Alzheimer's & Dementia
Translational Research
& Clinical Interventions

RESEARCH ARTICLE

Effects of water-soluble mangosteen extract on cognitive function and neuropsychiatric symptoms in patients with mild to moderate Alzheimer's disease (WECAN-AD): A randomized controlled trial

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Abstract

Introduction: The water-soluble mangosteen pericarp extract's (WME) effect was investigated in Alzheimer's disease (AD).

Methods: The participants received 4 mg/kg/day of WME for 24 weeks (low dose, n = 33), 4 mg/kg/day for 12 weeks and then 8 mg/kg/day for 12 weeks (high dose, n = 33); or a placebo (n = 42). The outcomes were neuropsychiatric test scores, safety, tolerability, and the blood 4-hydroxynonenal level.

Results: The proportion of participants who achieved the minimum clinically important difference for the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog; –2.6 points) at 24 weeks was significantly higher in the low-dose group (and a trend in the high-dose group) than in the placebo group. WME appeared safe and well tolerated. At 24 weeks, the 4-hydroxynonenal level declined in both intervention groups. The participants with a 5% reduction in this level showed greater ADAS-Cog improvements.

Conclusion: WME is a safe and well-tolerated cognitive enhancer in AD with varying benefits across individuals based on antioxidative response.

KEYWORDS
Alzheimer's disease, clinical trial, cognition, Garcinia mangostana, mangosteen extract, older adults, oxidative stress, safety

1 | BACKGROUND

Currently, there are no drugs that can reverse or delay the progression of Alzheimer's disease (AD).[1] Neuroinflammation could be one of the major underlying mechanisms of AD.[2] Oxidative stress can directly potentiate neurodegeneration via neuron cell damage[3] or indirectly activate the neuroinflammation process.[4] Additionally, pro-inflammatory cytokines, for example, interleukin 6 (IL-6), are upregulated in the brain of patients with AD, producing amyloid plaque and hyperphosphorylated tau, which are hallmark pathologies of AD,[5] and vice versa.[6] Thus, antioxidants may thwart or impede the injury of neuron cells.

Mangosteen pericarp extract is a potent natural antioxidant. The primary bioactive substances from pericarp extraction contain xanthones and derivatives (less polar substances) together with polyphenolic groups such as catechins and anthocyanidins (more polar substances).[8,9] Among the xanthone groups, the most abundant was α-mangostin. It showed both antioxidant activities[10,11] and a cytotoxic effect (by inducing apoptosis[7,12] and increasing reactive oxygen species [ROS] levels in various tumor cells).[7,13,14] Furthermore, investigations of mouse models reported colitis from α-mangostin.[15] A mangosteen extract dose not exceeding 200 mg/kg was safe in mice.[16] These controversial results could not exclude the possibility that α-mangostin might harm viable neuron cells. Consequently, the water-soluble ethyl acetate partitioned mangosteen pericarp ethanol extract (WME) should focus on the neurons' positive effect.[8,14,17] This solution yielded more polar substances described above, which preserve antioxidant activities and exhibit less cytotoxic activities.[11]

Two main mechanisms of action of WME in AD are antioxidant and acetylcholinesterase inhibitors. WME successfully inhibited the β_{1-42} amyloid peptide (Aβ_{1-42}) cytotoxic effect and minimized in vitro neuroblastoma cell model ROS levels.[18] Moreover, WME showed an inhibitory effect on acetylcholinesterase activities.[19,20] In an in vivo study, WME ameliorated scopolamine-induced memory-impaired mice.[19] It also increased the donepezil concentration in the brain of mice with minimal donepezil distribution to other tissue.[21]

WME was safe in healthy human subjects. A phase I trial was conducted in 11 healthy participants who took WME daily for 6 months. The results revealed no serious adverse events and no significant change in the blood safety check.[17] Additionally, WME significantly decreased the antioxidant activities, which was measured by blood 4-hydroxynonenal (HNE) level,[17] an end product generated by the ROS with polyunsaturated fats during oxidative stress.[22] These findings confirmed the antioxidative properties of WME in humans.[18,19]

The efficacy, tolerability, and safety of WME have never been thoroughly established in AD. Therefore, we aimed to discover the effects of WME on clinical outcomes (cognitive function and neuropsychiatric symptoms [NPS]), safety, tolerability, and antioxidant properties in mild to moderate AD in older people.

HIGHLIGHTS

- Mangosteen pericarp extract is a potent natural antioxidant with limited evidence in humans.
- Alzheimer's disease (AD) has multiple potential pathological etiologies, and oxidative stress is one of its mechanisms.
- Previously, there were no studies of the effect of water-soluble mangosteen pericarp extract (WME) in AD.
- WME was safe, enhanced the antioxidative effect, and slightly improved cognitive outcomes in older AD patients.

RESEARCH IN CONTEXT

1. Systematic Review: We reviewed the clinical trials in the MEDLINE database for the effects of mangosteen pericarp extract on cognition and neuropsychiatric symptoms. Research on this treatment among patients with Alzheimer's disease is insufficient.

2. Interpretation: This was the first 24-week, randomized, double-blind placebo-controlled trial that demonstrated the effect of water-soluble ethyl acetate partitioned mangosteen pericarp ethanol extract on a modest benefit in cognition (more patients experienced better clinically significant cognitive test scores). The intervention group whose blood biochemistry showed a 5% reduction in oxidative stress from baseline showed better cognitive outcomes than those who did not, implying that antioxidants had a role in the cognitive function of AD. This enhancement might differ across individuals based on their antioxidative response. Additionally, the extract was safe and well tolerated.

3. Future Directions: Long-term investigations of mangosteen pericarp extract could identify the factors influencing the relationship between antioxidants and cognition in AD.

