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Title: Antidepressant effect of cinnamon (*Cinnamomum burmanii*) bark extract in chronic stress induced rats
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Journal's name: Open Access Macedonian Journal of Medical Sciences

1. Proofreading service (October 10, 2019)
- Certificate of language service
2. Submission Acknowledgement (November 5, 2019)
3. Peer reviewer result (February 10, 2020)
4. Revision sent back to editor (March 14, 2020)
5. Editor decision (April 21, 2020)
6. Galleyproof (April 30, 2020)
7. Manuscript was published in journal website (May 21, 2020)

UNIVERSITY OF SKOPJE LANGUAGE INSTITUTE

Rajko 1000 Skopje, Republic of Macedonia

Certificate Proofreading Service Confirmation

University of Skopje Language Service provided comprehensive editing services for manuscript entitled **Antidepressant effect of cinnamon (*Cinnamomum burmanii*) bark extract in chronic stress induced rats** by Nita Parisa, Rachmat Hidayat, Ziske Maritska and Bintang Arroyantri Prananjaya.

The edit has achieved Grade A: priority publishing; no language polishing required after editing. Should you require any additional information, please do not hesitate to contact me.

Sincerely,
Anna Koneski, PhD
Head of Language Institute
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Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats

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Keywords: Antidepressant effect; Cinnamon bark; *Cinnamomum burmannii*

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Funding: The authors would like to deliver gratitude to the Department of Pharmacology, Department of Biology, and Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia, for the support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. An effective and commonly used drug is the selective serotonin reuptake inhibitor, namely, fluoxetine. However, this agent has so many side effects, one of them is erectile dysfunction. In order to find the better treatment, exploration and discovery of therapeutic modalities need to pursued using natural materials.

AIM: This study aimed to explore and evaluate antidepressant effects of cinnamon (*Cinnamomum burmannii*) extract (CE).

METHODS: A total of 30 male Wistar rats were obtained from Eureka Research Laboratory (Palembang, Indonesia). Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Rats were induced using chronic mild stress (CMS). CMS was a form of stress induction performed on experimental animals continuously, for 4 weeks. Forced swimming test (FST) was a test conducted to assess mobility in animal model. After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. After treatment and FST, organ evacuation was performed and followed by immunohistochemistry and enzyme-linked immunosorbent examination.

RESULTS: This study showed that CE with dose of 25 mg/kg BW to dose 100 mg/k BW could reduce the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression. Histologically, CE showed the potential to improve serotonin levels in the hippocampus with increasing doses. Tumor necrosis factor (TNF)-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group.

CONCLUSION: CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

Introduction

Depression is a mental disorder that is commonly found throughout the world. The depression could strike all ages, usually characterized by feeling of sadness, anxiety, guilty thought, insomnia, loss of interest in pleasure thing, and suicidal idea. According to the WHO, there are 300 million people in the world suffering from depression with nearly 800,000 people die due to suicide each year. Therefore, the depression is one of the mental disorders with the need of immediate proper treatment as early as possible [1].

To find the cause of depression, many studies had been conducted. It is believed that serotonin is a neurotransmitter that plays a role in the pathogenesis of depression. Inflammation is a major factor that relates in reducing the function of neurogenesis of neuronal cells in the brain. Decreased neurogenesis due to inflammation causes neuronal cell death which will lead to decreased neurotransmitter synthesis, including serotonin. Low levels of serotonin are a marker of depression, so the search for chemical compounds that can increase or maintain normal levels of serotonin is the main focus of pharmacotherapy. Today, an effective and commonly used drug is the selective serotonin reuptake inhibitor (SSRI), namely, fluoxetine. Even so, one of the side effects of this drug is erectile dysfunction, considering that depression often happen in productive age. The side effects caused by this drug cannot be ignored [2]. Therefore, exploration and discovery of therapeutic modalities need to be pursued using and utilizing natural materials.

Cinnamon (*Cinnamomum burmannii*) is one of the plants commonly found in Indonesia that is believed to have the potential to overcome various

health problems. In folk tradition, this plant was used hereditary to cure cough, fever, and joint pain. The main compound contained in this plant is cinnamaldehyde, which is a phenol group. Phenol compounds have lipophilic properties, so these compounds can penetrate the blood–brain barrier and are able to give effect to neuronal cells. Phenols have anti-inflammatory effects, which are believed to be able to inhibit the inflammatory cascade that forms the basis of the pathogenesis of depressive disorders [3], [4], [5].

This study was the first study to explore and evaluate antidepressant effects of cinnamon extract (CE) (*C. burmannii*). This study aimed to discover new modalities using and utilizing natural materials, CE (*C. burmannii*).

Methods

Animal

A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 h of light/dark cycles with temperatures of 22 ± 1°C and humidity of 40–60%), fed, and drank *ad libitum*. All animal treatments and experimental procedures were approved by the Ethics and Humanities Commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No.xxx/kptfkunsri-rsmh/2019).

CE preparation

Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Furthermore, extraction from cinnamon was done by maceration method. A total of 500 g of simplisia were macerated with 96% ethanol for 72 h. Then, it was separated to obtain the pulp and macerate. Macerate was then evaporated with a rotary evaporator (Heidolph) so that CE was obtained.

Animal model depression

Animal model depression was induced using chronic mild stress (CMS). CMS was a form of stress induction performed on the experimental animals continuously for 4 weeks. CMS procedures were performed with mild stressors such as repeated cold stress (4°C), space reduction in the homecage, changed cages within the CMS group, cage tilt, empty cage, intermittent air puff, wet bedding, white noise, overnight illumination, and social interaction with other animals of the CMS group. The particular context (stressor applied during preceding dark phase) of the water restriction intervals involved overnight illumination (Sunday-Monday), wet cage, and cage tilt (Tuesday-Wednesday and Thursday-Friday), and changed cages (Wednesday-Thursday), respectively [6]. As the following schedule:

Table 1: Procedure timeline of chronic mild stress

Day	Light phase		Dark phase	
	First half	Second half	First 2 h	Remaining 10 h
Monday	Cold stress (2×30 min)	Cold stress (30 min)	Water restriction	Space reduction
Tuesday	Changed room	Air puff (3×3 intermittent)	Wet cage	Wet cage
Wednesday	Wet cage	Social interaction	Foreign cage	Foreign cage
Thursday	Foreign cage	Social interaction	Water restriction	Cage tilt
Friday	Empty cage	Changed room	White noise	White noise
Saturday	White noise	Changed room	Changed room	Changed room
Sunday	Changed room	Changed room	Overnight illumination with 700×l	Overnight illumination with 700×l

Clinical evaluation: Forced swimming test (FST)

FST was a test conducted to assess mobility in animal model. First, the FST equipment was prepared, in the form of a cylindrical tube with a height of 60 cm and diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per mouse. Tests were conducted between 9 am and 3 pm. Each test animal was acclimatized for 10 min first, and then the mobility duration was measured in 5 min. Next, the mobility of the test animals was measured in seconds.

