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Pilot Study of the Tart Cherry Juice for the Treatment of Insomnia and Investigation of Mechanisms

Jack N. Losso, PhD,[1] John W. Finley, PhD,[1] Namrata Karki, PhD,[1] Ann G. Liu, PhD,[2] Alfredo Prudente, PhD,[3] Russell Tipton, MD,[2] Ying Yu, MS,[2] and Frank L. Greenway, MD[2]*

Background: Insomnia is common in the elderly and is associated with chronic disease, but use of hypnotics increases the incidence of falls. Montmorency tart cherry juice has improved insomnia by self-report questionnaire.

Study Question: Is insomnia confirmed by polysomnography and is tryptophan availability a potential mechanism for treating insomnia?

Study Design: A placebo-controlled balanced crossover study with subjects older than 50 years and insomnia were randomized to placebo (2 weeks) or cherry juice (2 weeks) (240 mL 2 times/d) separated by a 2-week washout.

Measures and Outcomes: Sleep was evaluated by polysomnography and 5 validated questionnaires. Serum indoleamine 2,3-dioxygenase (IDO), the kynurenine-to-tryptophan ratio, and prostaglandin E2 were measured. In vitro, Caco-2 cells were stimulated with interferon-gamma, and the ability of cherry juice procyanidin to inhibit IDO which degrades tryptophan and stimulates inflammation was measured. The content of procyanidin B-2 and other major anthocyanins in cherry juice were determined.

Results: Eleven subjects were randomized; 3 with sleep apnea were excluded and referred. The 8 completers with insomnia increased sleep time by 84 minutes on polysomnography ($P = 0.0182$) and sleep efficiency increased on the Pittsburgh Sleep Quality Index ($P = 0.03$). Other questionnaires showed no significant differences. The serum kynurenine-to-tryptophan ratio decreased, as did the level of prostaglandin E2 (both $P < 0.05$). In vitro, cherry juice procyanidin B-2 dose-dependently inhibited IDO.

[1]*Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge, LA;* [2]*Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA; and* [3]*BASF Corporation San Gabriel, LA.*

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**Address for correspondence: Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge, LA 70808. E-mail: Frank.Greenway@pbrc.edu*

Conclusions: Cherry juice increased sleep time and sleep efficiency. Cherry juice procyanidin B-2 inhibited IDO, increased tryptophan availability, reduced inflammation, and may be partially responsible for improvement in insomnia.

Keywords: procyanidin, indoleamine 2, 3-dioxygenase, montmorency tart cherry juice, sleep, tryptophan, kynurenine

INTRODUCTION

Insomnia is a common condition in the elderly and is associated with reduced quality of life and adverse outcomes. In a survey, 23%–34% of 9000 adults older than 65 years complained of insomnia.[1] Insomnia is associated with increased prevalence of medical disorders including hypertension,[2] type-2 diabetes,[3] exacerbation of chronic pain,[4] and a decline in cognitive function.[5] Cognitive behavioral therapy is not always effective for chronic primary insomnia, and the short-term use of hypnotics increases the risk of falls in the elderly by more than 4-fold.[6] Therefore, it is important to identify treatments for insomnia without apparent side effects.

Montmorency (*Prunus cerasus*) tart cherry juice has been reported to have a positive effect on insomnia in elderly people, as measured by the Insomnia Severity Index. The biggest effect seen was on the "waking after sleep onset" subscale.[7] Although the authors stated that the mechanism for the beneficial effect of tart cherry juice on insomnia was unknown, they suggested that melatonin contained in the cherries could be responsible. The effective dose of the cherry juice was derived from 100 g of cherries. The amount of melatonin in the dose of cherry juice used in the study was equivalent to 0.135 μg, and the dose of melatonin recommended for sleep is 0.5–5 mg.[8] Thus, it would seem that the effect of the tart cherry juice on sleep is due to more than its melatonin content.