2 | METHODS

2.1 | Experimental design

This study was a phase II, 24-week, randomized, double-blind placebo-controlled trial with three parallel arms conducted in a geriatric clinic at Siriraj Hospital in Bangkok, Thailand. The study was registered in the Thai Clinical Trial Registry (www.thaiclinicaltrials.org). The Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, approved the study protocol.

2.2 | Intervention and placebo capsule development

The WME extraction and encapsulation procedure were detailed in the previous studies[14,17] and described in the supporting information. There were three types of capsules: 220 mg of WME, 280 mg of WME, and placebo. All capsules had identical colors, sizes, weights, and packages.

2.3 | Participants

The full inclusion and exclusion criteria are described in the supporting information. Briefly, the inclusion criteria were adults aged > 50 years who were diagnosed with AD according to the Diagnostic and Statistical Manual of Mental Disorders-IV Text Revision (DSM-IV-TR) and National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorder Association's (NINCDS-ADRDA) criteria for probable AD. The Thai Mental State Examination (TMSE)[23] score ranged from 12 to 25, and there had to be a primary caregiver. If the participants were currently on antidementia medications, the dose had to be stable for 4 months with no plan to adjust the amount during the study period. The exclusion criteria were participants with other diagnoses apart from AD, unstable medical illness or disabilities which might affect the study protocol, and out-of-range blood panel abnormalities. For sedatives, antidepressants, or antipsychotic drugs, the dose must have been the same for 30 days before enrollment and during the study period. All eligible participants and their legal representatives were invited to the project without undue influence and provided written informed consent.

2.4 | Randomization and allocation

The study coordinator performed simple balloting randomization. The 1:1:1 ratio assigned participants to placebo, low-dose, and high-dose arms. The randomization results were kept by the same coordinator who was not involved in recruitment and primary outcome collection. After allocation, we performed the second round of 2:1 ratio simple balloting randomization of each group to sample candidates achieving the blood HNE test. The participants and the outcome assessors were blinded to both allocations. Blood HNE tests were taken together with other routine blood checks during the same period throughout the project.

2.5 | Procedures

All eligible participants and their primary caregivers met the geriatric neurologist for baseline medical history documentation and physical examination. All concurrent herbs and dietary supplements were advised to be discontinued and checked at every visit. Psychologists administered the neuropsychiatric assessments, including the

Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version (ADAS-Cog); the Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory 23-item Scale (ADCS-ADL); the Neuropsychiatric Inventory Questionnaire (NPI-Q), which includes the patient subscale (NPI) and caregiver distress subscale (NPI distress); the Clinical Dementia Rating Scale (CDR), which includes both the global rating scale and the sum of boxes (CDR-SB); and the TMSE[23] (see Table S1 in supporting information for more details). After excluding the participants that met the exclusion criteria and completing the randomization process, participants underwent a blood test and a blood HNE test (if allocated) between 8:00 and 9:00 a.m. The study coordinator gave patients an 8-week supply of capsules based on the study group. Participants and their primary caregiver were scheduled for face-to-face visits with the geriatric neurologist every week for 8 weeks (the 2nd week as the extra episode, 8th, 16th, and 24th week) and telephone interviews every other week of the 8 weeks (the 1st week as the additional episode, 4th, 12th, and 20th week). The interviews included assessing side effects, compliance checks (including pill counts), physical examination, neuropsychiatric tests, the routine 8:00 to 9:00 a.m. blood test, and 8-week allotments of intervention or placebo capsules for each face-to-face visit (except the 2nd week). In the telephone interview, there was a preplanned checklist conversation for a side-effect interview, increasing retention and compliance checks (pill count).

2.6 | Interventions and placebo

For both intervention arms, 50 to 100 mg/kg WME from a previously successful study in a mouse model[19] was converted to 4 to 8 mg/kg human equivalent doses,[24] and the US Food and Drug Administration was referenced for guidance.[25] This WME dosage was safe in the phase I trial among healthy volunteers.[17] Participants who weighed 55 kg or less received a 220 mg WME capsule, and those who weighed more than 55 kg received a 280 mg WME capsule. In the low-dose arm, the participants took one pill daily (4 mg/kg) until the end of the study. Participants in the high-dose arm took one pill daily for the first 12 weeks and then increased to two capsules daily (8 mg/kg) for the last 12 weeks. All participants in the placebo arm took the same placebo capsule throughout the study. All serious adverse events (SAEs) or suspected unexpected serious adverse reactions (SUSARs) were required to be reported to the human research ethics committee to evaluate whether the events were related to WME.

2.7 | Outcomes

2.7.1 | Primary endpoint

The primary endpoint (Figure S1 in supporting information) was the effectiveness of WME treatment versus placebo for enhancing cognitive function and NPS, as assessed by the neuropsychiatric tests at any visit. Improvements were measured by examining the mean score

change and the proportion of participants who reported beneficial score changes. The minimum clinically important difference (MCID) in cognitive efficacy was used for clinical significance, defined as a mean change of 2.6 points for the ADAS-Cog and 1.4 points for the TMSE.[26] Despite having less evidence regarding MCIDs for the remaining tests, were used cutoffs of 5 points for the ADCS-ADL,[27] 1 point for the CDR-SB,[28] and 8 points for the NPI.[29,37]

2.7.2 | Secondary endpoint

Secondary outcomes included safety and biochemistry (Figure S1). We evaluated the incidence of SAE as defined by (1) all-cause mortality, (2) intensive care unit admission, (3) intrahospital admission or prolonged hospitalization, (4) emergency department visits, and (5) disability. The monitored minor adverse events included self-reported and informant-reported symptoms, physical examination, and laboratory data for safety components. For the biochemistry component, the outcome was a decrement of normalized HNE level (HNE/actin ratio)[31] versus placebo, detailed in the supporting information. The association between this antioxidative effect and the primary outcome was investigated via post hoc analysis to prove the causation hypothesis.

