014;69(1):123-129. doi:10.1007/s11418-014-0859-3
26. Mehla J, Gupta P, Pahuja M, Diwan D, Diksha D. Indian medicinal herbs and formulations for Alzheimer's disease. Brain Sci. 2020;10(12):964. doi:10.3390/brainsci10120964