Tryptophan, a precursor of serotonin, reduces sleep latency in humans at doses of 1.2–2.4 g.[9] Because tart cherries contain only 9 mg of tryptophan per 100 g, one might presume that this small amount of tryptophan could not impact insomnia. Tryptophan degradation, however, parallels and predicts insomnia.[10–12] Tryptophan is degraded by the enzyme indoleamine 2,3-dioxygenase (IDO) to produce kynurenine. IDO is stimulated by inflammation, and inhibition of IDO not only increases serotonin and improves mood but also decreases inflammation.[13,14] Because tryptophan is degraded into kynurenine, the ratio of kynurenine to tryptophan is a measure of tryptophan degradation, a lower value suggesting decreased tryptophan degradation.

Tart cherry juice contains 0.2% procyanidins.[15] Because procyanidins can be detected in human serum 2 hours after ingestion,[16] we hypothesized that tart cherry juice standardized to a specific procyanidin content would decrease the ratio of kynurenine to tryptophan and contribute to the treatment of insomnia.

MATERIALS AND METHODS

Clinical trial

A randomized, double-blind, placebo controlled clinical trial was conducted to test the effectiveness of the tart cherry juice of known procyanidin content as a treatment for insomnia. This study was approved by the Institutional Review Board of Pennington Biomedical Research Center and registered on ClinicalTrials.gov under NCT01669317. Eleven healthy male or female subjects (age ≥50 years) with chronic insomnia and a usual bedtime between 9 PM and midnight were included in this study. Insomnia was defined as trouble sleeping on average more than 3 nights per week, with an Insomnia Severity Index score greater than or equal to 10 and meeting the International Classification of Sleep Disorders-2 (ICSD-2) criteria for insomnia.[17,18] ICSD-2 defines insomnia as a complaint of difficulty initiating sleep, difficulty maintaining sleep, waking up too early, and sleep that is chronically nonrestorative or poor in quality, despite adequate opportunity for sleep and circumstances conducive to sleep. Insomnia includes at least one of the following daytime complaints related to sleep difficulty: fatigue or malaise; poor attention, concentration, or memory impairment; social vocational dysfunction or poor school performance; mood disturbances or irritability; daytime sleepiness; motivation, energy, or initiative reduction; proneness for errors or accidents at work or while driving; tension, headaches, or gastrointestinal symptoms in response to sleep loss; or concerns or worries about sleep. Subjects who had diabetes mellitus, were taking sedating or hypnotic medications, or were taking chronic medication without a stable dose for 1 month or longer were excluded.

Subjects fasted for 10 hours except for water before screening, and blood was drawn for glucose, creatinine, potassium, uric acid, albumin, calcium, magnesium, creatine phosphokinase, alanine–leucine transaminase, alkaline phosphatase, iron, cholesterol, triglycerides, high density lipoprotein cholesterol, and low density lipoprotein cholesterol. A health questionnaire and 5 validated questionnaires were completed (Insomnia Severity Index, the Epworth Sleepiness Scale, the Pittsburgh Sleep Quality Index, the Beck Depression Inventory, and the State-Trait Anxiety Inventory).[19–22]

Subjects who passed screening drank 240 mL of the tart cherry juice containing a measured level of procyanidin (Table 4) or a placebo juice in the morning and 1–2 hours before bedtime for 14 days. All cherry juices were from the same batch and were analyzed for their content of phenolic compounds. The placebo juice was made of vapor-distilled water, fructose, dextrose, and lemon powder and looked and tasted like a cherry juice. After 2 weeks of cherry juice or placebo, subjects had an overnight polysomnographic sleep study, and blood was drawn that evening for measurement of the kynurenine-to-tryptophan ratio to evaluate tryptophan degradation and prostaglandin E2 (PGE2) to evaluate inflammation. On waking after the sleep study, the 5 validated questionnaires were repeated, and subjects were questioned about any adverse events. After a 2-week washout period, the subjects were crossed over to the tart cherry juice or the placebo they did not take in the first 2-week testing period, and the 2-week testing period was repeated. The order of the cherry juice was balanced and assigned randomly.

