

Original Research



Positive effects of anthocyanin-rich mulberry milk on mental health problems in the working population: an open-label study

Poonsri Rangseekajee ^{1,2,3}, Nawanant Piyavhatkul ^{1,2,3},
Jintanaporn Wattanathorn ^{1,3}, Wipawee Thukham-mee ^{1,3}, and
Pongsatorn Paholpak ^{1,2,3§}

¹Research Institute for High Human Performance and Health Promotion, Khon Kaen University, Khon Kaen 40000, Thailand

²Brain and Mind Wellness Research Group, Khon Kaen University, Khon Kaen 40000, Thailand

³Department of Psychiatry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40000, Thailand

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Corresponding Author:


Pongsatorn Paholpak


Department of Psychiatry, Faculty of Medicine,
Khon Kaen University, 123 Mittraphap Road,
Nai Mueang Subdistrict, Mueang Khon Kaen,
Khon Kaen 40002, Thailand.
Tel. +66-43-363027
Email. ppaholpak@kku.ac.th


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cited.


ORCID iDs

Poonsri Rangseekajee 
<https://orcid.org/0000-0001-8565-748X>

Nawanant Piyavhatkul 
<https://orcid.org/0000-0002-0041-4241>

Jintanaporn Wattanathorn 
<https://orcid.org/0000-0002-7383-2348>

Wipawee Thukham-mee 
<https://orcid.org/0000-0001-6923-396X>

Pongsatorn Paholpak 
<https://orcid.org/0000-0003-0408-8460>

ABSTRACT

BACKGROUND/OBJECTIVES: Depression and anxiety are common mental health problems. Anthocyanins from berries might have an inhibitory effect on monoamine oxidase (MAO) enzymes and alleviate various mood and anxiety symptoms. This study examined the effects of a daily supplement of an anthocyanin-rich product on mental health problems.

SUBJECTS/METHODS: This study was a secondary analysis from a randomized, 6-week, open-label trial in 300 healthy participants aged 18–60 years who consumed 1 or 2 servings of anthocyanin-rich mulberry milk daily. The General Health Questionnaire-28 (GHQ-28) and Hospital Anxiety and Depression Scale (HADS) were used to monitor mental health problems. In addition, the saliva activity levels of MAO-A, MAO-B, and cortisol were examined at the baseline and after 6 weeks.

RESULTS: The total scores of the GHQ-28 and HADS and all their subscales decreased in both groups (all $P < 0.05$). The cortisol, MAO-A, and MAO-B activities decreased significantly (all $P < 0.05$), but there were no significant differences between the groups (all $P > 0.05$). Significant correlations were noted between the decreased activity level of MAO-A enzyme and decreased scores from the GHQ-28 somatic subscale and the HADS depression subscale (all $P < 0.05$).

CONCLUSIONS: Daily consumption of anthocyanin-rich mulberry milk possibly improves mental health problems by reducing depressive and anxiety symptoms in the working population. The suppression of MAO-A activity is a possible underlying mechanism.

Trial Registration: Thai Clinical Trial Registration: #TCTR20201031002

Keywords: Depression; anxiety; anthocyanins; mulberry

INTRODUCTION

Depression, anxiety, and stress are common psychiatric symptoms in the working population. According to the Organization for Economic Co-operation and Development,

Trial Registration

Thai Clinical Trial Registration:
#TCTR20201031002

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Conflict of Interest

The authors declare no potential conflicts of interests.

