Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Wolffia globosa (Mankai)

Evidence Summary

Rich in polyphenols, mankai may benefit brain, metabolic, and cardiovascular health, though most studies have tested it as part of a Mediterranean diet. It may increase manganese intake, a potential concern.

Neuroprotective Benefit: In obese or dyslipidemic people, the green Mediterranean diet including mankai slowed the decline in hippocampal volume but did not affect cognition. No clinical trials have tested mankai alone in cognitive aging or dementia.

Aging and related health concerns: In diabetics, mankai decreased postprandial glucose. In obesity/dyslipidemia, a Mediterranean diet with mankai reduced body weight, waist circumference, LDL-c, blood pressure, intrahepatic fat, and CVD risk score.

Safety: No clinical trials have tested the long-term safety of mankai supplementation. Mankai contains high levels of manganese, which could be unsafe. Mankai contains phylloquinone, which could antagonize the effects of anticoagulants such as coumarins.

Availability: not much availability in the US; usually in powder form or frozen cubes for smoothies; In Thailand and other Asian countries, it is added to food (e.g., curry, salad, omelet).	**Dose**: A randomized controlled trial testing the green Mediterranean diet including mankai shakes used 100 grams of frozen plant cubes per day.	**Chemical formula:** N/A **MW**: N/A
Half-life: varies across compounds	**BBB**: polyphenols can cross the bbb	
Clinical trials: A randomized controlled trial testing the green Mediterranean diet including mankai shakes enrolled 294 participants.	**Observational studies**: None have evaluated mankai specifically.	

What is it?

Wolffia globosa, also known as mankai, is an aquatic plant of the duckweed family. It has a unique nutritional composition for a plant, with about 45% of its dry weight composed of protein including all 9 essential amino acids, 35-40% consisting of carbohydrates, and 9.5-12% consisting of fat (EFSA Panel). Mankai is also rich in omega-3 fatty acids, dietary fiber, polyphenols, iron, and several micronutrients including beta-carotene, riboflavin, vitamin B6, and folate. Total polyphenolic content ranges from 382 to 700 mg/100 g (gallic acid equivalents). One cup of mankai shake (equivalent to 20 g of dry matter) provides 18% of recommended protein intake, 75% of iron intake, 60% of folic acid intake, and 21% of vitamin B12 intake (Sela et al., 2020).

Mankai has been used as a vegetable and additives to food in northern Thailand, Myanmar, and Laos (EFSA Panel). In the US, mankai powder was on the market after 2019 as a food ingredient (e.g., plant-based meat alternatives, shakes, and baked goods); however, commercial availability is limited.

Neuroprotective Benefit: In obese or dyslipidemic people, the green Mediterranean diet including mankai slowed the decline in hippocampal volume but did not affect cognition. No clinical trials have tested mankai alone in cognitive aging or dementia.

Types of evidence:

- 1 clinical trial testing the effects of green Mediterranean diet that included mankai shakes
- No laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No studies have tested mankai by itself as an intervention for preventing dementia or cognitive decline.

In a randomized controlled trial (DIRECT PLUS), 224 participants with abdominal obesity or dyslipidemia were randomly assigned to one of three groups: 1) a control group that received nutritional counseling promoting a healthy diet, 2) a low-calorie Mediterranean diet group (men: 1500-1800 kcal/day; women: 1200-1400 kcal/day) with high amounts of vegetables, poultry/fish replacing beef/lamb, and 28 grams of walnuts/day (+440 mg/day of polyphenols), and 3) a low-calorie green Mediterranean diet group that followed the same instructions as the Mediterranean diet group, but in addition, consumed green tea (3-4 cups/day) and a green shake of mankai (*Wolffia globosa*, 100 grams, frozen plant cubes) as a dinner substitute, together adding 800 mg/day of polyphenols, while avoiding processed and red meat completely (Kaplan et al., 2022). Both the Mediterranean and green Mediterranean diet groups had ~40% of total fat from polyunsaturated fatty acids and monounsaturated fatty acids, with carbohydrates consisting of less than 40 grams per day in the first 2 months and increased gradually to 80 grams per day. All groups were given free gym membership and encouraged to get moderate-intensity physical activity with ~80% aerobic content. After 18 months, there was an overall decline in the volume of the hippocampus due to the normal changes that occur with aging, and this decline was more pronounced in participants over 50, consistent with previous findings showing that atrophy of the hippocampus accelerates at the age of 55 (Schmidt et al., 2018). However, in participants over the age of 50, there was less decline in the volume of the hippocampus in people eating the Mediterranean diet compared to those in the control group, with the best outcomes seen in people consuming the green Mediterranean diet (hippocampal occupancy: -0.8±1.6% in the green Mediterranean group vs. -1.3±1.4% in control; 95% CI, -1.5 to -0.02; p=0.042). Many metrics were associated with slower decline in the volume of the hippocampus. For people over the age of 50, weight loss, better insulin sensitivity

(measured by HOMA-IR), and lower triglyceride levels were associated with slower decline in hippocampus size.