2.8 | Statistical analysis

2.8.1 | Sample size calculation

Because WME's cognitive effects have never been studied in AD patients and WME could inhibit acetylcholinesterase activities in a mouse model, [22] we implied data from a prior investigation of the donepezil effect on ADAS-Cog score, which showed that those who received donepezil for 24 weeks had a higher proportion of stable or reduction of ADAS-Cog score at the end of the trial versus placebo (80% vs. 57%).[30] As we set the power of 80% and a two-sided significance level of 0.05 for three groups, 180 participants (60 in each group) were required to detect a difference.[32] Considering a potential loss to follow-up rate of 5%, we aimed to recruit 189 patients.

2.8.2 | Data analysis

Outcome analysis was the intention-to-treat method. Linear mixed-effects models were performed on the mean score change to evaluate the treatment effect (low dose, high dose, and placebo) over follow-up time (baseline, 8th, 16th, and 24th week) using their baseline score in each test as covariates. Model selection was based on the Bayesian information criterion. Assumptions for linear mixed models (e.g., normality of error terms) were checked thoroughly using the residual plots. For proportional efficacy analysis, safety, and post hoc categorical outcome, we used the Chi-square test, Fisher's exact test for univariate analysis, and logistic regression for multivariate analysis. For biochemistry, safety, and post hoc continuous endpoints, we used the independent samples t-test, linear regression, and one-way analysis of

variance for normally distributed data and the Kruskal–Wallis test for non-normally distributed data. We reported the estimated effect size in a mean or median difference for relevant data and odds ratio (OR) for binary outcomes with 95% confidence intervals. The Bonferroni method was used to counteract the problem of multiple comparisons. We adjusted P for age, sex, years of education (less than 6 years, 6–12 years, and above 12 years), baseline cognitive score, and the status of acetylcholinesterase inhibitor (AChEI) use (yes/no). Statistical analyses were conducted with PASW Statistics 18 (SPSS Inc.) and R programming language (version 4.1.2). All tests were two-tailed, and a two-sided P less than .050 was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

We screened 115 potential candidates for eligibility and excluded seven volunteers who met the exclusion criteria. Therefore, 108 participants were enrolled and randomly assigned to three groups (Figure 1). The study ended with 102 participants accomplishing the 24-week intervention (94.4% retention rate). Two cases were lost to follow-up at the 24th week in the low-dose group (metastatic cancer at the 16th week and transportation inconvenience), and three subjects were lost to follow-up at the 24th week of the placebo group (two participants denied due to active NPS, another was admitted for hip surgery). The baseline characteristics of the participants (Table 1) depicted a collation of three groups. Age, sex, education level, and TMSE were significant factors affecting follow-up cognition.[33,34]

3.2 | Efficacy outcome

A linear mixed model was used to examine the mean score change in the 8th, 16th, and 24th weeks (Figure 2). The high-dose group had a significantly different ADAS-Cog and TMSE score change than the placebo group (for P, see Table 2). Furthermore, the low-dose group had a marginally significant change in the ADAS-Cog score (adjusted P = .057). All models examined the interaction between treatment, time, along with the quadratic time term and showed no significant interaction. Additionally, the baseline score was an independent factor associated with the mean score change (Table 2).

Regarding the proportion of patients in each group who showed improvement (Tables S3, S4 in supporting information), the low-dose group had a greater proportion of participants who had an improved ADAS-Cog score at the 24th week than the placebo group (69.0% vs. 48.6%, respectively); this difference in proportions was statistically significant after adjusting for age, sex, education years, the status of AChEI use, and baseline cognitive score, with an OR 3.22 (95% confidence interval [CI]: 1.03–10.10, P = .045). The high-dose group had a greater proportion of participants who had an improved TMSE score at the 24th week than the placebo group (61.3% vs. 34.2%, respectively), with OR of 3.35 (95% CI: 1.21–9.32, P = .020). Concerning the minimum clinically important difference (Figure 3),[26] there was a signifi-

TABLE 1 Baseline characteristics of participants

Baseline Characteristics	Mean (SD)		
Participant profile	Placebo	Low dose	High dose
	n = 40	n = 31	n = 31
Age, years	78.0 (6.2)	77.2 (7.3)	78.0 (5.5)
Female (%)	25 (62.5)	24 (75.0)	21 (67.7)
Years of education (%)			
< 6 years	21 (52.5)	18 (58.1)	18 (58.1)
6-12 years	8 (20.0)	5 (15.6)	7 (22.6)
>12 years	11 (27.5)	8 (25.0)	6 (19.4)
Current smoker (%)	0 -	0 -	0 -
Current alcohol use (%)	1 (2.5)	1 (3.1)	0 -
BMI (kg/m^2)	22.8 (2.6)	22.9 (2.7)	22.8 (3.5)
Comorbidities (%)			
Diabetes mellitus	7 (17.5)	8 (25.8)	4 (12.9)
Hypertension	31 (77.5)	21 (67.7)	23 (74.2)
Hyperlipidemia	28 (70.0)	19 (61.3)	22 (71.0)
Cerebrovascular disease	4 (10.0)	3 (9.4)	3 (9.7)
Chronic kidney disease	10 (25.0)	4 (12.9)	7 (22.6)
Heart disease	7 (17.5)	3 (9.4)	3 (9.7)
Lung disease	1 (2.5)	1 (3.1)	0 -
Liver disease	0 -	1 (3.1)	1 (3.2)
Cancer	2 (5.0)	1 (3.1)	0 -
Depressive mood	0 -	2 (6.3)	2 (6.5)
Baseline ADAS-Cog (Median ± IQR)	26 (18-31)	22 (17-31)	23 (18-25)
Baseline TMSE (Median ± IQR)	22 (17-24)	22 (18-24)	22 (20-23)
Baseline CDR (%)			
0.5	11 (27.5)	14 (43.8)	14 (45.2)
1	20 (50.0)	14 (45.2)	15 (48.4)
2	9 (22.5)	2 (6.3)	2 (6.5)
3	0 -	1 (3.1)	0 -
Baseline CDR-SB (Median ± IQR)	6 (4-8)	5 (3-7)	5 (3-6)