Animal model treatment

After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. The normal control group was not induced by CMS. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. The extract was made into a suspension preparation with 1% carboxymethyl cellulose emulgator so that the volume of suspension for every rat was 3 ml. Furthermore, the animal model was performed with perfusion so when the organ evacuation process was started, cell damage did not occur. Before perfusion was performed, the animal was anesthetized with ketamine (5 mg/kg BW) and xylazine (0.2 mg/kg BW) intraperitoneally. Perfusion was done by entering perfusor fluid, paraformaldehyde 4%, and through the left ventricle of the heart, followed by a tear in the right atrium of the heart so that blood would come out of the right atrium replaced by perfusor fluid. Perfusion continues until the fluid coming out of the right atrium became clear. Evacuation of the animal's brain was done by performing a craniotomy, through sulcus coronarius from calvaria, so that the animal's brain organ was obtained and splitting was performed to divide the right and left cerebral hemispheres. The left

cerebral hemisphere was then inserted into a fixation fluid, neutral buffer formalin 10%.

Immunohistochemistry examination

The left cerebral hemisphere that had been inserted into the fixation fluid was dehydrated using gradient alcohol and xylene, then paraffinized and cut as thick as 5 um using a rotary microtome (Leica). Next, tissue was placed on coated-object glass. Then, rehydration was carried out on the tissue using xylene and alcohol with a concentration of 96%, 90%, 80%, and 70% and rinsed with tap water. The next stage, retrieval antigen was carried out by the heat-induced epitope retrieval method, where the slides were put into a citrate buffer solution, then heated at a temperature of 95°C for 60 min. Then, tumor necrosis factor (TNF)-alpha 1:700 (cloud clone) antibody was stained, followed by overnight incubation at 4°C. The next stage was to paint with a secondary antibody, biotinylated-horseradish peroxidase, and incubation for 1 h at room temperature. Next, chromogen was added to the slide. Furthermore, the dehydration process was again carried out using concentrated alcohol and xylene. The next step was mounting and evaluating the TNF-alpha expression using ImageJ Software so that the percentage of TNF-alpha expression was obtained.

Enzyme-linked immunosorbent assay (ELISA) examination

Serotonin levels in the hippocampus were examined by human serotonin ELISA kit (cloud clone), based on the protocol found in the manufacturer's protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that had been coated with anti-serotonin and incubated at 37°C for 30 min. After the plates were washed, 100 µl of biotinylated antibody solution was added and incubated for 30 min at 37°C. After washing 3 times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 min at 37°C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 min at 37°C. Finally, 50 ul stop solution was added to stop the reaction and optical density values were measured using an ELISA reader (Bio-Rad) at wavelength 450 nm.

Data analysis

All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 program. One-way ANOVA followed by *post hoc* analysis was conducted to assess the differences in the mean expression and levels of each protein as well as clinical data. $p < 0.05$ was determined as an indication that there were significant differences in the mean levels.

Results

Duration of immobility showed clinical symptoms of depression. The longer duration of immobilization indicated more depressed test animal. Figure 1 shows that CE with dose 25 mg/kg BW to 100 mg/kg BW reduced the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression.

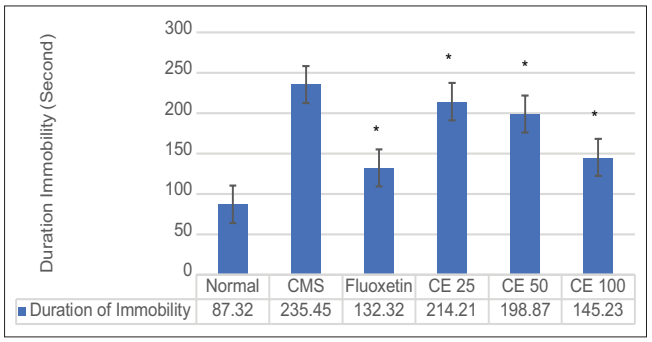

*Figure 1: Duration of immobility in rats for forced swimming test; *Versus chronic mild stress, $p < 0.05$*

Serotonin as the main neurotransmitter that plays a role in the pathophysiology of depressive disorders is an important marker in clinical depression. CE showed the potential to improve serotonin levels in the hippocampus with increasing doses (Table 1).

Table 1: Serotonin level in rats hippocampus

Group	Serotonin level (pg/mL) ± SD	p value*
Normal	178.86 ± 15.76	0.00
CMS	54.54 ± 3.12	-
Fluoxetine	112.21 ± 8.23	0.00
CE 25	65.23 ± 5.12	0.00
CE 50	87.12 ± 6.34	0.00
CE 100	98.76 ± 7.77	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

TNF-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group (Figure 2 and Table 2).

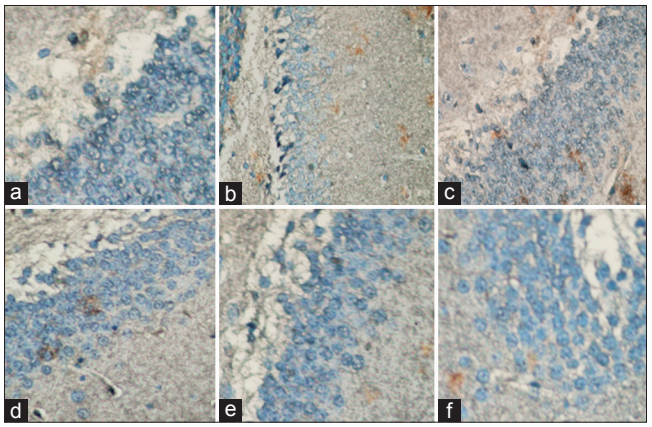

Figure 2: Tumor necrosis factor (TNF)-alpha expression in rats hippocampus. Black arrows: TNF-alpha expression. ×400. (a) Normal, (b) chronic mild stress, (c) fluoxetine, (d) cinnamon (CE) 25, (e) CE 50, (f) CE 100

Table 2: Tumor necrosis factor-alpha expression in rats hippocampus

Group	Tumor necrosis factor-alpha expression (%) Mean ± SD	p value*
Normal	2.86 ± 0.76	0.00
CMS	53.87 ± 3.62	-
Fluoxetine	19.11 ± 1.12	0.00
CE 25	45.56 ± 3.11	0.00
CE 50	37.31 ± 1.34	0.00
CE 100	28.76 ± 1.87	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

Discussion

Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. Increased mesolimbic dopamine will cause a decrease in dopamine in the mesocortical area so that it will cause the sufferer to experience a decrease in mood which results in depressive symptoms [7], [8], [9], [10].