Author Contributions

Conceptualization: Rangseekajee P, Wattanathorn J, Paholpak P; Formal analysis: Piyavhatkul N, Wattanathorn J, Paholpak P; Investigation: Rangseekajee P, Wattanathorn J, Thukham-mee W, Paholpak P; Methodology: Rangseekajee P, Wattanathorn J, Paholpak P; Writing - original draft: Rangseekajee P, Piyavhatkul N, Wattanathorn J, Paholpak P; Writing - review & editing: Rangseekajee P, Piyavhatkul N, Wattanathorn J, Thukham-mee W, Paholpak P.

the working population is defined as those aged 15 to 64 years. Both depressive and anxiety symptoms may lead to mental health problems, which are correlated with a poor quality of life and decreased productivity in this population. Stress from the coronavirus disease 2019 pandemic, social distancing, and the global financial crisis have been associated with an increased prevalence of depression and anxiety [1]. Stress stimuli induce glucocorticoid release, especially cortisol, and boost the activities of monoamine oxidase type A (MAO-A) and type B (MAO-B) [2]. These decreased levels of monoamine neurotransmitters, especially serotonin, dopamine (DA), and norepinephrine, in the synaptic cleft lead to a wide range of psychological symptoms, including depressed mood, anxiety, and somatic symptoms. Therefore, many dietary supplements and functional foods targeting the changes in the levels of these neurotransmitters have been investigated to improve psychological well-being. On the other hand, their clinical efficacy is still unclear owing to a lack of evidence-based findings from a large clinical trial.

Different berry fruits, including Mulberry or *Morus alba*, can be used as food and medicine. Berry fruits are enriched with polyphenols, particularly anthocyanins, and their extracts have been investigated for their inhibitory effect on MAO enzymes [3,4]. Previous studies of mulberry extracts showed they possessed antioxidant, anti-inflammatory, and cognitive-enhancing properties in animal models [5-8]. Another recent study also revealed the acute antistress and procognitive effects in children 1.5 hours after consuming anthocyanin-rich mulberry milk [9]. To the best of the authors' knowledge, there have been no clinical studies on the effects of mulberry milk on adult mental health. Therefore, this study examined the effects of anthocyanin-rich mulberry milk on adult psychological symptoms.

This study was a secondary analysis from the primary study that investigated the impacts of daily 6-week supplementation of anthocyanin-rich mulberry milk on the cognitive performance and changes in the levels of corresponding monoamine enzymes [10]. This study examined the changes in the severity of mental health problems, particularly in depressed mood and anxiety, along with their correlations with the changes in the monoamine enzyme activities.

SUBJECTS AND METHODS

Ethics

This study was approved by the Center for Ethics in Human Research, Khon Kaen University, Khon Kaen, Thailand (#HE631051). Written informed consent was obtained from all participants prior to participation, and the study was conducted in accordance with the Declaration of Helsinki. This study was registered in Thai Clinical Trial Registry #TCTR20201031002.

Study design, settings, and participants

Details of the primary study are described elsewhere [10]. This study was a randomized, open-label trial of standard versus double doses with a 1:1 allocation ratio. The patients were randomized to receive a single or double serving of anthocyanin-rich mulberry milk based on a computer-generated list from <https://www.randomizer.org>. The study was conducted in a single industrial district in Bangkok, Thailand. Healthy individuals provided written informed consent. The inclusion criteria were as follows: 1) age 18–60 years; 2) no severe or uncontrolled cardiovascular diseases, such as diabetes, hypertension, and ischemic heart

disease; 3) no history of uncontrolled respiratory disease, such as asthma, which required medical adjustment within recent 3 months; 4) no history of traumatic brain injury with loss of consciousness; and 5) no history of any herbal medicine or psychotropic medications. The exclusion criteria were as follows: 1) having any major psychiatric disorders or currently being treated; 2) diagnosis of liver disease; 3) lactose intolerance; and 4) drinking more than 4 standard alcoholic drinks per day or 14 standard drinks per week.

All participants agreed to 1) keep a food diary over the 6 weeks of the study, 2) not take any dietary supplements rich in anthocyanin during the study, and 3) be asked and reminded weekly via mobile phone or social media to adhere to the protocol by collecting a corner of each milk carton and completing a questionnaire on the adverse effects.

Anthocyanins-rich mulberry milk

The anthocyanin-rich mulberry milk (Memberry®) used in this study was supported by the Mark One Innovation Center Company Limited of MK Restaurant Group. Each serving (180 mL) contained the total phenolic compounds, flavonoids, and anthocyanins at concentrations of $1,415.17 \pm 1.85$ μg GAE/mg, 667 ± 1.07 μg quercetin/mg, and 34.30 ± 0.74 mg cyanidin glucoside equivalent/L, respectively.