Baseline characteristics	Mean (SD)		
Participant profile	Placebo	Low dose	High dose
	n = 40	n = 31	n = 31
Baseline ADCS-ADL (Median ± IQR)	52 (45-63)	55 (48-63)	59 (51-63)
Baseline NPI score (Median ± IQR)	3 (1-6)	2 (0-5)	3 (1-7)
Baseline NPI distress (Median ± IQR)	2 (1-4)	1 (0-3)	2 (1-4)
Medications used			
AChEI use (%)	36 (90.0)	31 (96.9)	29 (93.5)
Donepezil (%)	23 (74.2)	23 (74.2)	20 (64.5)
Donepezil dose (mg/day)	7.0 (2.5)	6.5 (2.4)	6.8 (2.4)
Rivastigmine patch (%)	13 (41.9)	7 (22.6)	10 (32.3)
Rivastigmine patch dose (mg/day)	7.6 (2.5)	8.8 (1.9)	7.5 (2.5)
Galantamine (%)	2 (6.5)	2 (6.5)	2 (6.5)
Galantamine dose (mg/day)	12.0 (5.7)	8.0 -	8.0 -
Memantine (%)	3 (9.7)	1 (3.2)	1 (3.2)
Memantine dose (mg/day)	10.0 -	10.0 -	10.0 -
Antipsychotic drug (%)	9 (22.5)	3 (9.4)	4 (12.9)
SSRIs (%)	12 (30.0)	9 (28.1)	12 (38.7)
Benzodiazepine (%)	1 (2.5)	1 (3.1)	0 -
Caregiver profile			
Female (%)	29 (72.5)	23 (74.2)	17 (54.8)
Relationship of primary caregiver (%)			
Child	26 (65.0)	17 (54.8)	26 (83.8)
Spouse	11 (27.5)	7 (22.6)	4 (12.9)
Grand child	1 (2.5)	4 (12.9)	1 (3.2)
Child-in-law	1 (2.5)	1 (3.2)	0 -
Sibling	1 (2.5)	1 (3.2)	0 -
Formal	0 -	0 -	1 (3.2)
Duration of primary caregiver role (%)			
<1 year	2 (5.0)	3 (9.4)	5 (16.1)
1-4 years	21 (52.5)	14 (45.2)	20 (64.5)
>4 years	17 (42.5)	14 (43.8)	6 (19.4)

Abbreviations: AChEIs, acetylcholinesterase inhibitors; ADAS-Cog, the Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version; ADCS-ADL, Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory 23-item Scale; BMI, body mass index; CDR, Clinical Dementia Rating Scale (global); CDR-SB, Clinical Dementia Rating Scale (sum of boxes); IQR, interquartile range; NPI distress, Neuropsychiatric Inventory Questionnaire (caregiver's distress level subscale); NPI, Neuropsychiatric Inventory Questionnaire (patient subscale); SD, standard deviation; SSRI, selective serotonin reuptake inhibitor; TMSE, Thai Mental State Examination.
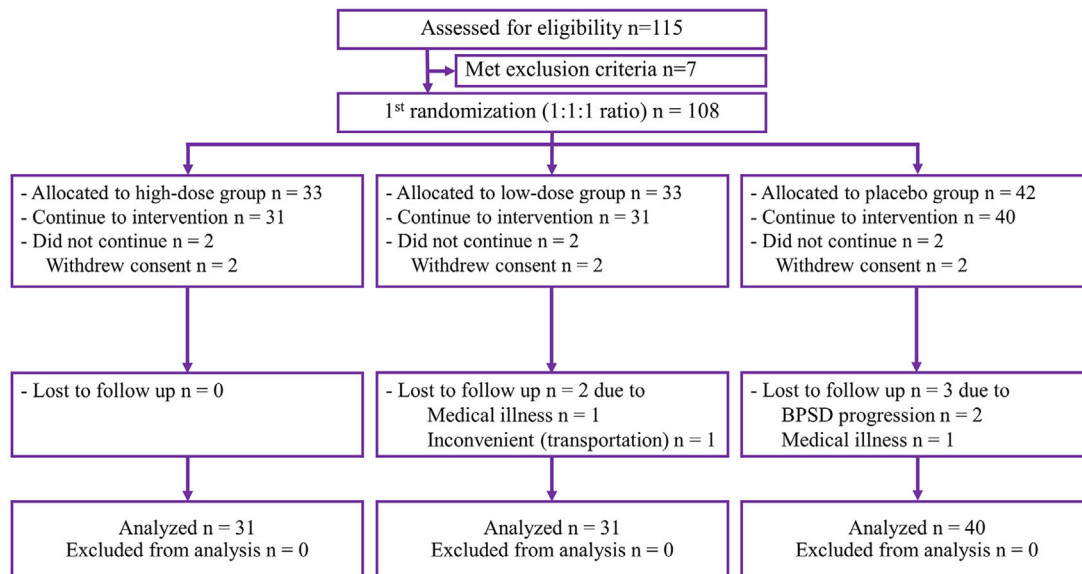
FIGURE 1 Flowchart of the study. BPSD, behavioral and psychological symptoms of dementia

cantly greater proportion of patients in the low-dose group than in the placebo group who achieved the MCID for the ADAS-Cog (51.7% vs. 29.7%, respectively) and the TMSE (37.9% vs. 15.8%, respectively). The OR and *P* are shown in Figure 3. Though there was a trend of worse TMSE between the low-dose and placebo group, this did not reach statistical significance. In subgroup analysis, the participants receiving the targeted dose of AChEI also received significant benefits (supporting information). There were no significant findings for the other neuropsychiatric tests (Table S3).