For all age groups, greater intake of mankai (p=0.043), green tea (p=0.016), and walnuts (p=0.023), and reduced intake of red and processed meat (p=0.047 and 0.042, respectively) were associated with slower decline in hippocampus size (Kaplan et al., 2022). This finding was partly confirmed by a urine analysis, where participants with high urine levels of specific polyphenols (urolithin A and tyrosol), reflecting high dietary intake of polyphenols, had slower decline in hippocampus size.

In a posthoc analysis of the DIRECT PLUS trial, improved glycemic control (measured by HbA1c, HOMA-IR, and fasting glucose) contributed to the neuroprotective benefit of both the Mediterranean and green Mediterranean diets on the slowing of age-related hippocampal volume loss (Pachter et al., 2024).

Interestingly, neither the Mediterranean diet nor the green Mediterranean diet were associated with benefits in cognitive functions in this study (Kaplan et al., 2022). This lack of cognitive effect may be explained by the relatively young age (average, 51 years old) and good baseline cognitive health of the study participants, the small size of the study, and the short intervention time. A future larger study with a longer intervention testing mankai specifically in an older population could potentially answer the question of whether mankai has benefits for neuroprotection and cognitive function.

Human research to suggest benefits to patients with dementia:

No clinical trials have tested mankai in dementia patients.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Mankai is rich in many nutrients and micronutrients that are important for optimal brain function, including omega-3 fatty acids, vitamin B12, folate, and polyphenols (Meir et al., 2021). About 200 different polyphenols and phenolic compounds have been detected in mankai. Examples of polyphenols detected in the mankai plant included quercetin, rutin, myricetin, apigenin, luteolin, epicatechin, caffeic acid, gallic acid, resveratrol, coumarin, and carnosol.

In the DIRECT PLUS randomized controlled trial described above, serum vitamin B12 levels increased by 5.2% (+1.25 ± 126.5 pg/mL) in the control group, 9.9% (+32.6 ± 76.2 pg/mL) in the Mediterranean diet group, and 15.4% (+48.8 ± 124.9 pg/mL) in the mankai-containing green Mediterranean/low-meat diets

(p=0.025 between control and green Mediterranean groups)(Sela et al., 2020). When statistically adjusted for age, sex, and baseline vitamin B12 levels, the significant difference remained (p=0.01).

Mankai cultured in greenhouse conditions contained a substantial level of bioactive forms of vitamin B12, and levels were stable across 4 seasons (Sela et al., 2020). Although vitamin B12 is typically rich in animal products like red meat, dairy, and eggs, the green Mediterranean diet discouraged meat intake, yet vitamin B12 levels increased significantly with the green Mediterranean diet. This increase is likely attributed to the high levels of vitamin B12 present in mankai.

APOE4 interactions: The randomized controlled trial evaluating the effects of the Mediterranean diet and the green Mediterranean diet (including mankai) against control reported no associations of APOE4 genotype with brain volumes or with trajectories of brain volumes after 18 months of diet-lifestyle intervention, but the study had a low number of APOE4 carriers (Kaplan et al., 2022). Also, because mankai was not tested by itself, APOE4 interactions could not be evaluated for mankai specifically.

Aging and related health concerns: In diabetics, mankai decreased postprandial glucose. In obesity/dyslipidemia, a Mediterranean diet with mankai reduced body weight, waist circumference, LDL-c, blood pressure, intrahepatic fat, and CVD risk score.

Types of evidence:

- 1 randomized controlled trial testing the effects of green Mediterranean diet that included mankai (numerous posthoc analyses of different outcomes presented across many publications)
- 1 randomized crossover controlled trial testing mankai drink in type 2 diabetes
- 1 randomized controlled trial comparing mankai green shake with yogurt shake
- A few laboratory studies

Obesity: BENEFIT WITH THE GREEN MEDITERRANEAN DIET
No clinical trials have tested mankai alone for obesity.

In the DIRECT PLUS randomized controlled trial, 294 participants with abdominal obesity or dyslipidemia were randomly assigned to one of three groups for 18 months: 1) a control group that received nutritional counseling promoting a healthy diet, 2) a low-calorie Mediterranean diet group with high amounts of vegetables, poultry/fish replacing beef/lamb, and 28 grams of walnuts/day (+440 mg/day of polyphenols), and 3) a low-calorie green Mediterranean diet group that followed the same instructions

as the Mediterranean diet group, but in addition, consumed green tea (3-4 cups/day) and a green shake of mankai (*Wolffia globosa*, 100 grams, frozen plant cubes) as a dinner substitute, together adding 800 mg/day of polyphenols, while avoiding processed and red meat completely (Tsaban et al., 2020). The co-primary outcomes were 18-month changes in abdominal fat, intrahepatic fat, and obesity. Weight loss was similar between the two Mediterranean diet groups; the green Mediterranean diet group lost 6.2±5.9 kg, the Mediterranean diet group lost 5.4±5.6 kg, and the control group lost 1.5±3.9 kg ($p<0.001$ for both comparisons with control). However, the green Mediterranean group had a greater reduction in waist circumference (-8.6±6.5 cm) than the Mediterranean (-6.8±5.9 cm; $p=0.033$) and control (-4.3±4.7 cm; $p<0.001$) groups.