Measurements

Characteristic data

After providing informed consent, the participants were asked to complete a baseline assessment for their characteristic data, including age, sex, years of education, marital status, income, level of exercise, and diet.

Psychological outcomes

The primary psychological outcomes in this study were scores from the GHQ-28 Thai. The questionnaire was developed by Goldberg *et al.* [11] and is used widely as a screening tool to detect and quantify symptoms of mental health problems from the following 4 domains: somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression. The questionnaire has 28 questions, each on a Likert scale. The questionnaire has been translated into many languages, including Thai. This study used the GHQ-28 Thai version with 2 different scoring systems. The first scoring system was the 0–1–2–3 rating, which was used to calculate sample size and quantify the severity of the psychological symptoms. Each question asks the participants to rate their recent experiences from 0–3 for each symptom (0 = not at all/better than usual; 1 = no more than usual/same as usual; 2 = rather more than usual/worse than usual; 3 = much more than usual/much worse than usual). A higher score reflects a greater severity of symptoms. The second scoring system was the 0–0–1–1 rating. This scoring system used the responses from each question in the first scoring system and converted the 0–1 scores into 0 and the 2–3 scores into 1. This scoring system is used frequently to screen for any mental health problem with a cut-off score of 6 or higher. Both scoring systems had an excellent correlation with each other [12]. Other psychological outcomes were scores from the Hospital Anxiety and Depression Rating Scale (HADS). The HADS is a 14-item Likert self-rating scale for screening and measuring the severity of depressive and anxiety symptoms. Each subscale has 7 questions, each rated from 0–3 (0 = none; 1 = sometimes/mild; 2 = frequent/moderately; 3 = very frequent/severe). The subscale was originally developed by Zigmond and Snaith *et al.* [13] and has been translated into many languages. This study used the HADS in the Thai language with a cut-off score of 11 or higher on any subscale as indicating clinically significant depression or anxiety [14].

Biological outcomes

Saliva samples were collected to quantify the MAO-A MAO-B activities and cortisol using a Salivette collection device kit (No. 51.1534; Sarstedt, Numbrecht, Germany). The samples were collected between 7.00 and 8.00 a.m. before breakfast. Each subject rested for one minute in a sitting position before a saliva sample was collected. A swab was placed directly into each subject’s mouth under the tongue and kept in the mouth for 2–3 minutes to ensure that the swab had been thoroughly soaked with enough saliva (a minimum of 1/4 teaspoon). The samples were immediately frozen and transported to the laboratory for assays. The saliva cortisol levels were measured using ELISA kits, and the MAO-A and MAO-B activities were determined using a modification of the method reported by Holt *et al.* [15].

Statistical analyses

All data are presented as median and interquartile range. The primary research question used the Wilcoxon Sign Rank Test to analyze differences in the GHQ-28 scores between the baseline and at 6 weeks. The secondary research questions used the Wilcoxon sign rank test, Mann–Whitney *U* test, and median test to compare the HADS scores, the MAO-A and MAO-B activities, and the cortisol levels. Correlation analyses between the psychometric measurements and enzyme levels were performed using Spearman’s rho statistic; *P*-values < 0.05 were considered significant.

RESULTS

Participants and demographic data

From 300 eligible participants for the study screening, 16 (5.3%) participants had either depression or anxiety subscale scores from the HADS ≥ 11 at the baseline. These participants were likely to have anxiety or depressive disorder and were excluded. Hence, 284 participants remained in the final analysis. **Fig. 1** shows a schematic diagram illustrating the subject intervention procedure.

