DUQUESNE UNIVERSITY
GRADUATE SCHOOL OF PHARMACEUTICAL SCIENCES

Cinnamon (Twak): a coumarin derivative, melatonin derivative, anti-oxidant or all the above?

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ABSTRACT

Bhavprakash Nighantu (v66-67) describes Cinnamon Zeylanica:

त्वक्स्वाद्वी तु तनुत्वक्स्यात्तथा दारुसिता मता ६६

उक्ता दारुसिता स्वाद्वी तिक्ताचानिलपित्तहृत्
सुरभिः शुक्रलाबल्या मुखशोषतृषापहा ६७

which translates to: "Tvak, Svadvi, Tanutvak and Darusita all are its Sanskrit name. It is bitter in taste, manages Vata and Pitta. It is aromatic. Promotes Shukardhatu in the body and complexion. It manages Dryness of mouth and thirst." Cinnamon comes in many forms but two of them, native to South Asia and Sri Lanka, are known as Ceylon and Cassia cinnamon. Cinnamaldehydes isolated from twak bark are responsible for the aroma of cinnamon and is the form commonly used in essential oils. Cinnamon's rasa is madhura, katu and tikta; its vipaka is katu and its virya is ushna and so it is used in Ayurveda to pacify vata and kapha but can be used in moderate amounts in pitta-dominant conditions. According to Aryabhishak, if prepared as an essential oil through boiling, this oil extract can be used to treat vomiting, nausea and diarrhea in pitta conditions. The essence of Ayurveda classics like Charak Samhita, Madhava Nidanam, Bhav Prakash and Sharngadhara Samhita are well documented in the new classic book "Aryabhishak", which describes a variety of uses for cinnamon including: basti, diseases of the mouth, throat and for cough possibly through its antibacterial and antifungal properties. Twak strengthens the heart and kidneys; thins the blood; helps treat respiratory, sinus congestion and bronchitis. It improves digestion, absorption and elimination; removes toxins and improves circulation. Twak increases the bioavailability of food, and increases rasa of all seven dhatus. Its anti-inflammatory properties may underlie its beneficial effects on arthritic joint pain. In Ayurvedic practice today, cinnamon is commonly used to reduce ama, treat high cholesterol, adrenal disorders, balance blood sugar levels and possibly balance hormonal levels. Twak may improve neurological conditions including Parkinson's and Alzheimer's disease and may improve memory through modulation of brain-derived neurotrophic factor (BDNF) to increase neurogenesis. Cassia cinnamon is commonly found in grocery stores and is gaining popularity for its high nutrient levels that include coumarin. Coumarin displays anti-oxidant properties which may underlie its reported anti-cancer and neuroprotective properties. Recent evidence suggests that derivatives derived from Cassia cinnamon can be neuroprotective and breast cancer-protective and bind to melatonin receptors.

Hypothesis: Cinnamon, particularly ferulic acid and coumarin derivatives, inhibit HER2+, ER+ and triple negative breast cancers by binding to and activating melatonin receptors.

Methods: Cinnamon-based derivatives were assessed for binding affinity to melatonin receptors using the radioligand, 2-[^{125}I]-iodomelatonin. In vitro cell culture assays were used to assess their efficacy to inhibit HER2+, ER+ and triple negative breast cancer cell viability and migration using MTT viability and "scratch" or wound assays, respectively.