In the same DIRECT PLUS randomized trial, the effects of the Mediterranean diet and the green Mediterranean diet on visceral adipose tissue were evaluated (Zelicha et al., 2022). Visceral adipose tissue is closely associated with the development of cardiovascular risk factors such as type 2 diabetes, dyslipidemia, hypertension, and mortality (Despres, 2012; Dhokte and Czaja, 2024; Saad et al., 2022). Visceral adipose tissue adipocytes produce more proinflammatory cytokines (reviewed in Kolb, 2022). In contrast, superficial subcutaneous adipose tissue is correlated with improved glycemic control and better cardiovascular health (Golan et al., 2012). In the DIRECT PLUS study, the green Mediterranean diet intervention had double the loss of visceral adipose tissue compared to the Mediterranean diet intervention and the control group (control, -4.2%; Mediterranean, -6.0%; green Mediterranean, -14.1%; $p<0.05$)(Zelicha et al., 2022). These differences in loss of visceral adipose tissue across the groups remained significant after adjusting for age, sex, and 18-month waist circumference change. Greater visceral adipose tissue was associated with higher cardiovascular risk score ($r=0.41$), systolic ($r=0.36$) and diastolic blood pressure ($r=0.28$), triglycerides ($r=0.21$), glucose ($r=0.30$), HOMA-IR ($r=0.47$), and the inflammation marker IL-6 ($r=0.30$), and lower HDL-c levels ($r=-0.18$). Deep subcutaneous adipose tissue accumulation was associated with higher HOMA-IR ($r=0.24$), weight ($r=0.60$), waist circumference ($r=0.63$), cardiovascular risk score ($r=0.20$), and IL-6 ($r=14$). In contrast, greater superficial subcutaneous adipose tissue was associated with lower cardiovascular risk score ($r=-0.36$), systolic blood pressure ($r=-0.13$), and triglycerides ($r=-0.21$). Changes in superficial subcutaneous adipose tissue and deep subcutaneous adipose tissue were not significantly different between the 3 groups after adjustment for waist circumference.

Higher dietary consumption of green tea, walnuts, and *Wolffia globosa*; lower red meat intake; higher total plasma polyphenols, and elevated urine urolithin A (polyphenol) were significantly related to greater visceral adipose tissue loss ($p<0.05$, multivariate models)(Zelicha et al., 2022). Within the green Mediterranean diet group, higher intake of *Wolffia globosa* was significantly associated with a greater

loss of visceral adipose tissue (26% visceral adipose tissue loss at the highest intake level of ≥ 3/week; p=0.04 compared to the lowest level, after adjusting for age). Higher intake of *Wolffia globosa* was also associated with lower cardiovascular disease risk and improved lipid profile.

After adjusting for age, sex, and waist circumference loss, total plasma polyphenol levels remained significantly associated with the loss of visceral adipose tissue (p=0.018)(Zelicha et al., 2022). Among plasma polyphenols examined, hippuric acid levels at 18 months were significantly higher in both Mediterranean diet interventions than in the control group ($p<0.05$) and were associated with loss of visceral adipose tissue in a multivariate model adjusted for age, sex, and waist circumference change. Higher urine levels of urolithin A was strongly correlated with lower visceral adipose tissue, even after adjusting for multiple comparisons for 139 identified metabolites ($r=-0.241$, $p<0.001$). Higher urolithin A levels were correlated with higher consumption of walnuts and mankai.

Ghrelin, known as the hunger hormone, is high during fasting and decreases after eating. Lower fasting ghrelin levels are associated with obesity and metabolic syndrome. In a secondary analysis of the above DIRECT PLUS trial, after 18 months of intervention, fasting ghrelin levels increased by 1.3%, 5.4%, and 10.5% in control, Mediterranean, and green Mediterranean groups, respectively (p=0.03 for green Med vs control groups)(Tsaban et al., 2022). Among men, an increase in fasting ghrelin levels was associated with favorable changes in insulin resistance profile (HbA1c and HOMA-IR) and visceral adipose tissue regression, after adjusting for relative weight loss. In contrast, female participants had a nonsignificant increase in fasting ghrelin levels after 6 months of intervention, but a significant reduction after 18 months that was numerically greater in the green Mediterranean group than the Mediterranean and control groups (control, −2.8±5.5%; Mediterranean, −5.8±7.3%; green Mediterranean, −20.1±7.1%). Because women were underrepresented in this trial, it is not clear if this is a true sex-specific difference.

