Clinical Science

Original Research Study
Acta Medica Academica 2022;51(1):xx-xx
DOI: 10.5644/ama2006-124.XX

The Potential of Cinnamon Extract (*Cinnamomum burmanii*) as Anti-insomnia Medication through Hypothalamus Pituitary Adrenal Axis Improvement in Rats

Rachmat Hidayat[1], Patricia Wulandari[2], Muhammad Reagan[3]

[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia, [2]Department of Psychiatry, Cattleya Mental Health Center, Palembang, Indonesia, [3]Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

Correspondence: *dr.rachmat.hidayat@gmail.com*; Tel.: + 62 882 25053819

Received: 16 January 2022; **Accepted:** 28 April 2022

Abstract

Objective. This study aimed to explore the efficacy of cinnamon extract as an anti-insomnia medication in experimental animals by evaluating the levels of hormones and neurotransmitters related to insomnia. **Materials and Methods.** A total of 30 male Wistar rats were divided into six groups. Induction of insomnia in animal models was done by administration of p-chloro-phenylalanine (PCPA) compounds. Estazolam was administrated to the positive control group. Cinnamon extract administration was divided into 3 doses, namely: 25 mg/kg BW, 50 mg/kg BW and 100 mg/kg BW. Evaluation of the organ coefficient was conducted to evaluate drug toxicity to the organs. The enzyme-linked-immunoassay method assessed hormones and neurotransmitters in the serum and hypothalamus related to insomnia. **Results.** There was a decrease in the adrenal coefficient in the cinnamon extract group compared to the PCPA group (0.011+0.001, P<0.05). In addition, there was a decrease in the corticotropin-releasing hormone, adrenocorticotropin hormone, and corticosterone levels in the serum of animals who received cinnamon extract. Our study found a dose of cinnamon extract of 50 mg/kg BW was the best dose to balance neurotransmitter levels in insomniac rats. **Conclusion.** The cinnamon extract increased serotonin and melatonin levels and decreased norepinephrine levels in the insomnia-induced group. Cinnamon extract has potential as an anti-insomnia medication through hypothalamus-pituitary-adrenal axis improvement and brain neurotransmitter regulation in an animal model of insomnia.

Key Words: *Cinnamomum burmanii* ▪ Corticosterone ▪ Corticotropin-Releasing Hormone ▪ Oxidative Stress ▪ Serotonin.

Introduction

Insomnia is a sleep disorder that can affect a person's quality of life, both physically and mentally (1). The increased frequency of insomnia in the last decade is influenced by various life stressors that are high in the millennial era (2). These life stressors are caused by job, school, or social problems. Insomnia is a clinical condition characterized by difficulty falling asleep and maintaining sleep, and decreased sleep quality (2). The diagnosis of insomnia also requires the presence of daytime impairment or consequences associated with the night time sleep complaints. Insomnia is reported to be experienced by nearly 30% or nearly a third of the world's population (3).

Disruption of neurotransmitters or endogenous sleep-regulating molecules is associated with insomnia. Neurotransmitters related to sleep regulation and circadian rhythms include gamma amino butyric acid (GABA), serotonin, melatonin, histamine, prostaglandins, and hypocretin or orexin (4). In addition, dysfunction of the hypothalamus-pituitary-adrenal gland (HPA) axis is thought to increase the disruption of the sleep-wake cycle, leading to insomnia (5). The HPA axis involves endogenous molecules such as corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (5).

Management of insomnia is carried out using two approaches, namely pharmacological, and non-pharmacological approaches according to the cognitive behavioral therapy (2). Drugs used in the pharmacological approach act on gamma-aminobutyric acid (GABA) receptors, melatonin, histamine or hypocretin (2). Drugs that act on these four receptors are currently a modality of insomnia therapy, but the available drugs have unwanted side effects and none of these drugs creates a sleep cycle that is similar to natural sleep (6). Of course, this is a new problem that will trigger new problems related to insomnia. Herbal medicine is one of the traditional treatments for insomnia. However, the efficacy of herbal remedies for treating this disorder are currently unknown.