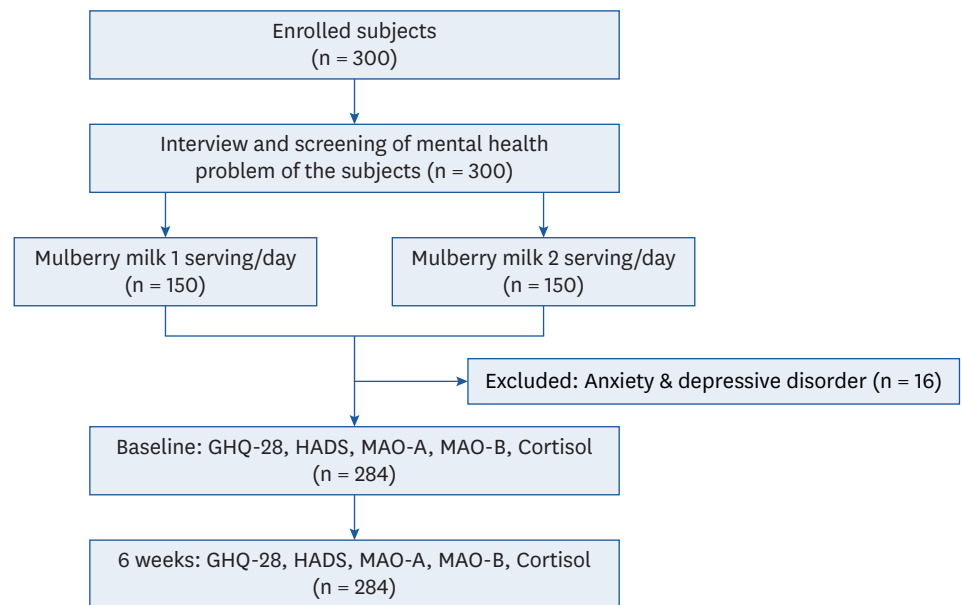


Fig. 1. Schematic diagram of the study. GHQ-28, General Health Questionnaire-28; HADS, Hospital Anxiety and Depression Scale; MAO, monoamine oxidase.

Table 1. Demographic data of the participants

Demographic	Single-serving group (n = 140)	Double-serving group (n = 144)	Both groups (n = 284)
Age (yrs)	35.8 (11.2)	35.6 (12.8)	35.7 (12.5)
Sex (female)	102 (72.9)	107 (73.4)	209 (73.6)
Education (yrs)	12.0 (4)	13.0 (4)	12 (4)
Alcohol drinking			
At least 1 standard drink a week	29 (20.7)	38 (26.6)	67 (23.7)
Intensity of exercise			
None or < 3 days a week	63 (45)	65 (45.1)	128 (45.1)
< 30 mins and > 3 days a week	59 (42.1)	62 (43.1)	121 (42.6)
> 30 mins and > 3 days a week	18 (12.9)	17 (11.8)	35 (12.3)

Values are presented as median (interquartile range) or number (%).

All characteristic data and outcomes were distributed non-normally according to the Shapiro–Wilk test ($P < 0.05$). The demographic data of the 16 excluded participants were not significantly different ($P > 0.05$) from those of the remaining participants. **Table 1** lists the demographic data of the participants. No significant differences in demographic factors existed between the single-serving and double-serving groups ($P > 0.05$).

Psychological outcomes

Table 2 lists the GHQ-28 and HADS scores at the baseline and at 6 weeks. The analyses of the primary outcome found significant differences between the GHQ-28 total scores at the baseline and at 6 weeks in the single-serving group, double-serving group, and entire population (all $P < 0.001$). Regarding the GHQ-28 subscales, significant differences between scores at baseline and at 6 weeks were observed in all 4 subscales (all $P < 0.001$) from the single-serving group, double-serving group, and entire population. When using the 0–0–1–1 scoring system, 55 participants (17.4%) had a total GHQ-28 score higher than the cut-off (≥ 6) at the baseline. The prevalence decreased to 15 (4.8%) at 6 weeks. The result from McNemar’s chi-square test found that the decreased percentage reached the level of statistical significance (chi-square = 19.12, $P < 0.001$).