In a secondary analysis of the DIRECT PLUS trial, the effects of the Mediterranean diet and the green Mediterranean diet on fasting morning cortisol levels were evaluated (Alufer et al., 2023). Cortisol levels are regulated by the central pacemaker in the suprachiasmatic nucleus in the brain and levels peak in the morning and are lowest at night (Spencer and Deak, 2017). Dysregulation of cortisol levels can lead to insulin resistance and diabetes (Ortiz et al., 2019). In the DIRECT PLUS trial, higher baseline fasting morning cortisol was associated with age, dysglycemia, visceral adiposity, fasting plasma glucose, high-sensitivity C-reactive protein (hsCRP), testosterone, progesterone, and TSH levels (Alufer et al., 2023). After 6 months of Mediterranean diet interventions, there were no significant differences in fasting morning cortisol levels across groups (control, +7.6%, 317.3 ± 126 nmol/L; Mediterranean diet, +12.4%, 328.1 ± 107.5 nmol/L; green Mediterranean diet, +11.6%, 313.5 ± 106.27 nmol/L). The authors speculate

that the increase in cortisol levels seen in all groups may be partly explained by the rapid weight loss that occurred in the first 6 months, which could have triggered a stress response. However, after 18 months of intervention, both Mediterranean diet interventions resulted in significantly reduced fasting morning cortisol levels (Mediterranean diet, -1.6%; -21.45 nmol/L; green Mediterranean diet, -1.8%; -26.67 nmol/L; $p<0.05$ vs baseline), while the control group showed no significant changes (+4% compared to baseline; -12 nmol/L). There was a significant difference between the green Mediterranean diet and control groups ($p=0.048$ multivariate model adjusted for age, sex, and weight loss from baseline). Fasting morning cortisol levels were significantly reduced in men ($p<0.01$), while in women, there was a trend for reduction ($p=0.08$). The decrease in fasting morning cortisol after 18 months of intervention was associated with favorable changes in fasting plasma glucose, HbA1c, hsCRP, TSH, testosterone and hepatosteatosis ($p<0.05$ for all).

In another secondary analysis of the DIRECT PLUS trial, the effects of the Mediterranean diet and the green Mediterranean diet on blood methylome and trascriptome were evaluated (Hoffman et al., 2023). Polyphenols can inhibit regulators of epigenetic processes such as DNA methyltransferases or methylenetetrahydrofolate reductase (MTHFR), which helps metabolize folate and homocysteine. The study found that the green Mediterranean diet appears to have a higher capacity to regulate blood epigenome than the Mediterranean diet or the control. In addition to the direct activation of the folate and methionine cycle by increasing its regulators, folate and vitamin B12, the green Mediterranean diet also modulated epigenetic regulators.

In another analysis of the DIRECT PLUS trial, both Mediterranean diets induced substantial changes in the gut microbiome, with the green Mediterranean diet leading to more prominent compositional changes, largely driven by the low abundant microorganisms (Rinott et al., 2022). The green Mediterranean diet was associated with enrichments in the genus *Prevotella* (genus associated with high fiber intake) and enzymatic functions involved in branched-chain amino acid degradation, and reductions in the genus *Bifidobacterium* and enzymatic functions responsible for branched-chain amino acid biosynthesis. Dysregulation of the branched-chain amino acid balance is a hallmark of obesity and insulin resistance (reviewed in Abdualkader et al., 2024). The intervention intensity of the Mediterranean and green Mediterranean diet was associated with an increase in branched-chain amino acid degradation pathways and increased insulin sensitivity measured by HOMA-IR (Rinott et al., 2022).

In a sub-study of the DIRECT PLUS trial, after 6 months of intervention, 90 participants provided fecal samples that were processed into frozen, opaque, and odorless capsules of autologous fecal microbiota transplantation (Rinott et al., 2021). Participants were assigned to either 100 capsules containing their

own fecal microbiota or placebo pills for 8 months, from month 6 to month 14. The primary outcome was weight regain during months 6-14, which were not significantly different across control, Mediterranean, and green Mediterranean groups. However, the autologous fecal microbiota transfer (aFMT) intervention significantly attenuated weight regain in the green-Mediterranean group (aFMT, 17.1%; placebo, 50%; p=0.02), but not in the control or Mediterranean diet groups. Similarly, aFMT treatment attenuated waist circumference gain (aFMT, 1.89 cm; placebo, 5.05 cm; p=0.01) and insulin rebound (aFMT, -1.46±3.6 µIU/mL; placebo, +1.64±4.7 µIU/mL; p=0.04) in the green Mediterranean group but not in the control or Mediterranean groups. The green-Mediterranean diet was the only intervention to induce a change in microbiome composition during the weight-loss phase, and to promote preservation of weight-loss-associated specific bacteria after the aFMT. In an exploratory analysis of mankai specifically, increased frequency of mankai intake at 6 months was associated with lower subsequent weight regain in people receiving aFMT treatment compared to placebo (p=0.04). A trend was also seen with increased green tea intake at 6 months (p=0.06).

Cardiovascular disease: BENEFIT WITH THE GREEN MEDITERRANEAN DIET
No clinical trials have tested mankai alone for the treatment or prevention of cardiovascular disease.