Conclusions: Oxyprenylated phenylpropanoids and their actions at melatonin receptors explain, in part, the health benefits of Cassia cinnamon to protect against neurodegenerative diseases and breast cancer and support the ancient wisdom of the *Rishis* about the health benefits of cinnamon as eloquently stated in the following *sloka*:

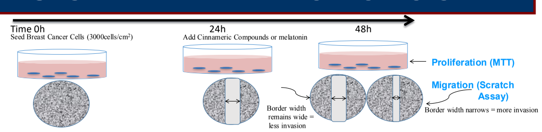
त्वच्यं लघु उष्णं कटुकं स्वादु तिकत्श्च रुक्षकं I
पित्तलं कफवातघ्नं कण्डु वामि अरुचि नाशनम् I

INTRODUCTION

- Bhavprakash Nighantu (59, 66, 67) states: "The quality (GUNA) of cinnamon is light and dry, potency is hot, and taste is pungent and bitter. It slightly increases Pitta, and balances Vata and Kapha. It relieves itching and distastefulness. Cinnamon Zeylanica is: Tvak, Svadvi, Tanutvak, Darusita as its Sanskrit name. It is bitter in taste, manages Vata and Pitta. It is aromatic. Promotes shukradhatu in the body and complexion. Manages dryness of the mouth and thirst."
- "Cinnamon is an effective herb for strengthening and harmonizing the flow of circulation (vyana vayu). It is a good diaphoretic and expectorant in colds and flu and is especially good for those of weak constitution. And a pain reliever for a toothache and muscle tension. It strengthens the heart, warms the kidneys and promotes Agni (digestion)." (Frawley and Lad, The Yoga of Herbs)
- Cinnamon is of 2 types: Cinnamon *zeylanicum* ("Sri Lanka cinnamon" or "true cinnamon") and Cinnamon *cassia* ("Cinnamon *aromaticum*" or "Chinese cinnamon").[6]
- Cinnamon cassia contains coumarin (*2H*-1-benzopyran-2-one) where 1kg cinnamon *cassia* contains 2.1-4.4 g coumarin which is equivalent to 1 tsp = 5.8-12.1mg coumarin. This amount of coumarin is greater than the Tolerable Daily Intake (TDI) of 0.1mg/kg body weight/day (German Federal Institute for Risk Assessment).[6]
- Cinnamon *zeylanicum* possesses anti-inflammatory and anti-microbial properties and is protective against cardiovascular disease and cancer and enhances cognitive function.[6]
- Cinnamonum *zeylanicum* (*trans*-cinnamaldehyde or cinnamtannin B1) demonstrates cardioprotection (↓ LDL, ↓triglycerides, ↓HbA1c, ↓lipid peroxidation, ↑HDL)[6] through its effects on glucose regulation (↓postprandial glucose absorption; ↓ fasting blood glucose, ↑insulin levels and ↑insulin sensitivity in skeletal muscle and adipose tissue via PPARγ, PPARδ and GLUT4) and lipid metabolism via activation of PPARδ, PPARγ, and retinoid X receptor (RXR) to enhance insulin sensitivity and fatty acid β-oxidation and energy coupling in skeletal muscle and adipose tissue perhaps through PPARγ and PPARδ-mediated activation of aP2 and CD36—genes involved in lipolysis and energy metabolism[2]; mechanism is different than thiazoladinediones.[1-,6] Water-soluble cinnamon extracts can act as a dual activator of PPARα mRNA (liver) and PPARγ mRNA (adipose tissue)[3]; also has anti-hyperglycemic and anti-hyperlipidemia in mice.[4]
- Cinnamon *zeylanicum* possesses anti-cancer activity in melanoma[7], cervical cancer[8] and breast cancer.[9] Mechanisms for anti-cancer actions include: free radical scavenging[6], anti-oxidant[6], by ↓ proangiogenic factors[7], ↑CD8+ T-cells to ↑cytotoxic activity[7], ↓MMP-2 and HER2 expression/activity[8], ↓ VEGF/VEGF rec 2 kinase activity-mediated angiogenesis[8], and melatonin/melatonin receptors[9].
- Research Question: Can cinnamon derivatives act in a similar manner as melatonin and, if so, should cinnamon use be modified to that of melatonin therapy to enhance its therapeutic efficacy to treat diseases associated with metabolism and cancer?