For the secondary outcomes, there were significant differences between the HADS total scores and the HADS depressive subscale score from the baseline and at 6 weeks in the single-serving group, double-serving group, and entire population (all $P < 0.001$). Significant differences between the baseline and the 6-week HADS anxiety subscale scores were found

Table 2. Severity of mental health problems at the baseline and after consuming anthocyanin-rich mulberry milk daily for 6 weeks

Psychometric questionnaire	Single-serving group (n = 140)				Double-serving group (n = 144)				Both group (n = 284)			
	Baseline (T0)	6-week (T1)	z	Comparison	Baseline (T0)	6-week (T1)	z	Comparison	Baseline (T0)	6-week (T1)	z	Comparison
GHQ-28												
Somatic symptom	4.0 (5)	2.0 (3)	6.1	T1 < T0***	4.0 (5)	2.0 (4)	-5.4	T1 < T0***	4.0 (5)	2.0 (3)	-8.2	T1 < T0***
Anxiety insomnia	3.0 (7)	2.0 (4)	-5.2	T1 < T0***	4.0 (6)	2.0 (5)	-4.9	T1 < T0***	4.0 (6)	2.0 (4)	-7.1	T1 < T0***
Social dysfunction	7.0 (2)	6.0 (3)	-4.8	T1 < T0***	7.0 (3)	6.0 (4)	-4.8	T1 < T0***	7.0 (2)	6.0 (3)	-6.8	T1 < T0***
Severe depression	0.0 (2)	0.0 (1)	-3.8	T1 < T0***	0.0 (1)	0.0 (1)	-2.0	T1 < T0*	0.0 (1)	0.0 (1)	-4.1	T1 < T0***
Total	14.0 (13)	10.0 (7)	-6.8	T1 < T0***	15.5 (12)	10.0 (10)	-6.1	T1 < T0***	15.0 (12)	10.0 (8)	-9.1	T1 < T0***
HADS												
Anxiety	7.0 (3)	6.0 (3)	-1.8	NS	7.0 (2)	7.0 (3)	-3.2	T1 < T0**	7.0 (3)	6.0 (4)	-3.5	T1 < T0**
Depression	3.0 (3)	1.0 (4)	-4.3	T1 < T0***	3.0 (3)	2.0 (3)	-4.2	T1 < T0***	3.0 (3)	2.0 (4)	-6.0	T1 < T0***
Total	10.0 (5)	8.0 (5)	-3.9	T1 < T0***	10.0 (4)	8 (5)	-4.5	T1 < T0***	10.0 (6)	8.0 (5)	-5.9	T1 < T0***

Values are presented as median (interquartile range).

GHQ-28, General Health Questionnaire 28-item; HADS, Hospital Anxiety Depression Scale; NS, non-significance.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ using Wilcoxon signed rank test.

in the double-serving group and the whole population ($P < 0.01$), while no significant differences were found in the single-serving group ($P > 0.05$). The analyses used different scores between the baseline and at 6 weeks from the GHQ-28 and HADS to assess the dose-related effects of mulberry milk. The analyses found no statistically significant differences in the score changes between the single-serving group and the double-serving group for the GHQ-28 total score, HADS total score, or their subscales (all $P > 0.05$).

Biological outcomes

Data on the MAO activities and cortisol levels were available in 275 participants. At 6 weeks, the MAO activity and cortisol levels decreased significantly from the baseline: MAO-A ($z = -2.21$, $P = 0.027$), MAO-B ($z = -2.41$, $P = 0.016$), and cortisol ($z = -13.82$, $P < 0.001$). The cortisol levels in both groups decreased significantly at 6 weeks ($P < 0.001$). In contrast, the MAO-A and MAO-B activities decreased significantly in the double-serving group only (both $P < 0.05$), as shown in **Table 3**. Neither the Mann–Whitney U nor median tests showed any statistically significant difference in the changes in levels between the single-serving group and the double-serving group (all $P > 0.05$). The results from correlation analyses between the different scores from the psychometric questionnaires and the changes in the enzyme levels at 6 weeks found a significant correlation between the decreased GHQ-28 total score and the decreased activity level of MAO-A ($\rho = 0.119$, $P = 0.049$). Regarding the changes in the GHQ-28 subscales, the only subscale that had significant correlations with the changes in enzyme levels was the somatic symptom subscale. The decreased score from the somatic symptom subscale was positively correlated with the decreased activity levels of MAO-A ($\rho = 0.225$, $P < 0.001$) and MAO-B ($\rho = 0.147$, $P = 0.014$). The decreased HADS total score was positively correlated with the decreased activity levels of both MAO-A ($\rho = 0.125$, $P = 0.039$). Regarding the changes in the HADS subscale scores, only the decreased HADS depressive subscale score was positively correlated with the decreased MAO-A activity ($\rho = 0.123$, $P = 0.041$). None of the correlations between the change in the cortisol levels and the changes in the psychometric scales reached the levels of the statistical trend or significance (all $P > 0.1$). **Table 4** lists the correlation analysis results.