In the DIRECT PLUS randomized controlled trial, after 6 months the green Mediterranean group achieved greater decrease in LDL cholesterol [green Mediterranean, -6.1 mg/dL (-3.7%), Mediterranean, -2.3 mg/dL (-0.8%), control -0.2 mg/dL (+1.8%); p=0.012 between green Mediterranean and control groups] and diastolic blood pressure (green Mediterranean, -7.2 mmHg; Mediterranean, -5.2 mmHg; control, -3.4 mmHg; p=0.005 between green Med and control groups)(Tsaban et al., 2020). The LDL-C/ HDL-C ratio decline was greater in the green Mediterranean group (-0.38) than in the Mediterranean (-0.21; p=0.021) and control (-0.14; p<0.001) groups. Triglyceride levels were reduced similarly in both Mediterranean diet groups (p=0.95) and significantly more than in the control group (p=0.008 and 0.11 for Mediterranean and green Mediterranean groups, respectively). Reduction in systemic inflammation, measured by high- hsCRP, was greater in the green Mediterranean group (-0.52 mg/L) than in the Mediterranean (-0.24 mg/L; p=0.023) and control (-0.15 mg/L; p=0.044) groups. Also, the green Mediterranean group achieved a better improvement in the 10-year Framingham Risk Score after 6 months of intervention (-3.7% absolute risk reduction, from 13.7 to 10.4%) compared to the Mediterranean group (-2.3%; p=0.073) and control group (-1.4%; p<0.001). The authors speculate that the reduction in LDL cholesterol with the green Mediterranean diet could be due in part to lower intake of meat and poultry and the high phytosterol content of the mankai shake which compete with cholesterol for absorption in the digestive system. The authors also discuss that the decrease in systolic

and diastolic blood pressures may be mediated by the increased fiber content and higher levels of nitric oxide from vegetables.

In the same DIRECT PLUS randomized trial, the effects of the Mediterranean diet and the green Mediterranean diet on proteome panels were evaluated (Zelicha et al., 2024). After 6 months of intervention, both the Mediterranean and the green Mediterranean interventions induced improvements in cardiovascular disease and proinflammatory risk proteins ($p<0.05$ compared to control), with the green Mediterranean diet producing more pronounced beneficial changes. These effects were driven by proinflammatory proteins (IL-1 receptor antagonist protein [IL-1RA; associated with leptin resistance in obesity], IL-16, IL-18, thrombospondin-2, leptin, prostasin, galectin-9, and fibroblast growth factor 21; adjusted for age, sex, and weight loss; $p<0.05$). Exclusively in the green Mediterranean diet were significant decreases in proinflammatory and cardiovascular disease risk proteins: CSTL1, HAOX1, IL-18, leptin, protein AMBP, PRSS8, spondin-2, IL-1 receptor-like 2, macrophage receptor MARCO, and FGF21. However, after 18 months of intervention, most proteins returned to levels similar to baseline levels and the significant changes seen across groups were attenuated.

Diabetes and metabolism: IMPROVED GLUCOSE LEVELS AND HOMA-IR
In a randomized crossover controlled trial of 45 patients with type 2 diabetes, mankai drink (~10 g of dry matter in 300 mL; 40 kcal) following dinner for 2 weeks significantly lowered postprandial glucose peak by 19.3% (Δpeak=24.3±16.8 vs 30.1±18.5 mg/dL; $p<0.001$) and delayed the time-to-peak by 20.0% (112.5 [interquartile range, 75 to135] vs 90 [60-105] min; $p<0.001$) compared with control (300 mL of water)(Tsaban et al., 2024). These changes in glucose levels are comparable to those after 4 days of treatment with SGLT2 inhibitors (dapagliflozin and canagliflozin) or sulphonylurea medications. The mankai drink contained 200 mL of freshly harvested mankai with 100 mL of water flavored with erythritol (apple, passion fruit, or coconut flavors), provided in frozen bottles. Each mankai drink contained 0.8 g of carbohydrates, 4.1 g of protein, 0.8 g of fats, 0.2 µg of vitamin B12, 113.9 µg of folic acid and 76.3 mg of polyphenols. Two thirds of participants responded favorably to mankai, while others showed neutral responses. Postprandial glucose was measured using continuous glucose monitoring. The mankai intervention lowered glucose levels starting from 30 minutes and until 135 minutes postprandially ($p<0.05$ for all time points). The postprandial glucose incline and decline slopes were shallower with mankai treatment compared to water ($p<0.001$ and $p<0.01$, respectively). Mean postprandial net incremental area-under-the-glucose curve was 20.1% lower with mankai compared with water (50.9 vs 63.7 mg/[dL min]; $p=0.03$). The mean glucose peak from baseline was 19.3% lower with mankai (+24.3±16.8 mg/dL) than with water (+30.1±18.5 mg/dL; $p<0.001$). Weight reduction was observed in both groups (mankai first, -0.5±1.0 kg; water first: −0.9±1.1 kg), but there were no

differences between groups. Reductions in the liver enzyme, alanine transaminase, was seen with both interventions, but parallel reduction in aspartate transaminase was only observed under the mankai intervention ($p<0.05$).