Blood testing and serum preparation and analysis

Blood drawn for testing of free tryptophan and kynurenine, and PGE2 was frozen and stored at −80°C until analysis by high-performance liquid chromatography (HPLC) with ultraviolet and fluorescence detection. The ratio of kynurenine/tryptophan as an index of IDO activity was calculated using an Agilent 1100 series HPLC with a diode array detector and a fluorescence detector.[23] The wavelength of the ultraviolet detector was set at 365 nm, and fluorescence excitation was at 254 nm with detection at 404 nm. A 6-methyltryptophan internal standard was used, and the fluorescence conditions were set to excitation at 220 nm, with detection at 354 nm.[24] The stock solutions of tryptophan or kynurenine were 1 mmol/L in the mobile phase and were prepared immediately before use. Serial dilutions of the serum samples were made to produce final (additional) concentrations of 6.25, 12.5, 25, 50, 75, and 100 μmol/L of tryptophan and 0.0625, 0.125, 0.25, 0.75, 1.5, and 6.25 μmol/L of kynurenine. After addition of the internal standard, the medium was precipitated with perchloric acid (8% final acid concentration) and centrifuged for 10 minutes at 64*g* in a refrigerated centrifuge, and 20 μL of the clear supernatant was used for testing. PGE2 levels in sera before and after cherry juice consumption were determined by enzyme-linked immunosorbent assay using a commercial kit from PeproTech (Rocky Hill, NJ).

Chemicals and reagents

The Cherry Marketing Institute provided Indian Summer cherry juice without vitamin C, which was stored at 20°C and used within 3 months. HPLC-grade acetonitrile, methanol, and trifluoroacetic acid were purchased from Fisher Scientific (Fair Lawn, NJ). C18 Sep-Pak cartridges were purchased from Waters (Milford, MA). Proanthocyanidin B-2 was purchased from ChromaDex (Irvine, CA). Dulbecco Modified Eagle Medium, fetal bovine serum, trypsin, and phosphate-buffered saline were obtained from Fisher Scientific (Pittsburgh, PA). Bio-Rad DC protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA). An enzyme-linked immunosorbent assay kit for PGE2 was purchased from PeproTech (Rick Hill, NJ). Polyvinylidene fluoride membranes and 4%–12% Bis-Tris gel were obtained from Invitrogen (Carlsbad, CA). Bovine serum albumin, primary antibodies against IDO, nuclear factor kappa light-chain enhancer of activated B-cell subunit (NF-κB-p65), and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Primary antibody against cyclooxygenase-2 (COX-2) was purchased from Cayman Chemical (Ann Arbor, MI). Peroxidase-conjugated secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA). X-ray film was purchased from Phoenix Research (Candler, NC).

Cell culture

Caco-2 colon cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA). The cells were propagated in Invitrogen's minimum essential media containing sodium bicarbonate, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid HEPES is a zwitterionic organic chemical buffering agent and one of the 20 Good's buffers, fetal bovine serum to a final concentration of 10%, Invitrogen Glutamax, and sodium pyruvate. All experiments were with 80%–90% confluent cultures. All cells were propagated at 37°C in 5% CO_2 in a humidified chamber.

In vitro effect of proanthocyanidin-B2

Caco-2 (1.8×10^5 cells/well) colon cancer cells were stimulated with 10 ng/mL interferon-gamma (IFN-γ) in the absence or presence of 0–50 μM of procyanidin B-2 at 37°C, 5% CO_2 for 24 hours. The levels of IDO,

Table 1. Demographics of the participants in the sleep study.

Number completed	8
Gender	
Female	5
Male	3
Race	
White	5
Black	3
Age average	68
SD	9.2
Height average, cm	167.4
SD	14.1
Weight average, kg	78.2
SD	11.3
SBP average, mm Hg	124
SD	4.8
DBP average, mm Hg	76
SD	4.1
Resting HR average, bpm	65
SD	6.1
BMI average, kg/m^2	28.1
SD	4.0

BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; NS, not significant; SD, standard deviation; SBP, systolic blood pressure.