Ref 1: Hosni, A. Biomed Pharmacother; 88: 52-80, 2017; Ref 2: Li, J. Amer J Chin Med, 43: 879-892, 2015; Ref 3: Sheng, X, PPAR Res, 2008: 581348, 2008; Ref 4: Kim, S., Arch Pharm Res, 33: 325-333, 2010; Ref 5: Ranasinghe, P. Diabetic Medicine, 2012; Ref 6: Ranasinghe, P., BMC CAM, 13: 275, 2013; Ref 7: Kwon, HK. Cancer Letters, 2015; Ref 8: Koppikar, BMC Cancer, 10: 210, 2010; Ref 9: Hasan, J Natural Product, 80: 3324-29, 2017

DESIGN AND METHODOLOGY



Cell Culture: Breast cancer cells, MMC (HER2+), MCF-7 (ER+/PR+), MDB-MB-231 (ER-/PR-/HER2- or triple negative) and the BT549 (triple negative), obtained from American Type Culture Collection, were cultured in DMEM:F12 medium supplemented with 10% fetal bovine serum and 1% penicillin/strepmycin antibiotic combination and maintained in a humidified 37ºC incubator with 5% CO_2/95% air.

Competition Binding for Melatonin Receptors:

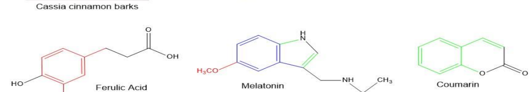
- Binding affinities of melatonin and the cinnameric derivatives for MT1 melatonin receptors were measured by competition binding analysis using 2-[^{125}I]-iodomelatonin on whole cell lysates prepared from human MT1 CHO cells.
- Cell lysates were prepared by first washing cells with 5 ml of PBS, lifting them into buffer (10mM KPO_4, 1mM EDTA, pH 7.4), pelleted by centrifugation (1500 rpm for 5 min) and then re-suspended in Tris-HCl buffer (50 mM, pH 7.4) to rupture the cells.
- Next, cells were added to tubes containing 100pM of 2-[^{125}I]-iodomelatonin and melatonin or cinnameric derivatives (1pM-100µM) and incubated for 1h at room temperature and then terminated by the addition of ice-cold Tris-HCl solution (50 mM) and rapid filtration over glass-fiber filters (0.22ìm; Schleicher & Schuell, Keene, NH) presoaked in 0.5% polyethylenimine solution (v/v) (Sigma). Each filter was washed twice with 5 ml of cold buffer. Radioactivity of 2-[^{125}I]-iodomelatonin was measured in a gamma counter.

Viability and Cell Migration Analyses:

- were plated in a 48-well plate (for MTT assay) or a 24-well plate (for scratch assay) 24 hours prior to starting the treatments.
- For the MTT (proliferation) assays, each well was exposed to either vehicle (0.01% ethanol), melatonin or the cinnamon derivatives (1pM, 1nM, 1µM, 10µM, 100µM) for 24 hours and then assayed using absorbance spectrophotometry (@570nm).
- For the wound/scratch (migration) assays, a small scratch was created in each cell culture well by dragging a pipette tip across the bottom of the well to remove a portion of cells (see image above). The widths of these channels were measured at time 0h. Next, cells were exposed to vehicle (0.01% ethanol), 100µM melatonin or 100µM cinnameric derivative 7 (NP-7) or 8 (NP-8) for 24h after which the border widths were remeasured. The border width at time 24h was subtracted from the border width at time 0h to calculate the migratory properties of the breast cancer cells in response to the various treatments. Calculation = (border $width_{24h}$ minus border $width_{0h}$ X 100). A more negative number indicates "greater invasiveness" or more cell migration while a more positive number indicates "less invasiveness" or less cell migration.
- For the MTT assays, data were fit by non-linear regression analysis to calculate potency (IC_{50}) and efficacy (maximum inhibition) values using GraphPad Prism (version 6) software. For the migration assays, data were analyzed by one-way ANOVA followed by Newman-Keuls post-hoc t-test where significance was defined as p<0.05 using GraphPad Prism (version 6).