In a randomized controlled crossover trial, 20 abdominally obese participants replaced dinner with a mankai shake (*Wolffia globosa* duckweed; 3 frozen cubes of 25 g each) or a yogurt shake (Zelicha et al., 2019). These participants were recruited from the DIRECT PLUS study. The 2-week crossover substudy was carried out in the initial phase of the DIRECT PLUS trial. Both shakes included 28 g of walnuts and one medium-sized banana. The two shakes were equivalent in calories (366 kcal in the Wolffia globosa Mankai shake; 351 kcal in the yogurt shake) and macronutrient contents (carbohydrates, 35 g in both the mankai and yogurt shakes; protein, 12 g in the mankai shake, 11 g in the yogurt shake; fat, 20 g in the mankai shake, 19 g in the yogurt shake). Mankai shake, compared to the yogurt shake, produced lower postprandial glucose peaks (mankai, 13.4±9.2 mg/dL; yogurt, 19.3±15.1 mg/dL; p= 0.044), more delayed glucose peaks (mankai, 77.5 ± 29.2 min; yogurt, 59.2±28.4 min; p=0.037), and a faster return to baseline glucose levels (mankai, 135.8±53.1 min; yogurt, 197.5±70.2 min; p=0.012). The mankai shake also resulted in lower next-morning fasting glucose levels (mankai, 83.2±0.8 mg/dL; yogurt, 86.6±13 mg/dL; p=0.041). Satiety rank was slightly higher for the mankai shake compared with the yogurt shake (7.5 vs. 6.5; p=0.035).

In the DIRECT PLUS randomized controlled trial, after 6 months, the green Mediterranean group achieved a greater decrease in homeostatic model assessment for insulin resistance compared to the control group (HOMA-IR; green Mediterranean, -0.77; Mediterranean, -0.46; control, -0.27; p=0.020 between green Med and control groups)(Tsaban et al., 2020). However, fasting plasma glucose was similarly decreased in all 3 groups.

Metabolic dysfunction-associated steatotic liver disease (MASLD): BENEFIT WITH THE GREEN MEDITERRANEAN DIET
MASLD is a condition where too much fat is stored in the liver (in people who drink little or no alcohol) and this condition is associated with elevated liver enzymes, insulin resistance, type 2 diabetes, cardiovascular disease risk, and cancers (formerly known as NAFLD). No clinical trials have tested mankai alone in people with MASLD.

In the DIRECT PLUS randomized controlled trial, after 18 months of intervention, the MASLD prevalence declined from 62% to 54.8% in the control group, 47.9% in the Mediterranean group, and 31.5% in the green Mediterranean group (p=0.012 between groups)(Meir et al., 2021). Adjustment for weight loss

resulted in a significant difference in MASLD prevalence between the two Mediterranean diet groups (p=0.024). The green Mediterranean group achieved approximately double the percentage of intrahepatic fat loss (-38.9% proportionally), as compared with the Mediterranean group (-19.6% proportionally; p=0.035, weight loss adjusted) and control group (-12.2% proportionally; p<0.001). After 18 months, both Mediterranean diet groups had significantly higher total plasma polyphenol levels (0.47±0.4 mg/L for both) as compared with the control group (0.35±0.4 mg/L; p<0.05 for both), with higher detection of naringenin (control, 4.4%; Med, 30.4%; green Med, 65.2%; p=0.001) and 2-5-dihydroxybenzoic-acid (control, 11.9%; Med, 37.4%; green Med, 50.7%; p<0.001) in the green Mediterranean diet group. Greater intrahepatic fat % loss was associated with each of the following: increased intake of mankai, increased intake of walnuts, decreased red/processed meat consumption, improved serum folate, a decline in diastolic blood pressure, decreased triglycerides/HDL ratio, and decreased cholesterol/HDL ratio, (p<0.05 for all).

The authors discuss that the green Mediterranean diet may reduce liver fat buildup (liver steatosis) through actions of abundant polyphenols, which reduce de novo lipogenesis, increase fatty acid oxidation, and reduce oxidative stress (Meir et al., 2021). Because of the numerous elements to the green Mediterranean diet, including increased green tea, mankai, walnuts, and decreased meat intake, it is not possible to pinpoint the effects of mankai alone.

Lifespan: UNKNOWN; HIGHER MANKAI INTAKE INVERSELY CORRELATED WITH AGING CLOCKS
No studies have tested the effects of *Wolffia globosa* on lifespan or mortality.