Cinnamon (*Cinnamomum burmanii*) is a plant that is well known by the Indonesian people as a cooking spice and has been used for generations in overcoming various health problems, including to help overcome insomnia (7, 8). Cinnamaldehyde is the main compound believed to play a role in improving sleep quality in insomnia (9). Cinnamon is also believed to be able to improve neurotransmitter activity in cases of insomnia.

This study is the first initial study to explore the efficacy of cinnamon extract as an anti-insomnia medication by evaluating the effect of cinnamon extract administration on experimental animals with induced insomnia. Exploration of serum levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), corticosterone (CORT), serotonin, norepinephrine and melatonin were carried out to test the efficacy of the cinnamon extract in sleep regulation in the animal model.

Methods

Cinnamon Extract Preparation

Cinnamon bark was obtained from the Tawangmangu Medicinal Plant Development Center, Karanganyar, Central Java, in September 2021, and was identified and classified at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia. The cinnamon bark was washed, cleaned and dried at 60° C for 36 hours. Further, the cinnamon was mashed so that it became dry simplicia. A total of 500 grams of dry simplicia were extracted by the maceration method using 70% ethanol (1:10) solvent for 36 hours. The macerate was then evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) to obtain a thick extract of 124 grams (24.8% yield).

Animals and Treatment

This study was conducted at the Eureka Research Laboratory, Palembang, Indonesia. The experimental animals used were 30 male Wistar rats of 150-200 grams weight and 3 months of age. This study was approved by the Animal Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya (Ref. No. 234/FKUNSRI/IX/2021). The animals were kept in a standardized rearing room (temperature 25 ± 2°C, humidity 50±5%, 12 hour light and dark cycle) with standard feed and water ad libitum. Induction of insomnia in the animal model was performed by administration of p-chloro-phenyl alanine (PCPA) compounds (Sigma, St. Louis, MO, USA; No: C6506) (10). PCPA was dissolved in weak alkaline saline (pH 7-8) and injected intraperitoneally (400 mg/kg BW) once daily for two days. After acclimatization, the experimental animals were grouped into six groups (N=5 per group): a control group (untreated, received only saline), the PCPA group (400 mg/kg BW), the PCPA + estazolam group (0.5 mg/kg BW), the PCPA + cinnamon extract group (50 mg/kg BW), the PCP + cinnamon extract group (100 mg/kg BW), and the PCPA + cinnamon extract group (200 mg/kg BW). The experimental animal treatment was conducted for 7 days. After injection with PCPA within 28-30 hours, there was a change in the circadian rhythm and sleep latency of the rats, and it was significantly different from the control group that was not injected with PCPA, which indicated the success of the induction method.

Organ Coefficient

The experimental animals were weighed for seven days of the treatment. Experimental animals were anaesthetized with 10% intraperitoneal chloral hydrate. Furthermore, the evacuation of the adrenal organs was carried out after taking blood from the abdominal aorta. The brain organs are then evacuated and put on ice, and the brain coefficient was determined. The organ coefficient is the ratio of organ weight to the bodyweight of the experimental animals. Changes in the value of organ coefficients indicate drug toxicity to the organs.

Assessment of CRH, ACTH and CORT Serum Levels

Before treatment, the rats were anaesthetized by injection of 10% intraperitoneal chloral hydrate. Blood was drawn through the abdominal aorta, then centrifuged at 3500 rpm at 4°C for 15 minutes. CRH), ACTH and CORT levels in the serum were assessed using the enzyme-linked immunoassay (ELISA) method according to the guidelines and kit instructions (CloudClone, Wuhan, China).

Assessment of Serotonin, Norepinephrine and Melatonin Levels in the Hypothalamus

The animals were anaesthetized by intraperitoneal injection of 10% chloral hydrate. Then the brain was evacuated and put on ice, and the hypothalamus evacuated with precision forceps. The supernatant from the hypothalamus was homogenized and centrifuged at 5000 g for 10 minutes, and then the levels of serotonin, norepinephrine and melatonin were measured by the ELISA method, according to the guidelines and kit instructions (CloudClone, Hanzhou, China).