3.3 | Safety outcome

A total of 93.3% of patients had a compliance rate above 90%. We observed no deaths or intensive care unit admissions, and the incidence of intrahospital admissions and emergency department visits were equal (Table S5 in supporting information). No reports of SAE or SUSAR were considered related to the intervention. None of the participants discontinued the intervention drug due to adverse events. At the end of the study, there was a statistically significant difference in total cholesterol and low-density lipoproteins (LDL) in the laboratory results (Table S6 in supporting information). However, the mean score changes from baseline were not significant (*P* = .077 and 0.254, respectively). No other clinically substantial changes in vital signs, body weights, electrocardiograms, or laboratory profiles were observed during the study in any group (Table S6).

3.4 | Biochemistry outcome

Seventy participants were allocated to perform the HNE blood test (Figure S1), including 23, 24, and 23 participants in the low-dose, high-dose, and placebo groups, respectively. A decreasing trend in oxidative

damage was observed and reached statistical significance at the 24th week in both the low-dose (median 0.93 [IQR: 0.71–1.03], *P* = .017) and high-dose groups (median 0.94 [IQR: 0.81–1.04], *P* = .018) compared to the placebo group (median 1.06 [IQR: 0.96–1.19]; Figure S2 in supporting information).

3.5 | Post hoc analysis

There were 15 (65.2%) participants from the low-dose group and 15 (62.5%) participants in the high-dose group who responded to WME (defined by a 5% reduction in blood HNE level). We categorized the intervention participants into WME antioxidative responders and nonresponders. The normalized blood HNE initiative level of the responders was significantly higher (median 1.10 [95% CI: 0.96–1.30] vs. 0.98 [95% CI: 0.82–1.08]) than that of the non-responders (*P* = .005; Figure S3 in supporting information). Baseline characteristics of the responders (Table S7 in supporting information) included a higher proportion of males (40% vs. 17.6%) and a higher proportion of comorbidities, for example, diabetes mellitus (20% vs. 11.8%) and chronic kidney disease (23.3% vs. 11.8%). The laboratory data showed non-significant differences.

Considering the mean score change of ADAS-Cog, the responders had a significantly better score at the 24th week (mean −3.63 [95% CI: −5.50, 1.77] in responders vs. −0.69 [95% CI: −3.73, 2.36] in non-responders, adjusted *P* = .049; Figure 4). However, there was no significant difference after classification based on the original intervention arm (low-dose and high-dose) and antioxidant responsiveness (Figure S4 in supporting information). As shown in Figure 4, there was also a higher proportion of responders than non-responders who achieved the MCID for the ADAS-Cog at the end of the trial (62.1% vs. 31.3%, OR 6.01 [95% CI: 1.17–30.89], *P* = .032, Figure 4). No significant differences were found for other neuropsychiatric tests.
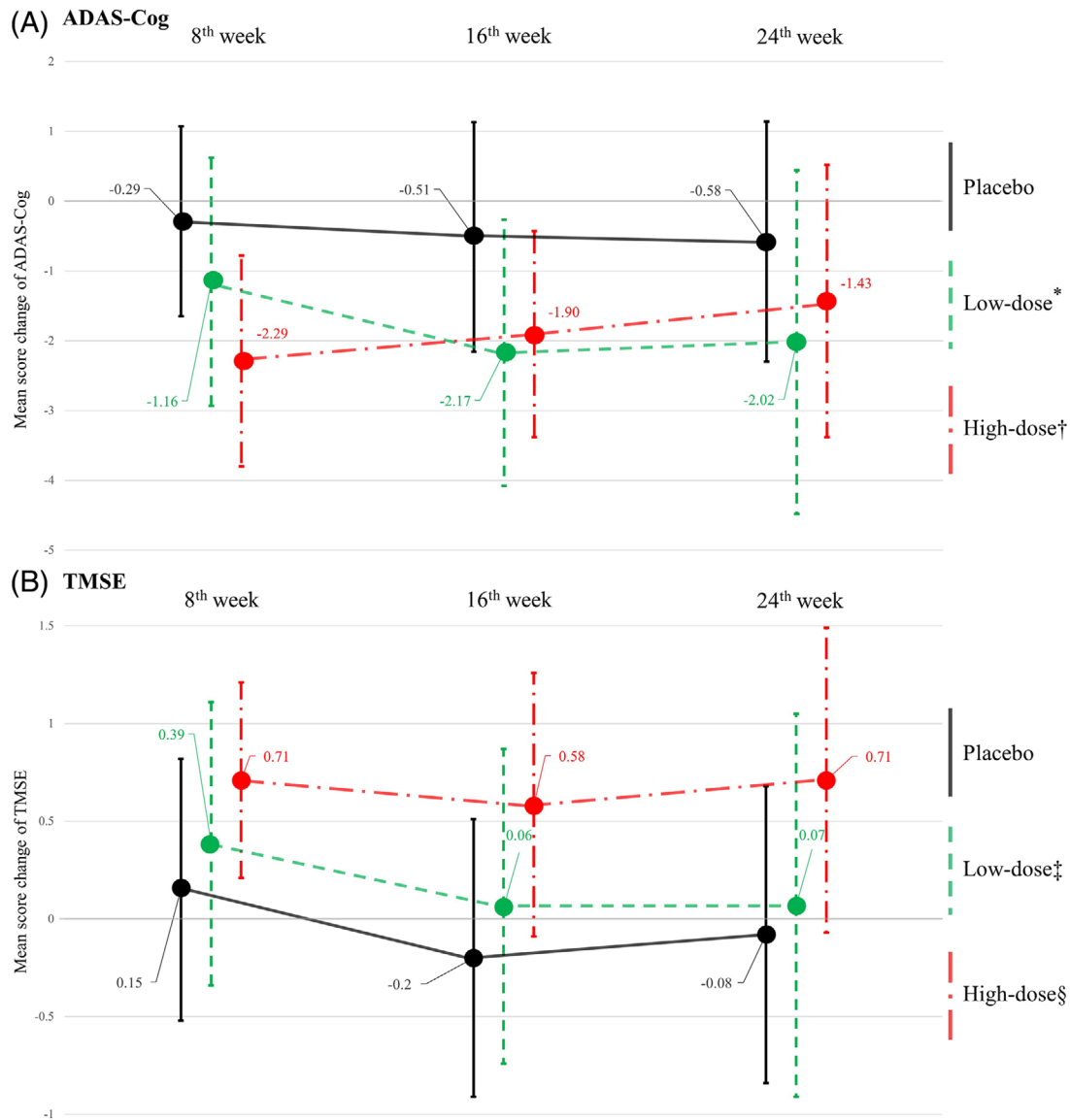
FIGURE 2 Primary endpoint: Mean score change analysis. The mean and 95% confidence interval were delineated for each follow-up time. The high-dose group's ADAS-Cog trend had a peak reduction at the 8th week but gradually increased at the 16th and 24th weeks. The low-dose group showed a constant diminishable trend but with an insignificant change. For details of other neuropsychiatric tests, see Table S2. All adjusted *P* attained from the linear mixed model after adjusting for age, sex, education years, AChEI use status, follow-up time, and baseline cognitive score of each test. *Adjusted *P* of the group = .057. †Statistically significant adjusted *P* of the group = .026. ‡Adjusted *P* of the group = .252. §Statistically significant adjusted *P* of the group = .049. AChEIs, acetylcholinesterase inhibitors; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version; TMSE, Thai Mental State Examination