Current management related to depressive disorders is aimed at maintaining optimum levels of serotonin in the synaptic cleft. Drugs, such as SSRI group and fluoxetine, play a role in inhibiting the uptake of serotonin in the synaptic cleft so that serotonin remains optimum and is able to improve depressive symptoms. Recent study was more focused on maintaining the viability of neuronal serotonin cells. Decreased neuronal serotonin cell viability will reduce serotonin levels so that it will cause symptoms of depression [11], [12], [13], [14], [15], [16].

Oxidative stress due to oxidants plays an important role as an initiator of inflammation. Inflammation is an important pathogenesis that plays a role in the initiation of cell death. Chronic inflammation will cause activation of the inflammatory cascade, which in turn activates the death receptor. Death receptor activation will then activate caspase which will cause apoptosis of neuronal serotonin cells [17], [18]. Therefore, it is important to make efforts to inhibit inflammation in neuronal cells. Phenol is a metabolite that is quite common in CE. Phenol has the potential to penetrate the blood–brain barrier, so it has the potential to have an effect on neuronal cells. Various studies show that phenol contained in cinnamon possesses antioxidant potential. Antioxidants are believed to be important compounds capable of neutralizing oxidants, to reduce the activation of inflammatory cascade and optimize the viability of serotonin neuronal cells [19], [20], [21], [22]. This study showed that CE improved clinical depression by inhibiting the expression of TNF-alpha in the hippocampus so that the viability of serotonin neuronal cells remained optimal and serotonin levels would increase compared to the group not administered with CE.

Conclusion

CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

References

1. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. https://doi.org/10.1037/e400972004-001. [Last accessed on 2019 Nov 01].
2. Du YH, Feng RZ, Li Q, Wei Q, Yin ZQ, Zhou LJ, *et al*. Anti-inflammatory activity of leaf essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao. Int J Clin Exp Med. 2014;7(12):5612-20.
   PMid:25664080
3. Partan RU, Hidayat R, Saleh MI, Parisa N, Tamzil NS. Effect of supplementation Kayu Manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy. Asian J Pharm Clin Res. 2018;11(2):363-5. https://doi.org/10.22159/ajpcr.2018.v11i2.22024
4. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, *et al*. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evid Based Complement Alternat Med. 2012;2012(429320):1-12.
5. Han X, Parker TL. Antiinflammatory activity of cinnamon (*Cinnamomum zeylanicum*) bark essential oil in a human skin disease model. Phytother Res. 2017;31(7):1034-8. https://doi.org/10.1002/ptr.5822
   PMid:28444928
6. Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: Of anhedonia, "anomalous anxiolysis" and activity. PLoS One. 2009;4(1):e4326. https://doi.org/10.1371/journal.pone.0004326
   PMid:19177164
7. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. Ups J Med Sci. 2013;118(1):3-8. https://doi.org/10.3109/03009734.2012.724118
   PMid:22970723
8. Amodeo G, Trusso MA, Fagiolini A. Depression and inflammation: Disentangling a clear yet complex and multifaceted link. Neuropsychiatry. 2017;7(4):448-57. Available from: http://www.jneuropsychiatry.org/peer-review/depression-and-inflammation-disentangling-a-clear-yet-complex-and-multifaceted-link.html. https://doi.org/10.4172/neuropsychiatry.1000236
9. Konsman JP. Inflammation and depression : A nervous plea for psychiatry to not become immune to interpretation. Pharmaceuticals (Basel). 2019;12(1):E29. https://doi.org/10.3390/ph12010029
   PMid:30769887
10. Köhler O, Krogh J, Mors O, Benrosc ME. Inflammation in depression and the potential for anti-inflammatory treatment. Curr Neuropharmacol. 2016;14(7):732-42.
    PMid:27640518
11. Abelaira HM, Réus GZ, Quevedo J. Animal models as

tools to study the pathophysiology of depression. Braz J Psychiatry. 2013;35(Suppl 2):S112-20. https://doi.org/10.1590/1516-4446-2013-1098

PMid:24271223

12. Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol. 2008;581(1-2):113-20. https://doi.org/10.1016/j.ejphar.2007.12.005

PMid:18184609

13. Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of depression: Neurotrophic and neuroinflammatory biomarkers. BioMed Res Int. 2014;2014(932757):932757. https://doi.org/10.1155/2014/932757

PMid:24999483

14. Zeldetz V, Natanel D, Boyko M, Zlotnik A, Shiyntum HN, Grinshpun J, *et al*. A new method for inducing a depression-like behavior in rats a new method for inducing a depression-like behavior in rats. J Vis Exp. 2018;132:57137. https://doi.org/10.3791/57137

PMid:29553503.

15. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34. https://doi.org/10.1038/nri.2015.5

PMid:26711676

16. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40-3. https://doi.org/10.1515/intox-2017-0006

PMid:30123035

17. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, *et al*. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200. https://doi.org/10.1186/1741-7015-11-200

18. Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars). 2004;64(4):439-48.

PMid:15586660

19. Ghaderi H, Rafieian M, Nezhad HR. Pharmacophore effect of hydroalcoholic *Cinnamomum zeylanicum* extract on reserpine-induced depression symptoms in mice. Pharmacophore. 2018;9(2):35-44. Available from: https://www.pharmacophorejournal.com/en/article/effect-of-hydroalcoholic-cinnamomum-zeylanicum-extract-on-reserpine-induced-depression-symptoms-in-mice. https://doi.org/10.4103/1735-5362.189308

20. Sharafeldin K, Raza M. Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin induced diabetic rats. J Basic Appl Zool. 2015;72:126-34. https://doi.org/10.1016/j.jobaz.2015.09.002

21. Chu X, Zhou Y, Hu Z, Lou J, Song W, Li J, *et al*. 24-hour-restraint stress induces phenotypes in mice. Sci Rep. 2016;6:32935.

22. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211-20. https://doi.org/10.1016/j.jfda.2017.03.006

Open Access Macedonian Journal of Medical Sciences

Submission Acknowledgment

November 5, 2019

Dear authors,

Thank you for submitting the manuscript "Antidepressant effect of cinnamon (*Cinnamomum burmanii*) bark extract in chronic stress induced rats" to Open Access Macedonian Journal of Medical Sciences.

Thank you for considering this journal as venue for your work.