NF-κB, or COX-2 in the control and procyanidin-treated cells were evaluated by Western blot using anti-IDO, anti–NF-κB, or anti-COX-2 antibodies. Beta actin served as a loading control. The data are representative of 3 separate experiments.

Determination of the procyanidin B-2 content

The method of Kennedy[25] was used to isolate and extract procyanidin-B2 from cherry juice. The method of Kim and Lee[26] was used to determine the levels of procyanidin B-2 in cherry juice. Procyanidin B-2 standards were used for quantitative analysis of procyanidin B-2 in cherry juice. The method of Kim and Lee[26] was used for the extraction, isolation, and analysis of cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, or cyanidin-3-O-glucosylrutinoside in cherry juice. These anthocyanins and procyanidins were selected because they represent the major anthocyanins and procyanidins present in tart cherries.[27] Analyses of procyanidin B-2 or anthocyanins were performed using an Agilent 1100 HPLC equipped with a photodiode detector. Procyanidin B-2 was detected at 280 nm, and anthocyanins were detected at 520 nm.

Table 2. Polysomnography.

	Difference (cherry juice − placebo)	*P*
No. awakenings	0.63 ± 6.09	0.78
Wake time after sleep onset	−4.9 min ± 42.2	0.75
Sleep onset latency	−0.56 min ± 28.38	0.96
Total sleep time	84 min ± 61.7	0.0182*
Sleep efficiency	0.046 ± 0.09	0.19
Stage REM latency	32.56 min ± 68.38	0.22

*clinically significant $p < 0.05$.

Table 3. Questionnaires.

Questionnaires	Difference (cherry juice − placebo)	*P*
Pittsburgh sleep quality index		
Habitual sleep efficiency	0.5 ± 0.5	0.0331*
Sleep duration	0.125 ± 0.083	0.6845
Other questions		NS
Insomnia Severity Index		
All questions		NS
Epworth Sleepiness Scale		
All questions		NS
Beck Depression Inventory II		
All questions		NS
State-Trait Anxiety Inventory		
All questions		NS

*clinically significant $p < 0.05$.
BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; NS, not significant; SD, standard deviation; SBP, systolic blood pressure.

Statistical analysis

Because there were no polysomnographic studies of cherry juice on which to base a power analysis, the clinical trial was designed as a pilot study to determine power for a larger, adequately powered study that would give statistically significant differences. The differences in the polysomnographic studies and questionnaires were compared by *t* test for paired observations. Categorical variables were compared by chi-squared test.

Proanthocyanidin-B2, Procyanidin-B2, anthocyanins, procyanidins, IDO, NF-κB, COX-2, PGE2, IFN-γ, tryptophan, kynurenine, and the kynurenine-to-tryptophan ratio were analyzed using analysis of variance or a nonparametric equivalent of analysis of variance. Comparisons between the active and the placebo conditions were made on days 35 and 70 using variance analysis

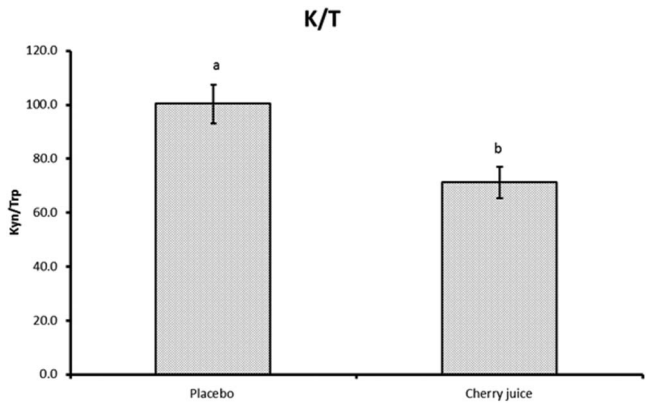

FIGURE 1. Serum kyurenine (kyn)-to-tryptophan (Trp) ratio (K/T) after placebo or cherry juice treatment. Different letters over the bars in the graph indicate significance at $P < 0.05$.

with the baseline values on day 0 as a covariate. Probability (P) values <0.05 were considered statistically significant.