Results

Legends:
---- Part of ferulic acid matches to the part of melatonin
---- Part of coumarin matches to the part of melatonin
---- Part of melatonin matches to the part of ferulic acid and coumarin



Cinnameric Derivatives Bind to Melatonin Receptors

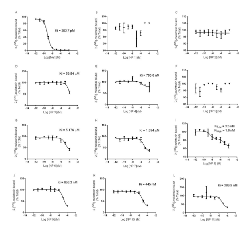


Figure 1: Competition of melatonin or **1-11** for 2-[^{125}I]-iodomelatonin binding to human MT1Rs expressed in CHO cells (1A-1L). Each point represents the mean ± SEM of 2-[^{125}I]-iodomelatonin binding normalized against vehicle (total) binding from 3 independent experiments.

Cinnameric Derivatives, Like Melatonin, Inhibit Breast Cancer Cell Proliferation

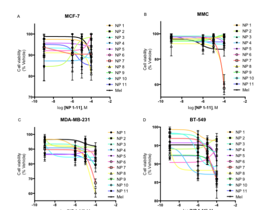


Figure 2: Cell proliferation in response to melatonin or compounds **1-11**. MTT assays were performed on (A) MCF-7 (ER+/PR+), (B) MMC (HER2+), (C) MDA-MB-231 (ER-/PR-/HER2-), or (D) BT-549 (ER-/PR-/HER2-) breast cancer cells. Each point represents the mean ± SEM of 3 independent experiments.

Cinnameric Derivatives, Like Melatonin, Inhibit Breast Cancer Cell Migration

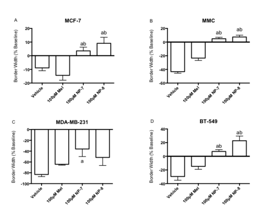
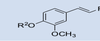
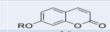


Figure 3: Breast cancer cell migration in response to vehicle or 100 µM **7 or 8** on (A) MCF-7 (ER+/PR+), (B) MMC (HER2+) (C) MDA-MB-231 (ER-/PR-/HER2-), or (D) BT-549 (ER-/PR-/HER2-) breast cancer cells. Each bar represents the mean ± SEM of the border width change that occurred over a 24h period normalized against baseline (0h). Change in border width after 24 h exposure to compound **7:** MCF=3%±3, MMC=5%±2, MDA-MB-231=-36%±14, BT-549=7%±3 and compound **8**: MCF=9%±4, MMC=7%±3, MDA-MB-231= -52%±15, BT-549=23%±7. Data were analyzed by one-way ANOVA followed by Newman-Keuls post-hoc t-test where significance was defined as p<0.05. "a" denotes p<0.05 vs. vehicle; "b" denotes p<0.05 vs Mel.

RESULTS

Table 1: Natural product composite binding affinity to melatonin receptors and potency and efficacy to inhibit breast cancer cell proliferation.

NP	Compound	Structure	Affinity to MT1R (Ki)	Proliferation							
				Potency (IC_{50})				Efficacy (% Veh.)			
				MCF-7	MMC	MDA-MB-231	BT-549	MCF-7	MMC	MDA-MB-231	BT-549
											
1	Ferulic Acid	R^1 = COOH, R^2 = H	3.419µM	0.6M	23.5M	0.5µM	1.2µM	96±8	100±1	92*±2	99±5
2	Boropinic Acid	R^1 = COOH, R^2 = 3,3-dimethylallyl	1.376nM	0.7µM	6.1µM	0.8pM	15µM	102±5	98±3	89*±3	97±0.7
3	4'-Gernayloxyferulic acid	R^1 = COOH, R^2 = geranyl	59.54µM	N.C.	2.5µM	21.8µM	5.6µM	102±11	99±7	79*±5	93±1
4	Boropinal	R^1 = CHO, R^2 = 3,3-dimethylallyl	785.8nM	N.C.	0.22µM	63.1µM	45nM	94±4	96±4	79*±1	83*±2
											