Regards,

Prof. Dr. Mirko Spiroski

Editor in Chief

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Nita Parisa[1], Rachmat Hidayat[2*], Ziske Maritska[2], Bintang Arroyantri Prananjaya[3]

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Abstract

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Funding: The authors would like to deliver gratitude to the Department of Pharmacology, Department of Biology, and Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia, for the support.

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Animal model depression was induced using chronic mild stress (CMS). CMS was a form of stress induction performed on the experimental animals continuously for 4 weeks. CMS procedures were performed with mild stressors such as repeated cold stress (4°C), space reduction in the homecage, changed cages within the CMS group, cage tilt, empty cage, intermittent air puff, wet bedding, white noise, overnight illumination, and social interaction with other animals of the CMS group. The particular context (stressor applied during preceding dark phase) of the water restriction intervals involved overnight illumination (Sunday-Monday), wet cage, and cage tilt (Tuesday-Wednesday and Thursday-Friday), and changed cages (Wednesday-Thursday), respectively [6]. As the following schedule:

Table 1: Procedure timeline of chronic mild stress

Day	Light phase		Dark phase	
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Tuesday	Changed room	Air puff (3×3 intermittent)	Wet cage	Wet cage
Wednesday	Wet cage	Social interaction	Foreign cage	Foreign cage
Thursday	Foreign cage	Social interaction	Water restriction	Cage tilt
Friday	Empty cage	Changed room	White noise	White noise
Saturday	White noise	Changed room	Changed room	Changed room
Sunday	Changed room	Changed room	Overnight illumination with 700×l	Overnight illumination with 700×l

Clinical evaluation: Forced swimming test (FST)

FST was a test conducted to assess mobility in animal model. First, the FST equipment was prepared, in the form of a cylindrical tube with a height of 60 cm and diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per mouse. Tests were conducted between 9 am and 3 pm. Each test animal was acclimatized for 10 min first, and then the mobility duration was measured in 5 min. Next, the mobility of the test animals was measured in seconds.

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After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. The normal control group was not induced by CMS. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. The extract was made into a suspension preparation with 1% carboxymethyl cellulose emulgator so that the volume of suspension for every rat was 3 ml. Furthermore, the animal model was performed with perfusion so when the organ evacuation process was started, cell damage did not occur. Before perfusion was performed, the animal was anesthetized with ketamine (5 mg/kg BW) and xylazine (0.2 mg/kg BW) intraperitoneally. Perfusion was done by entering perfusor fluid, paraformaldehyde 4%, and through the left ventricle of the heart, followed by a tear in the right atrium of the heart so that blood would come out of the right atrium replaced by perfusor fluid. Perfusion continues until the fluid coming out of the right atrium became clear. Evacuation of the animal's brain was done by performing a craniotomy, through sulcus coronarius from calvaria, so that the animal's brain organ was obtained and splitting was performed to divide the right and left cerebral hemispheres. The left

cerebral hemisphere was then inserted into a fixation fluid, neutral buffer formalin 10%.

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The left cerebral hemisphere that had been inserted into the fixation fluid was dehydrated using gradient alcohol and xylene, then paraffinized and cut as thick as 5 um using a rotary microtome (Leica). Next, tissue was placed on coated-object glass. Then, rehydration was carried out on the tissue using xylene and alcohol with a concentration of 96%, 90%, 80%, and 70% and rinsed with tap water. The next stage, retrieval antigen was carried out by the heat-induced epitope retrieval method, where the slides were put into a citrate buffer solution, then heated at a temperature of 95°C for 60 min. Then, tumor necrosis factor (TNF)-alpha 1:700 (cloud clone) antibody was stained, followed by overnight incubation at 4°C. The next stage was to paint with a secondary antibody, biotinylated-horseradish peroxidase, and incubation for 1 h at room temperature. Next, chromogen was added to the slide. Furthermore, the dehydration process was again carried out using concentrated alcohol and xylene. The next step was mounting and evaluating the TNF-alpha expression using ImageJ Software so that the percentage of TNF-alpha expression was obtained.

Enzyme-linked immunosorbent assay (ELISA) examination

Serotonin levels in the hippocampus were examined by human serotonin ELISA kit (cloud clone), based on the protocol found in the manufacturer's protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that had been coated with anti-serotonin and incubated at 37°C for 30 min. After the plates were washed, 100 µl of biotinylated antibody solution was added and incubated for 30 min at 37°C. After washing 3 times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 min at 37°C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 min at 37°C. Finally, 50 ul stop solution was added to stop the reaction and optical density values were measured using an ELISA reader (Bio-Rad) at wavelength 450 nm.

Data analysis

All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 program. One-way ANOVA followed by *post hoc* analysis was conducted to assess the differences in the mean expression and levels of each protein as well as clinical data. $p < 0.05$ was determined as an indication that there were significant differences in the mean levels.

Results

Duration of immobility showed clinical symptoms of depression. The longer duration of immobilization indicated more depressed test animal. Figure 1 shows that CE with dose 25 mg/kg BW to 100 mg/kg BW reduced the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression.

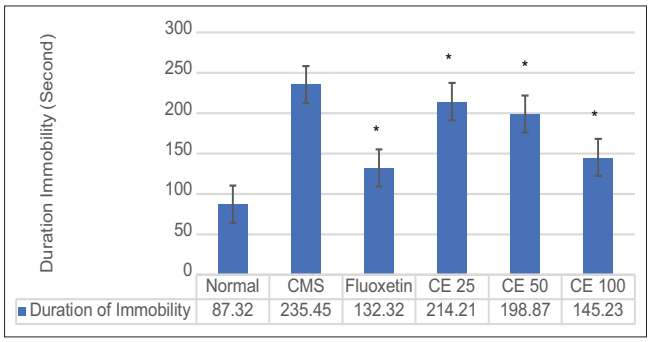

*Figure 1: Duration of immobility in rats for forced swimming test; *Versus chronic mild stress, $p < 0.05$*

Serotonin as the main neurotransmitter that plays a role in the pathophysiology of depressive disorders is an important marker in clinical depression. CE showed the potential to improve serotonin levels in the hippocampus with increasing doses (Table 1).

Table 1: Serotonin level in rats hippocampus

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*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

TNF-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group (Figure 2 and Table 2).