Statistical Analysis

The results of the study are presented as mean ± standard deviation (SD). One way ANOVA was used to compare the results between multiple groups. $P<0.05$ showed a significant difference, and all data processing was carried out using the SPSS 25.0 program (IBM, Armonk, USA).

Results

The results showed a difference in the addition of the adrenal coefficient between the control and PCPA groups (Table 1). The increase in body weight and brain coefficient was significantly different from the treatment group that received cinnamon extract. The decrease in adrenal coefficient in the group that received cinnamon extract compared to the PCPA group was also quite significant.

Table 1. Effect of Cinnamon Extract on Brain Coefficient and Adrenal Coefficient in PCPA-induced Insomnia

Group	Brain coefficient (Mean±SD)	Adrenal coefficient (Mean±SD)
Control	0.850±0.030	0.01±0.003
PCPA*	0.830±0.040	0.016±0.002
PCPA + estazolam	0.900±0.040†	0.014±0.002
PCPA + cinnamon extract (25 mg/kg BW‡)	0.870±0.030	0.014±0.002
PCPA+ cinnamon extract (50 mg/kg BW‡)	0.890±0.030†	0.011±0.001†
PCPA+ cinnamon extract (100 mg/kg BW‡)	0.890±0.020	0.011±0.001†

*P-chloro-phenyl alanine; †P<0.05 compared with PCPA group. ‡Body weight.

The PCPA group showed a significant increase in CRH, ACTH, and CORT serum levels compared to the control group (Table 2). Giving cinnamon extract for seven days could reduce levels of CRH, ACTH and CORT. As a comparison, this study used estazolam, a benzodiazepine group that works by binding to the benzodiazepine receptor and strengthening the effect of GABA.

The PCPA-induced group showed decreased serotonin and melatonin levels in the hypothalamus compared to the control group (Table 3). Norepinephrine levels in the group receiving PCPA showed a significant increase compared to the control group. The cinnamon extract increased serotonin and melatonin levels and decreased

Table 2. Effect of Cinnamon Extract on Serum Levels of CRH, ACTH and CORT in PCPA-induced Insomnia

Group	CRH[†] (ηg/mL) (Mean±SD)	ACTH[‡] (ρg/mL) (Mean±SD)	CORT[§] (ηg/mL) (Mean±SD)
Control	8.150±0.400	423.100±15.140	30.100±2.140
PCPA[*]	12.120±0.900	587.500±23.210	46.100±3.650
PCPA + estazolam	8.130±0.400[‖]	442.600±18.670[‖]	38.100±2.140[‖]
PCPA + cinnamon extract (25 mg/kg BW[¶])	9.870±0.300[‖]	436.700±16.740[‖]	41.600±1.540[‖]
PCPA + cinnamon extract (50 mg/kg BW[¶])	7.900±0.300[‖]	433.700±19.720[‖]	33.400±2.120[‖]
PCPA + cinnamon extract (100 mg/kg BW[¶])	7.540±0.200[‖]	429.500±17.180[‖]	32.500±1.640[‖]

[*]P-chloro-phenyl alanine; [†]Corticotropine releasing hormone; [‡]Adrenocorticotropine hormone; [§]Cortisone; [‖]P<0.05 compared with PCPA group; [¶]Body weight.

Table 3. Effects of Cinnamon Extract on Serotonin, Norepinephrine and Melatonin Levels in PCPA-induced Insomnia

Group	Serotonin (ηg/mL) (Mean±SD)	NE[*] (ηg/mL) (Mean±SD)	MT[†] (ρg/mL) (Mean±SD)
Control	6.150 ±0.400	0.320±0.010	6.100±0.400
PCPA[‡]	3.120±0.200	0.650±0.040	3.400±0.200
PCPA + Estazolam	6.130±0.400[§]	0.350±0.010[§]	5.100±0.400[§]
PCPA + cinnamon extract (25 mg/kgBW[‖])	7.170±0.300[§]	0.470±0.020[§]	4.600±0.400[§]
PCPA + cinnamon extract (50 mg/kgBW[‖])	7.850±0.300[§]	0.400±0.020[§]	5.400±0.100[§]
PCPA + cinnamon extract (100 mg/kgBW[‖])	7.930±0.200[§]	0.380±0.010[§]	6.200±0.300[§]