4 | DISCUSSION

This trial is the first study evaluating WME in AD patients. WME showed modest cognitive benefits and was safe and well tolerated in older patients with AD. This study demonstrated no SAEs or SUSARs. Moreover, WME could reduce blood HNE levels compared to placebo, analogous to the phase I study findings in healthy adults.[17] In the proportion efficacy analysis, more patients in the low-dose arm showed improved outcomes and achieved the MCID for the ADAS-Cog and TMSE. This benefit was in addition to the gold standard treatment (AChEIs), which the participants previously experienced (93.5%). A

previous study of the cognitive effects of pure-water mangosteen pericarp extraction performed in a younger population (mean age 39 years) in different groups of patients (patients with schizophrenia and schizoaffective disorder) and different cognitive tests (CogState Brief Battery test) did not demonstrate a significant change in the outcomes.[35]

The benefit of WME might be individualized, depending on the antioxidative response in each patient. In a post hoc analysis, the responders showed an enhanced effect on the cognitive outcome of ADAS-Cog mean score change and the proportion of patients who achieved ADAS-Cog MCID levels (Figure 4) compared to
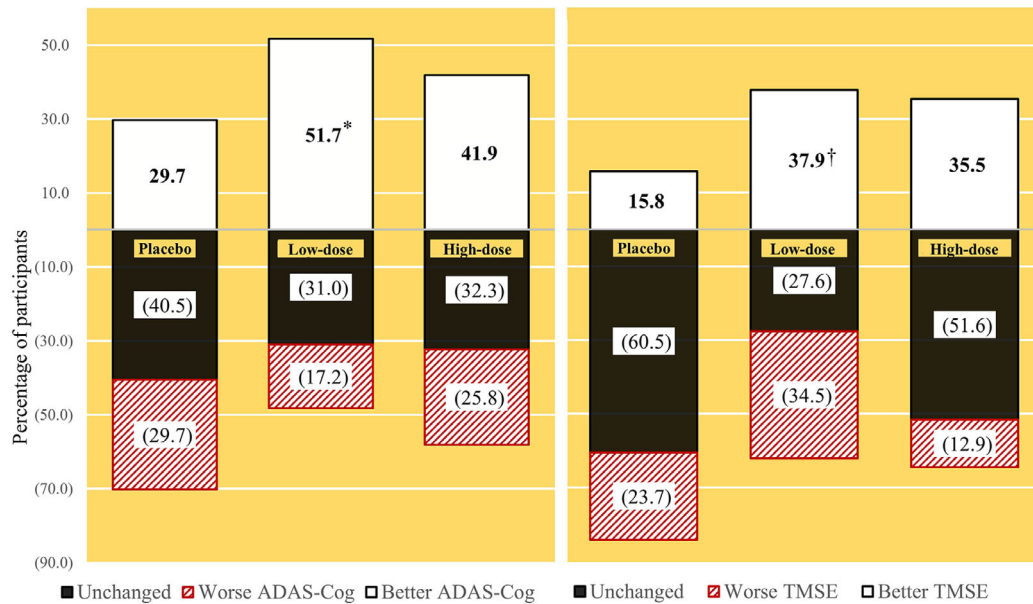
FIGURE 3 Primary endpoint: the proportion of participants who achieved better MCID scores on the neuropsychiatric tests at the end of the study (−2.6 for ADAS-Cog and 1.4 for TMSE). A, ADAS-Cog; (B) TMSE. All adjusted P attained from logistic regression adjusted by age, sex, education years, the status of AChEI use, and baseline cognitive score of each test (for more detail, see Tables S3, S4). * Statistically significant adjusted P = .026 OR 3.70 (95% CI: 1.17–11.68). † Statistically significant adjusted P = .034 OR 3.69 (95% CI: 1.11–12.31). The proportion of participants who experienced the stable or better outcome of MCID level was