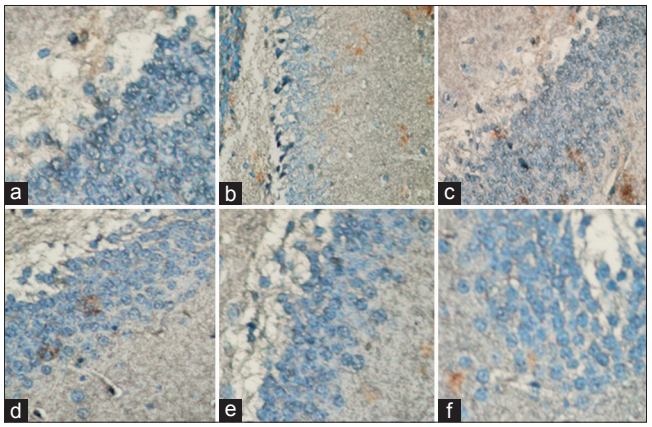

Figure 2: Tumor necrosis factor (TNF)-alpha expression in rats hippocampus. Black arrows: TNF-alpha expression. ×400. (a) Normal, (b) chronic mild stress, (c) fluoxetine, (d) cinnamon (CE) 25, (e) CE 50, (f) CE 100

Table 2: Tumor necrosis factor-alpha expression in rats hippocampus

Group	Tumor necrosis factor-alpha expression (%) Mean ± SD	p value*
Normal	2.86 ± 0.76	0.00
CMS	53.87 ± 3.62	-
Fluoxetine	19.11 ± 1.12	0.00
CE 25	45.56 ± 3.11	0.00
CE 50	37.31 ± 1.34	0.00
CE 100	28.76 ± 1.87	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

Discussion

Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. Increased mesolimbic dopamine will cause a decrease in dopamine in the mesocortical area so that it will cause the sufferer to experience a decrease in mood which results in depressive symptoms [7], [8], [9], [10].

Current management related to depressive disorders is aimed at maintaining optimum levels of serotonin in the synaptic cleft. Drugs, such as SSRI group and fluoxetine, play a role in inhibiting the uptake of serotonin in the synaptic cleft so that serotonin remains optimum and is able to improve depressive symptoms. Recent study was more focused on maintaining the viability of neuronal serotonin cells. Decreased neuronal serotonin cell viability will reduce serotonin levels so that it will cause symptoms of depression [11], [12], [13], [14], [15], [16].

Oxidative stress due to oxidants plays an important role as an initiator of inflammation. Inflammation is an important pathogenesis that plays a role in the initiation of cell death. Chronic inflammation will cause activation of the inflammatory cascade, which in turn activates the death receptor. Death receptor activation will then activate caspase which will cause apoptosis of neuronal serotonin cells [17], [18]. Therefore, it is important to make efforts to inhibit inflammation in neuronal cells. Phenol is a metabolite that is quite common in CE. Phenol has the potential to penetrate the blood–brain barrier, so it has the potential to have an effect on neuronal cells. Various studies show that phenol contained in cinnamon possesses antioxidant potential. Antioxidants are believed to be important compounds capable of neutralizing oxidants, to reduce the activation of inflammatory cascade and optimize the viability of serotonin neuronal cells [19], [20], [21], [22]. This study showed that CE improved clinical depression by inhibiting the expression of TNF-alpha in the hippocampus so that the viability of serotonin neuronal cells remained optimal and serotonin levels would increase compared to the group not administered with CE.

Conclusion

CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

References

1. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. https://doi.org/10.1037/e400972004-001. [Last accessed on 2019 Nov 01].
2. Du YH, Feng RZ, Li Q, Wei Q, Yin ZQ, Zhou LJ, *et al*. Anti-inflammatory activity of leaf essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao. Int J Clin Exp Med. 2014;7(12):5612-20.
   PMid:25664080
3. Partan RU, Hidayat R, Saleh MI, Parisa N, Tamzil NS. Effect of supplementation Kayu Manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy. Asian J Pharm Clin Res. 2018;11(2):363-5. https://doi.org/10.22159/ajpcr.2018.v11i2.22024
4. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, *et al*. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evid Based Complement Alternat Med. 2012;2012(429320):1-12.
5. Han X, Parker TL. Antiinflammatory activity of cinnamon (*Cinnamomum zeylanicum*) bark essential oil in a human skin disease model. Phytother Res. 2017;31(7):1034-8. https://doi.org/10.1002/ptr.5822
   PMid:28444928
6. Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: Of anhedonia, “anomalous anxiolysis” and activity. PLoS One. 2009;4(1):e4326. https://doi.org/10.1371/journal.pone.0004326
   PMid:19177164
7. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. Ups J Med Sci. 2013;118(1):3-8. https://doi.org/10.3109/03009734.2012.724118
   PMid:22970723
8. Amodeo G, Trusso MA, Fagiolini A. Depression and inflammation: Disentangling a clear yet complex and multifaceted link. Neuropsychiatry. 2017;7(4):448-57. Available from: http://www.jneuropsychiatry.org/peer-review/depression-and-inflammation-disentangling-a-clear-yet-complex-and-multifaceted-link.html. https://doi.org/10.4172/neuropsychiatry.1000236
9. Konsman JP. Inflammation and depression : A nervous plea for psychiatry to not become immune to interpretation. Pharmaceuticals (Basel). 2019;12(1):E29. https://doi.org/10.3390/ph12010029
   PMid:30769887
10. Köhler O, Krogh J, Mors O, Benrosc ME. Inflammation in depression and the potential for anti-inflammatory treatment. Curr Neuropharmacol. 2016;14(7):732-42.
    PMid:27640518
11. Abelaira HM, Réus GZ, Quevedo J. Animal models as

tools to study the pathophysiology of depression. Braz J Psychiatry. 2013;35(Suppl 2):S112-20. https://doi.org/10.1590/1516-4446-2013-1098

PMid:24271223

12. Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol. 2008;581(1-2):113-20. https://doi.org/10.1016/j.ejphar.2007.12.005

PMid:18184609

13. Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of depression: Neurotrophic and neuroinflammatory biomarkers. BioMed Res Int. 2014;2014(932757):932757. https://doi.org/10.1155/2014/932757

PMid:24999483

14. Zeldetz V, Natanel D, Boyko M, Zlotnik A, Shiyntum HN, Grinshpun J, *et al*. A new method for inducing a depression-like behavior in rats a new method for inducing a depression-like behavior in rats. J Vis Exp. 2018;132:57137. https://doi.org/10.3791/57137

PMid:29553503.

15. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34. https://doi.org/10.1038/nri.2015.5

PMid:26711676

16. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40-3. https://doi.org/10.1515/intox-2017-0006

PMid:30123035

17. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, *et al*. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200. https://doi.org/10.1186/1741-7015-11-200

18. Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars). 2004;64(4):439-48.