[*]Norepinephrine; [†]Melatonin; [‡]P-chloro-phenyl alanine; [§]P<0.05 compared with PCPA group; [‖]Body weight.

norepinephrine levels in the insomnia-induced group. Our study found a dose of cinnamon extract of 50 mg/kg BW was the best dose to balance neurotransmitter levels in insomnia rats.

Discussion

Our study showed that in the treatment group that received cinnamon extract, there was an increase in the adrenal coefficient, indicating the cellular repair of the brain and adrenals. In accordance with this finding, a previous study found improvement and reduction of inflammation in the rats' brains after treatment with cinnamon extract (11). Another study revealed cinnamaldehyde in cinnamon extract has beneficial effects against oxidative stress and nitric oxide metabolites in rats' adrenal glands and brains (12).

Injection of p-chloro-phenyl alanine (PCPA) is the most commonly used model to establish an insomnia model (13). In our study, induction of insomnia by administration of PCPA caused a significant increase in hypothalamus-pituitary-adrenal gland axis (HPA axis) hormone levels compared to the control group. Furthermore, cinnamon extract treatment in our study caused a decrease in HPA axis hormone levels (cortisol, ACTH and CRH), which showed the efficacy of the cinnamon extract in improving regulation of the HPA axis. Another study found a decrease in the hormone levels of the HPA axis after administration of cinnamon extract (14).

Our results further demonstrated an increase in serotonin levels and decreased norepinephrine levels in the insomnia-induced group after cinnamon extract treatment. Serotonin and melatonin are essential neurotransmitters that play a role in the initiation of sleep, while norepinephrine is a neurotransmitter that keeps a person alert. A PCPA injection in animal models causes a decrease in serotonin and melatonin activity which causes sleep disturbances, and a PCPA injection causes an increase in norepinephrine activity, which stimulates the rats to remain awake. A previous study showed increased melatonin activity can improve sleep quality in human (15). Disruption of neurotransmitters is one of the factors that play a role in the pathogenesis of insomnia. Several

neurotransmitters, including serotonin, norepinephrine, dopamine and GABA, play an essential role in regulating sleep and wakefulness. In our study cinnamon extract treatment was shown to increase serotonin and melatonin activity, and reduce norepinephrine activity. Our study also revealed the efficacy of cinnamon extract as anti-insomnia medication in an animal model.

Conclusion

On the basis of our results, it was concluded that cinnamon extract has potential as an anti-insomnia medication through HPA axis improvement and regulation of brain neurotransmitters in an animal model of insomnia. To understand further and evaluate the active compound of *Cinnamomum burmanii* extract for insomnia, isolation of the active compound, and toxicity and safety tests are needed.

What Is Already Known in This Topic:

Cinnamon (Cinnamomum burmanii) is a plant that is well known as a cooking spice and has been used for generations in overcoming various health problems, including to help overcome insomnia. Cinnamaldehyde is the main compound believed to play a role in improving sleep quality in insomnia. Cinnamon is believed to be able to improve neurotransmitter activity in cases of insomnia.

What This Study Adds:

This study is the first, initial study to explore the efficacy of cinnamon extract as an anti-insomnia medication by evaluating the effect of cinnamon extract administration on experimental animals with induced insomnia. In this study, cinnamon extract treatment for seven days reduced levels of corticotropin hormone, adrenocorticotropin hormone and cortisone. The cinnamon extract increased serotonin and melatonin levels, and decreased norepinephrine levels in insomnia-induced animals.