Table 3. Level of cortisol and monoamine oxidase enzyme activities at the baseline and after consuming anthocyanin-rich mulberry milk daily for 6 weeks

Level of hormone and enzyme activity	Single-serving group (n = 134)				Double-serving group (n = 141)				Both group (n = 276)			
	Baseline (T0)	6-week (T1)	z	Comparison	Baseline (T0)	6-week (T1)	z	Comparison	Baseline (T0)	6-week (T1)	z	Comparison
Cortisol (ng/mL)	8.49 (2.3)	6.82 (2.01)	-9.8	T1 < T0***	8.32 (2.4)	6.66 (1.92)	-9.7	T1 < T0***	8.35 (2.3)	6.77 (1.9)	-13.8	T1 < T0***
MAO-A (μmol/h/mg protein)	0.12 (0.12)	0.11 (0.14)	-0.4	T1 = T0	0.13 (0.14)	0.12 (0.11)	-2.6	T1 < T0*	0.13 (0.12)	0.11 (0.12)	-2.2	T1 < T0***
MAO-B (μmol/h/mg protein)	0.1 (0.14)	0.07 (0.13)	-1.0	T1 = T0	0.11 (0.14)	0.1 (0.12)	-2.3	T1 < T0*	0.1 (0.14)	0.09 (0.13)	-2.4	T1 < T0***

Values are presented as median (interquartile range).

MAO, monoamine oxidase.

* $P < 0.05$, *** $P < 0.001$ using Wilcoxon signed rank test.

Table 4. Correlation coefficients between changes in both enzyme level/activity and changes in the psychometric score after consuming anthocyanin-rich mulberry milk for 6 weeks (n = 275)

Level of hormone and enzyme activity	General Health Questionnaire 28-item					Hospital Anxiety Depression Scale			
	Somatic symptom	Anxiety	Insomnia	Social dysfunction	Severe depression	Total score	Anxiety	Depression	Total score
Cortisol level	-0.047		0.045	-0.063	0.027	0.015	0.04	-0.055	-0.01
MAO-A	0.225***		0.084	0.04	-0.003	0.119*	0.073	0.123*	0.125*
MAO-B	0.147**		0.083	0.061	0.017	-0.005	0.053	0.114	0.091

MAO, monoamine oxidase.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ using Spearman rank correlation.

DISCUSSION

To the best of the authors' knowledge, this is the first open-label study to report the positive benefits of daily consumption of anthocyanin-rich mulberry milk for 6 weeks on mental health using standardized psychometric scales in a working population. The major benefits of mulberry milk from this study were significantly improved well-being and mental health. Decreased cortisol levels and MAO enzyme activities were observed, and there were correlations between the decreased MAO enzyme activities and psychological symptoms. The interpretations and possible mechanisms of action are discussed below.