5	Umbelliferone	R = H	16.1pM	2.9µM	10.3M	3.7µM	1mM	124±31	93±4	88*±1	91±4
6	7-iso-pentenyloxycoumarin	R = 3,3-dimethylallyl	5.176µM	13.7M	N.C.	N.C.	36.8µM	105±2	98±0.05	83*±4	94±1
7	Auraptene	R = geranyl	1.894µM	N.C.	56.9mM	80.2µM	24.3µM	90±3	57*±5	67*±3	86*±2
8	Umbellinprenin	R = farnesyl	Ki_{Low} = 3.3nM Ki_{High} = 1.6nM	30M	88.2mM	30.2µM	1.1pM	81±3	61*±7	60*±3	85*±1
9	7-(pent-2'-ynil) coumarin	R = CH_2-C≡C-CH_2CH_3	888.3nM	50µM	24.1µM	N.C.	74.3µM	89±7	101±3	87±3	86*±1
10	7-Benzyloxycoumarin	R = CH_2Ph	445nM	7.1µM	0.4µM	0.4nM	5.4µM	89±4	91±2	82±2	88*±0.4
11	7-Styryloxycoumarin	R = CH=CH-Ph	369.9nM	2.2µM	N.C.	6.6µM	0.14mM	87±5	93±4	82±3	86*±1
	Melatonin		303.7pM	18.2mM	0.2µM	0.9µM	30.4mM	87±4	91±8	92*±2	91±1

Data represent the mean ± SEM of 3 independent experiments. Data were analyzed by one-way ANOVA followed by Newman-Keuls post-hoc t-test where significance was defined as p<0.05. * denote significance vs. vehicle. N.C. = not converged.

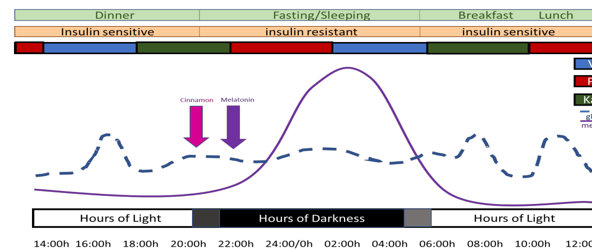


Fig. 5. Schematic showing natural physiological rhythms throughout a 24-hour cycle. Melatonin achieves its maximum therapeutic efficacy (e.g., anti-cancer effect; bone-protective effect) when dosed in sync with its natural endogenous rhythm to bump up the natural nocturnal surge. Giving cinnamon closer to bedtime may enhance its anti-oxidant, detoxification and/or adrenal supportive actions.

SUMMARY AND CONCLUSIONS

- ❑ Derivatives of cinnamon are structurally similar to coumarin and melatonin
- ❑ Derivatives of cinnamon can bind to melatonin receptors and inhibit cancer similar to melatonin; however, the mechanism is unclear.
- ❑ Cinnamon derivatives may be working through both coumarin- and melatonin-dependent mechanisms that include anti-oxidant pathways and melatonin receptor-dependent pathways to protect the body against disease.
- ❑ As shown in the figure above, melatonin displays a circadian rhythm coincident with the light/dark cycle where levels peak during the hours of darkness.
- ❑ Insulin resistance also follows a circadian rhythm coincident with the melatonin rhythm where insulin resistance occurs during the night when melatonin levels are highest.
- ❑ Melatonin achieves maximal therapeutic efficacy when dosed in sync with the endogenous melatonin rhythm with respect to cancer- and bone-protection.
- ❑ Cinnamon being structurally similar to melatonin may achieve greater therapeutic efficacy when dosed according to the endogenous melatonin rhythm.

Ayurveda

- Based on these data, giving cinnamon closer to one's bedtime may provide greater benefit to the body to protect against cancer, diabetes and adrenal fatigue.

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