The study was conducted at the Eureka Research Laboratory, Palembang and Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

Authors' Contributions: Conception and design: RH and PW; Acquisition, analysis, and interpretation of data: RH, PW and MR; Drafting the article: RH, PW and MR; Revising it critically for important intellectual content: RH, PW and MR; Approved final version of the manuscript: RH, PW and MR.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Mariani D, Muzasti RA, Thamrin A. The Relationship Between Quality of Sleep and Quality of Life of Patients in Medan, Indonesia. Open Access Maced J Med Sci. 2019;7(11):1794-7. doi: 10.3889/oamjms.2019.353.
2. Dopheide JA. Insomnia overview: epidemiology, pathophysiology, diagnosis and monitoring, and nonpharmacologic therapy. Am J Manag Care. 2020;26(4 Suppl):S76-84. doi: 10.37765/ajmc.2020.42769.
3. Kalmbach DA, Pillai V, Drake CL. Nocturnal insomnia symptoms and stress-induced cognitive intrusions in risk for depression: A 2-year prospective study. PLoS One. 2018;13(2):e0192088. doi: 10.1371/journal.pone.0192088.
4. Levenson JC, Kay DB, Buysse DJ. The pathophysiology of insomnia. Chest. 2015;147(4):1179-92. doi: 10.1378/chest.14-1617.
5. Hirotsu C, Tufik S, Andersen ML. Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. Sleep Sci. 2015;8(3):143-52. doi: 10.1016/j.slsci.2015.09.002.
6. Watson CJ, Baghdoyan HA, Lydic R. Neuropharmacology of Sleep and Wakefulness. Sleep Med Clin. 2010;5(4):513-28. doi: 10.1016/j.jsmc.2010.08.003.
7. Kawatra P, Rajagopalan R. Cinnamon: Mystic powers of a minute ingredient. Pharmacognosy Res. 2015;7(Suppl 1):S1-6. doi: 10.4103/0974-8490.157990.
8. Ribeiro-Santos R, Andrade M, Madella D, Martinazzo AP, Moura LAG, Melo NR, et al. Revisiting an ancient spice with medicinal purposes: Cinnamon. Trends Food Sci Tech. 2017;62(C):154-69. doi: 10.1016/j.tifs.2017.02.011.
9. Sharifian FE, Bahrami F, Zekri S, Sahraei H. Cinnamaldehyde antagonizes REM sleep reduction induced by immobilization stress in rats [in Persian]. J Mazandaran Univ Med Sci. 2019;29(175):14-24.
10. Ren XJ, Wang QQ, Zhang XP, Wang GY, Liu T, Deng N, et al. Establishment of a rat model with ageing insomnia induced by D-galactosef and para-chlorophenylalanine. Exp Ther Med. 2020;20(4):3228-36. doi: 10.3892/etm.2020.9080.
11. Sayad-Fathi S, Zaminy A, Babaei P, Yousefbeyk F, Azizi N, Nasiri E. The metanolic extract of Cinnamomum zeylanicum bark improves formaldehyde-induced neurotoxicity through reduction of phospho-tau (Thr231), inflammation and apoptosis. EXCLI J. 2020;19:671-86. https://pubmed.ncbi.nlm.nih.gov/32536837
12. Ataie Z, Mehrani H, Ghasemi A, Farrokhfall K. Cinnamaldehyde has beneficial effects against oxidative stress and nitric oxide metabolites in the brain of aged rats fed with long-term, high fat diet. J Funct Food. 2019; 52:545-51. Doi: 10.1016/j.jff.2018.11.038
13. Si Y, Wang L, Lan J, Li H, Guo T, Chen X, at al. Lilium davidii extract alleviates p-chlorophenylalanine-induced insomnia in rats through modification of the

hypothalamic-related neurotransmitters, melatonin and homeostasis of the hypothalamic pituitary adrenal axis. Pharmaceutical Biol. 2020;58(1):915-24. Doi: 10.1080/13880209.2020.1812674

14. Parisa N, Hidayat R, Maritska Z, Prananjaya BA. Antidepressant effect of cinnamon (Cinnamomum burmanii) bark extract in chronic stress-induced rats. Open Access Maced J Med Sci. 2020;8(A):273-7. doi: https://doi.org/10.3889/oamjms.2020.3995.
15. Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol.2018;175(16):3190-9. Doi:10.1111/bph.14116