The effects of reduced psychological symptoms from mulberry milk were observed in all 4 symptom domains from GHQ-28 and both subscales from HADS. The median decrease in the GHQ-28 total score was 4 (10) when using the 0–1–2–3 scoring system and 1 (4) when using the 0–0–1–1 scoring system. The interpretation of the change in the GHQ-28 total score is difficult, given that the scale has been used more often as a major screening tool for detecting mental health problems than as a symptom monitoring tool. No clinically meaningful difference score (CMD) of the total GHQ-28 score has been reported. On the other hand, a median one-point reduction (4 points in symptom severity) could remove one symptom completely, which is likely to affect an individual's perception of their mental well-being. For the HADS score, the median decrease in the HADS total score was 1 (5) point. The CMD of HADS total score has been reported at 1.5–2 points in patients with respiratory or cardiovascular disease who received antidepressants, suggesting a twenty-percent decrease in overall anxiety and depressive symptoms [16,17]. Although the median of decreased HADS total score from the entire population did not reach the CMD threshold of 2-point reduction, there were 137 participants (48.2%) who met the CMD. These findings suggested that the supplement may be useful in alleviating symptoms of anxiety and depression in approximately half of the population at clinically relevant levels. Nevertheless, the study failed to show a dose-dependent effect of the supplement. This may be because the product contains many ingredients. Hence, the effects of active substances may be masked by other ingredients.

The significantly decreased cortisol levels and MAO-A and MAO-B activities after supplementation were likely due to the anthocyanins in the mulberry milk. Many studies showed that anthocyanins from various berry fruits had a high affinity for MAO-A and MAO-B. *In vitro*, anthocyanins exhibited inhibitory effects on MAO enzymes [4,18]. *In vivo*, a group of aged mice that received high-dose anthocyanin had significantly higher DA levels in the brain than a group of control mice, indicating an affinity toward DA activity [19]. Anthocyanins have benefits in reducing anxious behavior (elevated plus-maze task) in mice from uncontrolled studies [3] and controlled studies with saline as a comparator [20]. Moreover, antidepressant-like effects comparable to imipramine were also observed in mice that received anthocyanin when they performed tasks [21]. Previous studies of anthocyanin-rich supplements in humans also aligned well with data from animal studies. One study reported that the daily consumption of wild blueberry juice for 12 weeks was associated with significantly reduced depressive symptoms [22], and other studies using purple grape juice had mood-calming properties in healthy young adults [23]. Moreover, correlation analyses in the present study supported the above speculation. The significant correlations between the decreased MAO-A activity and the decreased GHQ-28 total score, GHQ-28 somatic symptom subscale, HADS total score, and HADS depressive subscale strongly suggested that the decreased severity of both depression and somatic symptoms might be explained by the decreased MAO-A enzyme activity. While a significant decrease in the cortisol level

was also found, it was less likely to explain decreased psychological symptoms because of a lack of correlation. Overall, the antidepressant and anxiolytic effects observed in this study were possibly explained by the decreased MAO-A and MAO-B activities from anthocyanin in mulberry milk. These factors supposedly led to increased availability of monoamine neurotransmitters in the central nervous system.

In conclusion, this study showed that the daily consumption of anthocyanin-rich mulberry milk for 6 weeks had benefits on mental health, particularly in decreased anxiety, depression, social dysfunction, and somatic symptoms. The possible mechanism involved decreased MAO activities from anthocyanin in mulberry milk, which led to increased monoamine neurotransmitter levels. This study is the first large, open-label trial to examine the effects of anthocyanin-rich mulberry milk on psychological symptoms in the Asian population. The results also added to the growing literature on the potential use of anthocyanins from mulberry for their anxiolytic and antidepressant properties. This study also explained the possible neurobiological mechanisms using measurable biomarkers. On the other hand, the results from this study should be interpreted with caution because of certain limitations. First, exposure biases unavoidably affected the results because of its open-label design without a placebo-controlled arm. Second, generalization of the results to age groups other than the working population may be limited. Last, the changes in the cortisol levels and MAO activities may also be affected by other ingredients in the product. Therefore, the current data suggest that daily consumption of anthocyanin-rich mulberry milk benefits mental health because of its inhibitory effect on MAO enzymes. Further investigation to explore its effects on other areas in mental health and psychiatry, such as stress-related disorders and neurocognitive disorders, is required.

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