not statistically significant in either ADAS-Cog (82.8% of patients in the low-dose group and 74.2% of patients in the high-dose group vs. 70.3% of patients in the placebo group, adjusted P = .139 for low-dose and .635 for high-dose, respectively) or TMSE (65.5% of patients in the low-dose group and 87.1% of patients in the high-dose group vs. 76.3% of patients in the placebo group, adjusted P = .412 for low-dose and .317 for high-dose consecutively). AChEIs, acetylcholinesterase inhibitors; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version; CI, confidence interval; MCID, minimum clinically important difference; OR, odds ratio; TMSE, Thai Mental State Examination
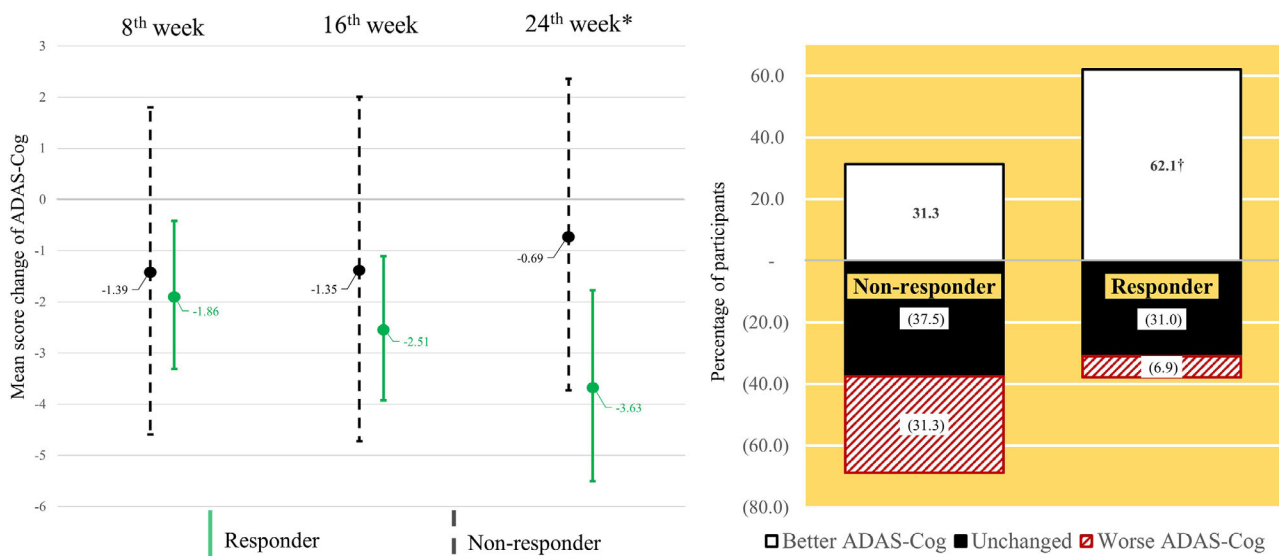

FIGURE 4 Post hoc analysis of the participants in the intervention group categorized by the responsiveness of antioxidant activity (5% reduction of normalized blood HNE level from baseline categorized for responder), (A), mean score analysis; (B) proportion analysis. A, Mean score change of ADAS-Cog, adjusted P attained from the linear regression after adjusting for age, sex, education years, AChEI use status, follow-up time, and baseline ADAS-Cog score. B, Proportion of participants who encountered the MCID level score of the ADAS-Cog. Adjusted P attained from logistic regression after adjusting for age, sex, education years, AChEI use status, and baseline ADAS-Cog score. *Statistically significant adjusted P = .049 mean −3.63 (95% CI: −5.50, −1.77) versus −0.69 (95% CI: −3.73, 2.36) of non-responders. †Statistically significant adjusted P = .032 OR 6.01 (95% CI: 1.17–30.89). AChEIs, acetylcholinesterase inhibitors; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version; CI, confidence interval; HNE, 4-hydroxynonenal; MCID, minimum clinically important difference; OR, odds ratio;

TABLE 2 Linear mixed model analysis of mean score change, the interaction between treatment versus time, and quadratic time term[a]