PMid:15586660

19. Ghaderi H, Rafieian M, Nezhad HR. Pharmacophore effect of hydroalcoholic *Cinnamomum zeylanicum* extract on reserpine-induced depression symptoms in mice. Pharmacophore. 2018;9(2):35-44. Available from: https://www.pharmacophorejournal.com/en/article/effect-of-hydroalcoholic-cinnamomum-zeylanicum-extract-on-reserpine-induced-depression-symptoms-in-mice. https://doi.org/10.4103/1735-5362.189308

20. Sharafeldin K, Raza M. Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin induced diabetic rats. J Basic Appl Zool. 2015;72:126-34. https://doi.org/10.1016/j.jobaz.2015.09.002

21. Chu X, Zhou Y, Hu Z, Lou J, Song W, Li J, *et al*. 24-hour-restraint stress induces phenotypes in mice. Sci Rep. 2016;6:32935.

22. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211-20. https://doi.org/10.1016/j.jfda.2017.03.006

Open Access Macedonian Journal of Medical Sciences

Peer Reviewer Results

February 10, 2020

Dear authors,
We have reached decision regarding your manuscript "Antidepressant effect of cinnamon (Cinnamomum burmanii) bark extract in chronic stress induced rats".
Our decision is: **Revised your manuscript** until February 20, 2020.

The details of revision has been attached below.

Regards,
Prof. Dr. Mirko Spiroski
Editor in Chief

Reviewer A;
- In the article abstract, the introduction section is too long and incomprehensible. Please rewrite the section and be precise in preferred language and terminology used for this topic.
- Please add citation for marked text.
- I suggest the manuscript should be proofread by Native speaker familiar to Academic English.

Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats

Nita Parisa[1], Rachmat Hidayat[2*], Ziske Maritska[2], Bintang Arroyantri Prananjaya[3]

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Keywords: Antidepressant effect; Cinnamon bark; *Cinnamomum burmannii*

***Correspondence**: Rachmat Hidayat; email: yolandaevita@gmail.com

Funding: The authors would like to deliver gratitude to the Department of Pharmacology, Department of Biology, and Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia, for the support.
Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. An effective and commonly used drug is the selective serotonin reuptake inhibitor, namely, fluoxetine. However, this agent has so many side effects, one of them is erectile dysfunction. In order to find the better treatment, exploration and discovery of therapeutic modalities need to pursued using natural materials.

AIM: This study aimed to explore and evaluate antidepressant effects of cinnamon (*Cinnamomum burmannii*) extract (CE).

METHODS: A total of 30 male Wistar rats were obtained from Eureka Research Laboratory (Palembang, Indonesia). Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Rats were induced using chronic mild stress (CMS). CMS was a form of stress induction performed on experimental animals continuously, for 4 weeks. Forced swimming test (FST) was a test conducted to assess mobility in animal model. After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. After treatment and FST, organ evacuation was performed and followed by immunohistochemistry and enzyme-linked immunosorbent examination.

RESULTS: This study showed that CE with dose of 25 mg/kg BW to dose 100 mg/k BW could reduce the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression. Histologically, CE showed the potential to improve serotonin levels in the hippocampus with increasing doses. Tumor necrosis factor (TNF)-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group.

CONCLUSION: CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

Introduction

Depression is a mental disorder that is commonly found throughout the world. The depression could strike all ages, usually characterized by feeling of sadness, anxiety, guilty thought, insomnia, loss of interest in pleasure thing, and suicidal idea. According to the WHO, there are 300 million people in the world suffering from depression with nearly 800,000 people die due to suicide each year. Therefore, the depression is one of the mental disorders with the need of immediate proper treatment as early as possible [1].

To find the cause of depression, many studies had been conducted. It is believed that serotonin is a neurotransmitter that plays a role in the pathogenesis of depression. Inflammation is a major factor that relates in reducing the function of neurogenesis of neuronal cells in the brain. Decreased neurogenesis due to inflammation causes neuronal cell death which will lead to decreased neurotransmitter synthesis, including serotonin. Low levels of serotonin are a marker of depression, so the search for chemical compounds that can increase or maintain normal levels of serotonin is the main focus of pharmacotherapy. Today, an effective and commonly used drug is the selective serotonin reuptake inhibitor (SSRI), namely, fluoxetine. Even so, one of the side effects of this drug is erectile dysfunction, considering that depression often happen in productive age. The side effects caused by this drug cannot be ignored [2]. Therefore, exploration and discovery of therapeutic modalities need to be pursued using and utilizing natural materials.

Cinnamon (*Cinnamomum burmannii*) is one of the plants commonly found in Indonesia that is believed to have the potential to overcome various

health problems. In folk tradition, this plant was used hereditary to cure cough, fever, and joint pain. The main compound contained in this plant is cinnamaldehyde, which is a phenol group. Phenol compounds have lipophilic properties, so these compounds can penetrate the blood–brain barrier and are able to give effect to neuronal cells. Phenols have anti-inflammatory effects, which are believed to be able to inhibit the inflammatory cascade that forms the basis of the pathogenesis of depressive disorders [3], [4], [5].

This study was the first study to explore and evaluate antidepressant effects of cinnamon extract (CE) (*C. burmannii*). This study aimed to discover new modalities using and utilizing natural materials, CE (*C. burmannii*).

Methods

Animal

A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 h of light/dark cycles with temperatures of 22 ± 1°C and humidity of 40–60%), fed, and drank *ad libitum*. All animal treatments and experimental procedures were approved by the Ethics and Humanities Commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No.129/kptfkunsri-rsmh/2019).

CE preparation

Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Furthermore, extraction from cinnamon was done by maceration method. A total of 500 g of simplisia were macerated with 96% ethanol for 72 h. Then, it was separated to obtain the pulp and macerate. Macerate was then evaporated with a rotary evaporator (Heidolph) so that CE was obtained.

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FST was a test conducted to assess mobility in animal model. First, the FST equipment was prepared, in the form of a cylindrical tube with a height of 60 cm and diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per mouse. Tests were conducted between 9 am and 3 pm. Each test animal was acclimatized for 10 min first, and then the mobility duration was measured in 5 min. Next, the mobility of the test animals was measured in seconds.

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All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 program. One-way ANOVA followed by *post hoc* analysis was conducted to assess the differences in the mean expression and levels of each protein as well as clinical data. $p < 0.05$ was determined as an indication that there were significant differences in the mean levels.

Results

Duration of immobility showed clinical symptoms of depression. The longer duration of immobilization indicated more depressed test animal. Figure 1 shows that CE with dose 25 mg/kg BW to 100 mg/kg BW reduced the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression.