In a secondary analysis of the DIRECT PLUS trial, various biological aging epigenetic clocks were used to measure biological aging in 256 participants, and showed that both Mediterranean diet interventions had ~8.9 months of slowed Li methylation age clock at the end of the intervention compared to the expected methylation age (p=0.02), while no significant slowing was observed in the control group (Meir et al., 2023). Higher methylation age is associated with all-cause mortality (Christiansen et al., 2016). The findings are consistent with other studies showing a healthy lifestyle and diet decreases methylation age (Gensous et al., 2020; Fitzgerald et al., 2021). Various epigenetic clocks of different generations were used: Horvath2013, Hannum2013, Li2018, Horvath skin and blood2018, PhenoAge2018, PCGrimAge2022. Although the intervention groups did not significantly differ compared to control in terms of changes in methylation age clocks, greater green Mediterranean diet adherence was associated with a lower 18-month relative change in Li and Hannum clocks (p=0.004 and 0.03, respectively; multivariate models)(Meir et al., 2023). The green Mediterranean diet adherence score was based on the intake of 9 items: walnuts, vegetables, processed meat, red meat, legumes, fruits, fish, green tea,

and mankai. The Li methylation age remained significantly associated with greater green Mediterranean diet adherence after controlling for age, sex, baseline methylation age, and 18-month weight loss (p=0.004). The food items associated with lower methylation age change were mankai and green tea intake (p=0.061 and 0.0016, respectively, after adjusting for age, sex, baseline Li methylation age, and weight loss). Higher green Mediterranean diet adherence was also associated with Hannum methylation age relative change, Hannum age acceleration, and intrinsic epigenetic age acceleration changes (p=0.04 and 0.04, respectively), but not with the Horvath relative methylation age changes, Horvath skin and blood, PhenoAge, and DunedinPACE.

Baseline chronological age (51.3±10.6 years) was significantly correlated with all methylation age clocks with correlations ranging from 0.83 to 0.95 ($p < 2.2e^{-16}$ for all)(Meir et al., 2023). Greater Li clock slowing, after adjusting for age, sex, baseline methylation age clock, and weight loss, corresponded with elevated urine polyphenols: hydroxytyrosol (p=0.003), tyrosol (p=0.03), urolithin C (p=0.012), and a trend for elevation in urolithin A (p=0.08), highly common in green plants.

Safety: No clinical trials have tested the long-term safety of mankai supplementation. Mankai contains high levels of manganese, which could be unsafe. Mankai contains phylloquinone, which could antagonize the effects of anticoagulants such as coumarins.

Types of evidence:

- 1 safety assessment by the European Food Safety Authority Panel
- 1 randomized controlled trial testing a mankai drink in type 2 diabetes
- 1 randomized controlled trial comparing mankai, soft cheese, and green peas
- Several laboratory studies

No clinical trials have tested the long-term safety of mankai treatment on its own. The clinical trials that included mankai as part of a dietary intervention were not designed to investigate safety. In a randomized controlled trial of 36 men, effects of 3 iso-protein (30 g) meals were compared: soft cheese, green peas, and mankai (Kaplan et al., 2019). Blood concentrations of essential amino acids were significantly increased in all 3 iso-protein meals. Liver enzyme levels (ALT) remained unchanged after 180 minutes compared to baseline, with no significant differences between the 3 iso-protein meals.

In a randomized crossover controlled trial of 45 patients with type 2 diabetes, mankai drink (~10 g of dry matter in 300 mL; 40 kcal) following dinner for 2 weeks did not result in any side effects related to the

intervention (Tsaban et al., 2024). No participant withdrew from the study as a result of intolerance to interventions or an inability to consume mankai. Participants reported high personally perceived satiety after dinner, which did not differ during the mankai or water interventions.

In a safety assessment by the European Food Safety Authority Panel (EFSA Panel) on Nutrition, Novel Foods and Food Allergens, *Wolffia globosa* powder (cultivated mankai plant that is washed with hot water and dried) as a novel food was deemed a safety concern due to a potential increase in manganese intake (EFSA Panel). Intake of mankai was calculated for adults at 286 mg/kg bodyweight per day. The EFSA Panel noted that the safety of *Wolffia globosa* powder as a novel food could not be established; however, with the exception of concerns related to the manganese intake, considering the composition of mankai, consumption is "not nutritionally disadvantageous".

The concentration of manganese in the *Wolffia globosa* powder may reach 116.5 mg/kg, which is higher compared to foods rich in manganese, including nuts (24.9 mg/kg), dried fruit, nuts, and seeds (11.9 mg/kg), and chocolate (8.9 mg/kg)(EFSA Panel). The EFSA Panel noted that exposure to high levels of manganese may be neurotoxic. However, there is not a set upper limit for manganese intake. The EFSA has previously reported that estimated mean manganese intakes of adults in Europe ranged from 2 to 6 mg/day, with the majority of values around 3 mg/day. Mankai supplement alone (2.33 mg/day) could increase manganese intake by 39% as compared to the highest manganese intake estimates for adults.

The analyzed *Wolffia globosa* powder had concentrations of pesticides, mycotoxins, and cyanotoxins which were below the quantification limit (EFSA Panel). Stability testing showed that the powder is stable for 16 months from manufacturing date under recommended storage conditions (in nitrogen-sealed packs at room temperature and humidity below 60%). EFSA calculated the intake of heavy metals and trace elements from the powder, which are dependent on the cultivation conditions. The panel considers that levels of heavy metals and trace elements are not expected to exceed established maximum levels and upper levels for any population group.