Mean score change	Baseline cognitive score		Time		Low dose		High dose	
	Coef. ± SE	P	Coef. ± SE	P	Coef. ± SE	P	Coef. ± SE	P
ADAS-Cog								
Unadjusted[b]	−0.117 ± 0.044	.009[d]	−0.002 ± 0.034	.958	−1.500 ± 0.927	.109	−2.078 ± 0.934	.028[d]
Adjusted[c,d]	−0.107 ± 0.044	.018[d]	−0.001 ± 0.034	.966	−1.806 ± 0.935	.057	−2.095 ± 0.926	.026[d]
ADCS-ADL								
Unadjusted[b]	−0.185 ± 0.035	<.001[c,d]	−0.056 ± 0.036	.120	−0.200 ± 1.022	.845	1.408 ± 1.037	.1780
Adjusted[a,c]	−0.177 ± 0.038	<.001[c,d]	−0.056 ± 0.036	.122	−0.205 ± 1.051	.845	1.355 ± 1.051	.2070
NPI								
Unadjusted[b]	−0.616 ± 0.063	<.001[c,d]	−0.044 ± 0.028	.117	−0.218 ± 0.650	.738	−0.885 ± 0.649	.1760
Adjusted[c,d]	−0.613 ± 0.063	<.001[c,d]	−0.044 ± 0.028	.118	−0.141 ± 0.654	.830	−0.874 ± 0.641	.1760
TMSE								
Unadjusted[b]	−0.126 ± 0.053	.020[d]	−0.009 ± 0.017	.620	0.482 ± 0.423	.258	0.867 ± 0.426	.044[d]
Adjusted[c]	−0.114 ± 0.055	.039[d]	−0.009 ± 0.017	.624	0.501 ± 0.435	.252	0.853 ± 0.429	.049[d]
CDR-SB								
Unadjusted[b]	−0. 291 ± 0.051	<.001[d]	−0.004 ± 0.014	.793	−0.220 ± 0.351	.532	−0.201 ± 0.357	.5740
Adjusted[c]	−0.296 ± 0.051	<.001[d]	−0.004 ± 0.014	.797	−0.180 ± 0.354	.612	−0.219 ± 0.353	.6120

Abbreviations: ADAS-Cog, the Alzheimer's Disease Assessment Scale–Cognitive Subscale, 11-task version; ADCS-ADL, Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory 23-item Scale; CDR-SB, Clinical Dementia Rating Scale (sum of boxes); Coef., the regression coefficients; NPI, Neuropsychiatric Inventory Questionnaire (patient subscale); P, P-value; SD, standard deviation; SE, standard error; TMSE, Thai Mental State Examination.
[a]All models meet the assumptions. For more details about mean score changes, see Table S2.
[b]Unadjusted P from linear mixed model.
[c]Adjusted P attained from the linear mixed model after adjusting for age, sex, years of education, AChEI use status, follow-up time, and baseline cognitive score of each test.
[d]Statistically significant.

non-responders. This might expound on the controversial result of the primary endpoint between mean score change and proportion efficacy analysis. The principal difference in baseline characteristics between the responders and non-responders was the responder's significantly higher outset of initial blood normalized HNE levels (Figure S3). These results concluded that WME could perform better in participants previously exposed to additional oxidative stress. Our findings are consistent with previous studies of the elevation of cerebrospinal fluid (CSF)[36] or plasma HNE[37] as a biomarker of oxidative stress in AD. These were connected with the pathophysiology of increment in oxidative substance production of AD brain due to $A\beta_{1-42}$, activated microglia, and dysfunctional mitochondria.[38] As AD was the disease with multifactorial etiologies,[39,40] our finding could link to the concept that oxidative damage was one of the primary etiologies of AD, explaining the selective effect of WME in each patient.

The strength of this study was the confidence of the data collected from the same identified primary caregiver throughout the trial. There was also a high adherence and compliance rate for the investigation in AD patients. Other co-interventions that could interfere with antioxidative therapy were checked, advised to stop, and reiterated at every visit. To minimize inter-rater reliability, the geriatric neurologist and psychologist who conducted the neuropsychiatric test were unchanged for each patient until the end of the trial.

There were several limitations in this study. First, although the baseline age, sex, education, and TMSE were similar, the placebo group tended to start with higher ADAS-Cog and CDR scores but lower ADCS-ADL scores. Second, this study was confined to mild to moderate AD patients with an initial stable dose of antidementia or psychotropic medications and no dose adjustment plan to control the potential cognitive and NPS effects. Therefore, participants were more likely to complete the study. However, this might limit generalization. Third, the slight advantages of WME in our research might result from several reasons. The small sample size could diminish the power of the study. Furthermore, the limited duration for the study of antioxidants with cognitive outcomes might hinder the WME benefit. Antioxidant mechanisms in antioxidative non-responders may require more time to reveal clinical outcomes. In investigating mild to moderate AD for the efficacy of potent antioxidants, α-tocopherol (vitamin E), on slow progression of ADCS-ADL sequelae,[41] took a mean follow-up of 2.27 years to obtain a positive result. However, the 24-week study period resembled the mean period of other investigations of AChEIs in cognition.[42] Nevertheless, the positive finding of the proportion analysis of participants who achieved the MCID response at the end of the study ensures the benefit of the WME. Last, the paucity of pharmacokinetic (PK) and pharmacodynamic information of herbal products was one common issue in clinical research. Based on current evidence, there was

no PK study for the bioactive substance of WME owing to the problem of bioactive polyphenol substance identification of WME (supporting information). WME contained several organic polyphenols, which were not yet all identified.[11] We found no dose-dependent response of WME. Evidence from the preclinical and phase I trial demonstrated a dose-dependent antioxidant effect.[17,20] In the vivo mice model, a higher WME dose yielded better benefits in enhancing the memory and antagonizing the anticholinergic effect.[3] However, no dose-dependent response was observed in the acetylcholinesterase inhibiting effect.[23] In the current study, no dose response was demonstrated in clinical measures despite the dose response of the antioxidant effects. One possible explanation might be from a short period of increased high dose in the last 12 weeks of the study.[20]

In conclusion, we demonstrated slightly improved cognitive outcomes in older AD patients after 6 months of WME use. Post hoc analysis proved that the benefit of WME might be individual, depending on the antioxidative response in each patient. WME was safe, enhanced the antioxidative effect, and might be considered an adjuvant therapy.

HUMAN ETHICS APPROVAL DECLARATION

This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. On-site monitoring was conducted regularly for all patient data. Every participant or his or her legal representative signed written informed consent.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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