RESULTS

Clinical trial

Eleven subjects were randomized, but 8 subjects completed both arms of the study, and data relating to those 8 subjects were analyzed. Three subjects had moderate-to-severe sleep apnea by polysomnography. They were eliminated from the analysis and referred for evaluation and treatment. The 8 subjects who completed both arms of the study are described in Table 1. The polysomnographic results are presented in Table 2. The sleep time was extended in the cherry juice condition by 84 minutes ($P = 0.0182$). The sleep efficiency improved in the cherry juice condition but did not reach statistical significance. The results of the Pittsburgh Sleep Quality Index are presented in Table 3. The Habitual Sleep Efficiency improved ($P = 0.03$), and the sleep duration also improved on the PSQI but did not reach statistical significance. There were no statistically significant differences on the Insomnia Severity Index, the Epworth Sleepiness scale, the Beck Depression Inventory II, or the State-Trait Anxiety Inventory. There were no adverse events.

Serum kynurenine

The kynurenine-to-tryptophan ratio was reduced in the cherry juice condition ($P < 0.05$), indicating an inhibition of IDO with a reduction in the degradation of tryptophan (Figure 1). The level of PGE2, a marker of inflammation, was also dose-dependently reduced ($P < 0.05$) (Figure 2).

Procyanidin B-2 and anthocyanin contents of tart cherry juice

The tart cherry juice contained procyanidin B-2, cyanidin-3-O-glucosylrutinoside, cyanidin-3-O-glucoside, and cyanidin-3-O-rutinoside (Table 4). The presence of these bioactives in cherries has been reported.[15]

Interaction of procyanidin B-2 and inflammatory biomarkers

The efficacy of procyanidin B-2 against IDO was demonstrated by stimulating Caco-2 cells with IFN-γ. The IDO, NF-κB, and COX-2 levels in cancer cells decreased with progressively higher concentrations of procyanidin B-2. We found that procyanidin B-2 at 24–50 μM inhibited IFN-γ–induced IDO in human Caco-2 colon cancer cell lines (Figure 3).

DISCUSSION

Pigeon et al performed a pilot cross-over study exploring the effect of tart cherry juice (240 mL twice a day) or placebo over 2 weeks on insomnia in individuals older than or aged 65 years with a 2-week washout period between cross-over arms.[7] They reported statistically significant improvement during the cherry juice consumption in the Insomnia Severity Index and a 62-minute improvement in waking after sleep onset. Our study design was patterned after that of Pigeon et al, but unlike that study, ours used formal polysomnography testing, the gold-standard method for evaluating sleep pathology. Sleep questionnaires used in the Pigeon et al study represent a weakness because they rely on self report to evaluate a condition in

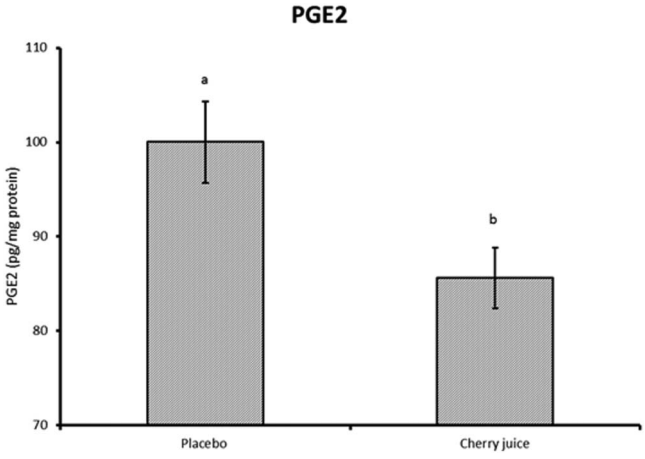

FIGURE 2. Serum PGE2 levels after placebo or cherry juice treatments. Different letters over the bars in the graph indicate significance at $P < 0.05$.

Table 4. Contents of procyanidin B-2 and major anthocyanins in Indian Summer tart cherry juice.