In a subacute toxicity study (4-day repeated dosing) in rats, mankai treatment (0, 1700, 2500, and 3400 mg/kg/day) did not significantly affect body weight or result in abnormal findings (EFSA Panel). Clinical chemistry and hematology analysis showed no major differences between control and mankai-treated rats except for a highly significant ~50% reduction in concentrations of lactate dehydrogenase (LDH) and creatine kinase (CPK) with mankai. No gross pathology abnormalities were observed in the animals.

In a 28-day subchronic toxicity study, rats given mankai in chow (5, 10, or 20 g mankai/kg feed), corresponding to mean daily intake of 300, 600, and 1200 mg/kg bw in females and 250, 500, and 1000 mg/kg bw in males, did not result in any mortalities and there were no statistically significant differences in bodyweight, feed consumption, or clinical or histopathological observations compared to control rats (EFSA Panel). Another 28-day subchronic toxicity study was conducted in male rats given a rodent diet with 20 g/kg feed or a diet without mankai, and no significant differences between mankai and control were seen for bodyweight, feed consumption, hematological findings, and histopathological findings.

In a 90-day toxicity study, rats were fed 0, 5, 10, or 20% mankai (w/w) in a powdered diet (EFSA Panel; Kawamata et al., 2020). There were no treatment-related effects in clinical observations, body weight, food consumption, or ophthalmology. The mean mankai intakes were 3,176, 6,491, and 13,164 mg/kg bw per day in males and 3,583, 7,423, and 15,027 mg/kg bw per day in females.

Urinalysis data showed that mankai treatment significantly increased water intake and decreased urinary sodium in high-dose males (EFSA Panel). In female rats, no difference in water intake was observed. Mankai treatment was not associated with any findings regarding weight and histopathology of kidneys in male or female rats.

Hematology data showed a decrease in fibrinogen in mid- and high-dose female rats, which were considered as treatment-related (EFSA Panel). No changes were seen in male rats. No histopathological changes in the liver were observed with mankai treatment.

In high-dose male rats, there was a statistically significant decrease in the relative prostate weight (14% decrease), though there were no histopathological findings seen for prostate (EFSA Panel). In female rats receiving low-dose mankai, there was a significant decrease in heart weight, though no differences in histopathology were observed across groups.

Based on the totality of evidence from the rat toxicity studies, the EFSA Panel considers the middle dose tested for males (6.5 g/kg bw per day) as the overall no observed adverse effect level (NOAEL) of this study (EFSA Panel).

In a genotoxicity study, dry mankai was not genotoxic in a bacterial reverse mutation test and in vitro micronucleus assay (Kawamata et al., 2020).

In human cell lines (HUVEC, K-562, and HeLa cells), extracts from duckweed species, including *Wolffia globosa*, did not exert any anti-proliferative or cytotoxic effects (Sree et al., 2019).

Wolffia globosa grown in contaminated water could be of concern as this plant species has shown significant uptake and accumulation of cadmium, arsenic, and chromium (Boonyapookana et al., 2002; Zhang et al., 2009; Xie et al., 2013). For this reason, *Wolffia globosa* has been considered as having potential for phytofiltration of contaminated water and paddy soil.

Drug interactions: Drug interactions with mankai have not been well studied. In a safety assessment by the European Food Safety Authority Panel (EFSA Panel) on Nutrition, Novel Foods and Food Allergens, *Wolffia globosa* powder contained phylloquinone concentrations (2-12 mg/100 g), which when consumed as food supplement, may reach 2.4 mg/day for adults (EFSA Panel). This level of phylloquinone may antagonize the effects of anticoagulants such as coumarins, so people taking such therapies need to consult with their healthcare provider on whether mankai is safe for them.

Sources and dosing:

Mankai (*Wolffia globosa*) is usually in powder form or frozen cubes for smoothies. In Thailand and other Asian countries, it is added to food (e.g., curry, salad, omelet). Mankai powder is manufactured by cultivating *Wolffia globosa* plants in greenhouses or in a semi-open mesh construction under controlled conditions, with water and fertilizers used for the growth of the plant (EFSA Panel). Fresh plant material is washed with hot water and dried using a dehydrator or freeze-dryer.

Research underway:

Based on ClinicalTrials.gov, there is one ongoing clinical trial testing the effects of *Wolffia globosa*. A randomized controlled trial is testing the effects of *Wolffia globosa* on glycemic control in 104 people with type 2 diabetes (NCT06416475). The intervention is a mankai beverage (10 grams of dry matter per day dissolved in water) or control (water) 3 times per day, postprandially for 3 months. The primary outcome is HbA1c and fasting glycemic and insulin resistance profiling. This study was scheduled to be completed in August 2024; results have not been posted to date.

Search terms:

Pubmed, Google: *Wolffia globosa* and mankai

Websites visited for *Wolffia globosa* and mankai:

- ClinicalTrials.gov
- NIH RePORTER (0)
- Examine.com (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- Labdoor.com (0)
- Eatmankai.com
- Wannagreens.com

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