Major compounds in cherry juice	Content, μg/mL
Procyanidin B-2	451.56
Cyanidin-3-O-glucosylrutinoside	123.33
Cyanidin-3-O-rutinoside	20.26
Cyanidin-3-O-glucoside	3.51

which people are not conscious of their surroundings, and sleep questionnaires do not identify participants with sleep apnea. Sleep apnea is a mechanical condition that results in poor sleep quality but would not be expected to improve without reversal of airway obstruction. Because 3 of 11 subjects in our study meeting the Pigeon et al screening criteria had sleep apnea rather than insomnia, being able to identify and eliminate sleep apnea is important. Using polysomnography gives a more accurate assessment of sleep and allows selection of subjects with insomnia.

Howatson et al[24] evaluated sleep quality in 20 healthy exercising volunteers between the ages of 18 and 40 years (26 ± 4.6, mean ± SD). They used actigraphy to access the endpoints and gave 30 mL of tart cherry juice concentrate twice a day for 7 days or placebo in a cross-over trial with a 2-week washout period between study arms. The cherry juice condition had a statistically significant increase of time in bed (25 minutes), total sleep time (34 minutes), and sleep efficiency (5%–6%). The urinary degradation product of melatonin increased statistically (~17%) in the cherry juice condition, but there was no change in the circadian rhythm of melatonin. Melatonin is postulated to treat insomnia, by restoring disturbed circadian rhythms. Cherry juice increased melatonin intake

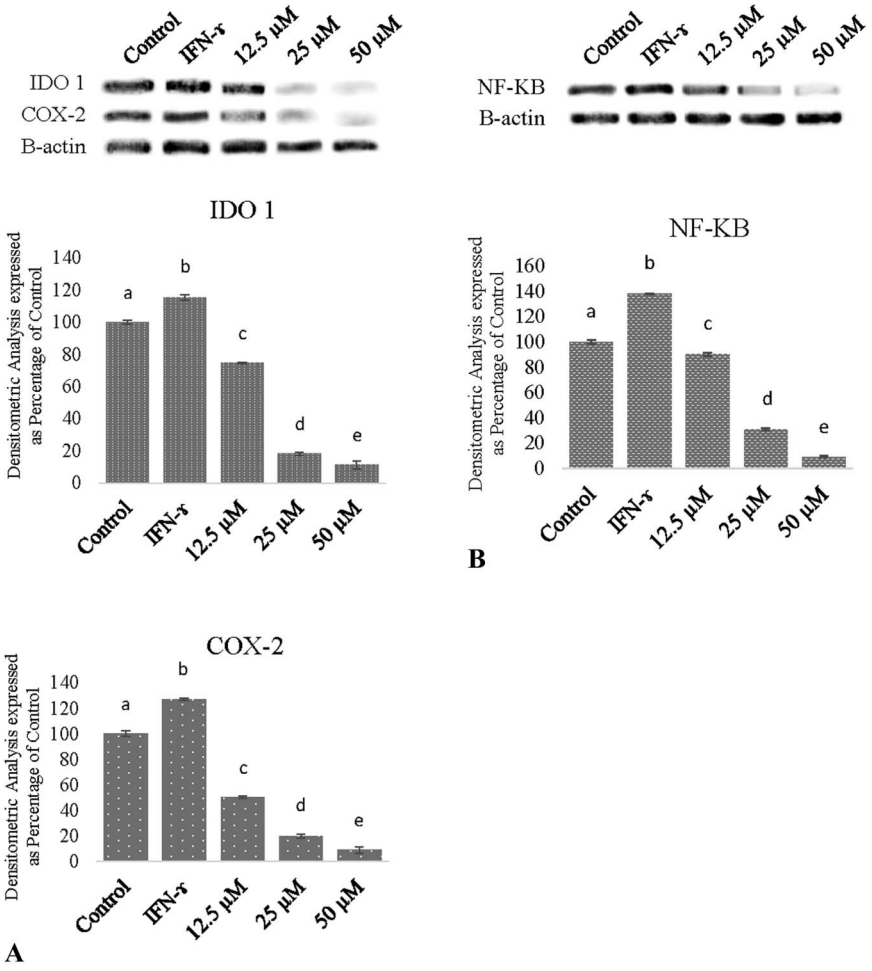

FIGURE 3. (A) Relative induction of IDO and COX-2 in Caco-2 cells stimulated with IFN-γ and decrease of IDO and COX-2 in cells treated with procyanidin B-2. (B) Relative induction of NF-κB in Caco-2 cells stimulated with IFN-γ. a = control, b = IFN-γ, c = 12.5 μM procyanidin B-2, d = 25 μM procyanidin B-2, and e = 50 μM procyanidin B-2.

by 85 μg/d, although the melatonin dose shown to treat insomnia ranges between 0.5 and 5 mg/d, a dose that is 6- to 60-fold higher. Thus, the increase in melatonin may not be the major mechanism responsible for the improving sleep quality. The Howatson et al[24] study does, however, give support to the beneficial effects of tart cherry juice on sleep quality in a younger group of volunteers than those tested in this study.

Our study included healthy volunteers who were older than or aged 50 years. We eliminated 3 of 11 subjects with sleep apnea who were diagnosed by polysomnography, something that the studies by Pigeon et al and Howatson et al did not do. The polysomnography was performed on only 2 nights, and some acclimation to the procedure might have been desirable. This weakness should have been overcome by the balanced order of the sleep studies and the cross-over design of the trial. Although the prevalence of sleep apnea may have been lower in the younger subjects studied by Howatson, the subjects studied by Pigeon et al were all older than or aged 65 years. One strength of our study was its accuracy through the use of polysomnography, but because it was a small pilot study, many of the parameters we measured did not demonstrate a statistically significant change. This is a weakness that can be addressed by a larger future study with adequate power to detect the other endpoints. Despite the small size of our pilot study, we demonstrated a statistically significant 84 minutes increase in sleep time by polysomnography and reduced plasma levels of kynurenine with increased tryptophan levels. The kynurenine–tryptophan ratio (K/T) is the gold standard for determining IDO activity.[28,29] The in vitro and in vivo results obtained suggest that the procyanidin B-2 in cherry juice is an inhibitor of IDO and part of the mechanism by which tart cherry juice improves sleep efficiency. These findings suggest that procyanidin B-2–rich cherry juice can improve tryptophan bioavailability for serotonin synthesis and might theoretically contribute to mood-enhancing effects, although this was not detected on the questionnaires evaluated in this study.[30] These findings suggest that of all the procyanidins that reach the gastrointestinal tract, the oligomeric procyanidins are converted into dimeric procyanidins and absorbed. The polymeric procyanidins are not bioavailable. Because of the high content of procyanidin B-2 in the tart cherry juice used in this study, we used procyanidin B-2 as a standard for the study.

The other major phenolic compounds in tart cherries such as cyanidin-3-O-glucosylrutinoside are deglycosylated before absorption and metabolized into phenolic acids. We did not evaluate the effects of the phenolic acids on IDO. IFN-γ upregulates COX-2 and IDO and has been shown to correlate with increased production of PGE2 and kynurenine.[31] IDO overexpression is linked to COX-2 expression.[32] Inhibition of IDO is associated with COX-2 suppression.[33] Our in vitro results showed inhibition of IDO and COX-2 (Figure 3), and our in vivo results showed inhibition of PGE2 (Figure 2).

We conclude that tart cherry juice is an effective treatment for insomnia. It has no adverse events as demonstrated in our study and in prior studies by others. Procyanidin B-2 is a likely active ingredient in tart cherry juice acting through plasma kynurenine reduction, tryptophan enhancement, and inhibition of IDO. Procyanidin B-2 may be an effective ingredient for improving insomnia. Thus, tart cherry juice and its active ingredients may offer a safe, yet effective, improvement in insomnia that will not increase the prevalence of falls or other side effects associated with hypnotic medications.

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