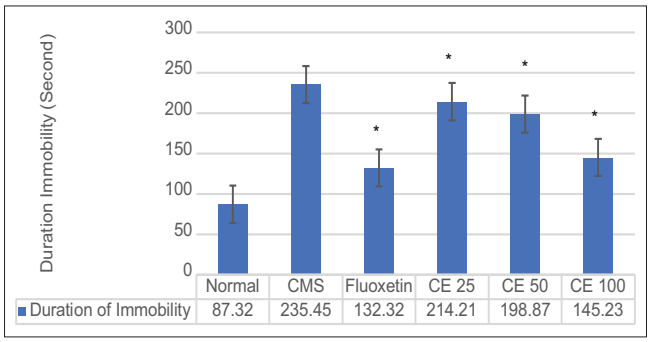

*Figure 1: Duration of immobility in rats for forced swimming test; *Versus chronic mild stress, $p < 0.05$*

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TNF-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group (Figure 2 and Table 2).

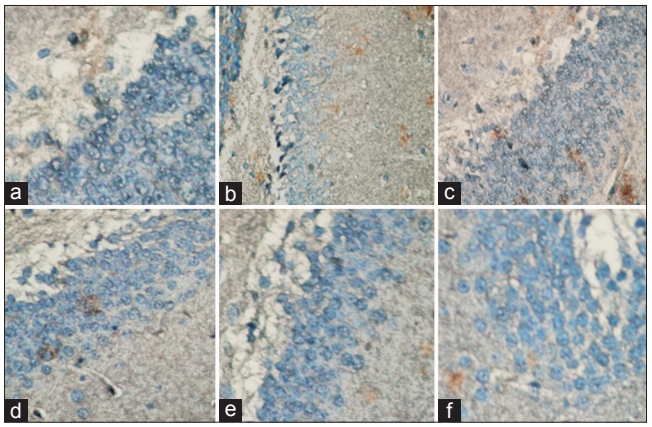

Figure 2: Tumor necrosis factor (TNF)-alpha expression in rats hippocampus. Black arrows: TNF-alpha expression. ×400. (a) Normal, (b) chronic mild stress, (c) fluoxetine, (d) cinnamon (CE) 25, (e) CE 50, (f) CE 100

Table 2: Tumor necrosis factor-alpha expression in rats hippocampus

Group	Tumor necrosis factor-alpha expression (%) Mean ± SD	p value*
Normal	2.86 ± 0.76	0.00
CMS	53.87 ± 3.62	-
Fluoxetine	19.11 ± 1.12	0.00
CE 25	45.56 ± 3.11	0.00
CE 50	37.31 ± 1.34	0.00
CE 100	28.76 ± 1.87	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

Discussion

Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. Increased mesolimbic dopamine will cause a decrease in dopamine in the mesocortical area so that it will cause the sufferer to experience a decrease in mood which results in depressive symptoms [7], [8], [9], [10].

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Oxidative stress due to oxidants plays an important role as an initiator of inflammation. Inflammation is an important pathogenesis that plays a role in the initiation of cell death. Chronic inflammation will cause activation of the inflammatory cascade, which in turn activates the death receptor. Death receptor activation will then activate caspase which will cause apoptosis of neuronal serotonin cells [17], [18]. Therefore, it is important to make efforts to inhibit inflammation in neuronal cells. Phenol is a metabolite that is quite common in CE. Phenol has the potential to penetrate the blood–brain barrier, so it has the potential to have an effect on neuronal cells. Various studies show that phenol contained in cinnamon possesses antioxidant potential. Antioxidants are believed to be important compounds capable of neutralizing oxidants, to reduce the activation of inflammatory cascade and optimize the viability of serotonin neuronal cells [19], [20], [21], [22]. This study showed that CE improved clinical depression by inhibiting the expression of TNF-alpha in the hippocampus so that the viability of serotonin neuronal cells remained optimal and serotonin levels would increase compared to the group not administered with CE.

Conclusion

CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

References

1. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. https://doi.org/10.1037/e400972004-001. [Last accessed on 2019 Nov 01].
2. Du YH, Feng RZ, Li Q, Wei Q, Yin ZQ, Zhou LJ, *et al*. Anti-inflammatory activity of leaf essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao. Int J Clin Exp Med. 2014;7(12):5612-20.
   PMid:25664080
3. Partan RU, Hidayat R, Saleh MI, Parisa N, Tamzil NS. Effect of supplementation Kayu Manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy. Asian J Pharm Clin Res. 2018;11(2):363-5. https://doi.org/10.22159/ajpcr.2018.v11i2.22024
4. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, *et al*. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evid Based Complement Alternat Med. 2012;2012(429320):1-12.
5. Han X, Parker TL. Antiinflammatory activity of cinnamon (*Cinnamomum zeylanicum*) bark essential oil in a human skin disease model. Phytother Res. 2017;31(7):1034-8. https://doi.org/10.1002/ptr.5822
   PMid:28444928
6. Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: Of anhedonia, "anomalous anxiolysis" and activity. PLoS One. 2009;4(1):e4326. https://doi.org/10.1371/journal.pone.0004326
   PMid:19177164
7. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. Ups J Med Sci. 2013;118(1):3-8. https://doi.org/10.3109/03009734.2012.724118
   PMid:22970723
8. Amodeo G, Trusso MA, Fagiolini A. Depression and inflammation: Disentangling a clear yet complex and multifaceted link. Neuropsychiatry. 2017;7(4):448-57. Available from: http://www.jneuropsychiatry.org/peer-review/depression-and-inflammation-disentangling-a-clear-yet-complex-and-multifaceted-link.html. https://doi.org/10.4172/neuropsychiatry.1000236
9. Konsman JP. Inflammation and depression : A nervous plea for psychiatry to not become immune to interpretation. Pharmaceuticals (Basel). 2019;12(1):E29. https://doi.org/10.3390/ph12010029
   PMid:30769887
10. Köhler O, Krogh J, Mors O, Benrosc ME. Inflammation in depression and the potential for anti-inflammatory treatment. Curr Neuropharmacol. 2016;14(7):732-42.
    PMid:27640518
11. Abelaira HM, Réus GZ, Quevedo J. Animal models as

tools to study the pathophysiology of depression. Braz J Psychiatry. 2013;35(Suppl 2):S112-20. https://doi.org/10.1590/1516-4446-2013-1098

PMid:24271223

12. Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol. 2008;581(1-2):113-20. https://doi.org/10.1016/j.ejphar.2007.12.005

PMid:18184609

13. Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of depression: Neurotrophic and neuroinflammatory biomarkers. BioMed Res Int. 2014;2014(932757):932757. https://doi.org/10.1155/2014/932757

PMid:24999483

14. Zeldetz V, Natanel D, Boyko M, Zlotnik A, Shiyntum HN, Grinshpun J, *et al*. A new method for inducing a depression-like behavior in rats a new method for inducing a depression-like behavior in rats. J Vis Exp. 2018;132:57137. https://doi.org/10.3791/57137

PMid:29553503.

15. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34. https://doi.org/10.1038/nri.2015.5

PMid:26711676

16. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40-3. https://doi.org/10.1515/intox-2017-0006

PMid:30123035

17. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, *et al*. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200. https://doi.org/10.1186/1741-7015-11-200

18. Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars). 2004;64(4):439-48.

PMid:15586660

19. Ghaderi H, Rafieian M, Nezhad HR. Pharmacophore effect of hydroalcoholic *Cinnamomum zeylanicum* extract on reserpine-induced depression symptoms in mice. Pharmacophore. 2018;9(2):35-44. Available from: https://www.pharmacophorejournal.com/en/article/effect-of-hydroalcoholic-cinnamomum-zeylanicum-extract-on-reserpine-induced-depression-symptoms-in-mice. https://doi.org/10.4103/1735-5362.189308

20. Sharafeldin K, Raza M. Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin induced diabetic rats. J Basic Appl Zool. 2015;72:126-34. https://doi.org/10.1016/j.jobaz.2015.09.002

21. Chu X, Zhou Y, Hu Z, Lou J, Song W, Li J, *et al*. 24-hour-restraint stress induces phenotypes in mice. Sci Rep. 2016;6:32935.

22. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211-20. https://doi.org/10.1016/j.jfda.2017.03.006

Open Access Macedonian Journal of Medical Sciences

Letter of Acceptance

April 21, 2020

Dear authors,
We have reached decision regarding your manuscript "Antidepressant effect of cinnamon (Cinnamomum burmanii) bark extract in chronic stress induced rats".

Our decision is: Accept your manuscript for publication in OAMJMS

Regards,
Prof. Dr. Mirko Spiroski
Editor in Chief

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 May 21; 8(A):273-277.
https://doi.org/10.3889/oamjms.2020.3995
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Physiology*

Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats

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Edited by: Ksenija Bogoeva-Kostovska
Citation: Parisa N, Hidayat R, Maritska Z, Prananjaya BA. Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats. Open Access Maced J Med Sci. 2020 May 21; 8(A):273-277. https://doi.org/10.3889/oamjms.2020.3995
Keywords: Antidepressant effect; Cinnamon bark; *Cinnamomum burmannii*
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Received: 05-Nov-2019
Revised: 06-Mar-2020
Accepted: 21-Apr-2020

Funding: The authors would like to deliver gratitude to the Department of Pharmacology, Department of Biology, and Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia, for the support.
Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. An effective and commonly used drug is the selective serotonin reuptake inhibitor, namely, fluoxetine. However, this agent has so many side effects, one of them is erectile dysfunction. In order to find the better treatment, exploration and discovery of therapeutic modalities need to pursued using natural materials.

AIM: This study aimed to explore and evaluate antidepressant effects of cinnamon (*Cinnamomum burmannii*) extract (CE).

METHODS: A total of 30 male Wistar rats were obtained from Eureka Research Laboratory (Palembang, Indonesia). Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Rats were induced using chronic mild stress (CMS). CMS was a form of stress induction performed on experimental animals continuously, for 4 weeks. Forced swimming test (FST) was a test conducted to assess mobility in animal model. After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. After treatment and FST, organ evacuation was performed and followed by immunohistochemistry and enzyme-linked immunosorbent examination.

RESULTS: This study showed that CE with dose of 25 mg/kg BW to dose 100 mg/k BW could reduce the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression. Histologically, CE showed the potential to improve serotonin levels in the hippocampus with increasing doses. Tumor necrosis factor (TNF)-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group.

CONCLUSION: CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

Introduction

Depression is a mental disorder that is commonly found throughout the world. The depression could strike all ages, usually characterized by feeling of sadness, anxiety, guilty thought, insomnia, loss of interest in pleasure thing, and suicidal idea. According to the WHO, there are 300 million people in the world suffering from depression with nearly 800,000 people die due to suicide each year. Therefore, the depression is one of the mental disorders with the need of immediate proper treatment as early as possible [1].

To find the cause of depression, many studies had been conducted. It is believed that serotonin is a neurotransmitter that plays a role in the pathogenesis of depression. Inflammation is a major factor that relates in reducing the function of neurogenesis of neuronal cells in the brain. Decreased neurogenesis due to inflammation causes neuronal cell death which will lead to decreased neurotransmitter synthesis, including serotonin. Low levels of serotonin are a marker of depression, so the search for chemical compounds that can increase or maintain normal levels of serotonin is the main focus of pharmacotherapy. Today, an effective and commonly used drug is the selective serotonin reuptake inhibitor (SSRI), namely, fluoxetine. Even so, one of the side effects of this drug is erectile dysfunction, considering that depression often happen in productive age. The side effects caused by this drug cannot be ignored [2]. Therefore, exploration and discovery of therapeutic modalities need to be pursued using and utilizing natural materials.

Cinnamon (*Cinnamomum burmannii*) is one of the plants commonly found in Indonesia that is believed to have the potential to overcome various

health problems. In folk tradition, this plant was used hereditary to cure cough, fever, and joint pain. The main compound contained in this plant is cinnamaldehyde, which is a phenol group. Phenol compounds have lipophilic properties, so these compounds can penetrate the blood–brain barrier and are able to give effect to neuronal cells. Phenols have anti-inflammatory effects, which are believed to be able to inhibit the inflammatory cascade that forms the basis of the pathogenesis of depressive disorders [3], [4], [5].

This study was the first study to explore and evaluate antidepressant effects of cinnamon extract (CE) (*C. burmannii*). This study aimed to discover new modalities using and utilizing natural materials, CE (*C. burmannii*)**.**

Methods

Animal

A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 h of light/dark cycles with temperatures of 22 ± 1°C and humidity of 40–60%), fed, and drank *ad libitum*. All animal treatments and experimental procedures were approved by the Ethics and Humanities Commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No.129/kptfkunsri-rsmh/2019).

CE preparation

Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Furthermore, extraction from cinnamon was done by maceration method. A total of 500 g of simplisia were macerated with 96% ethanol for 72 h. Then, it was separated to obtain the pulp and macerate. Macerate was then evaporated with a rotary evaporator (Heidolph) so that CE was obtained.

Animal model depression

Animal model depression was induced using chronic mild stress (CMS). CMS was a form of stress induction performed on the experimental animals continuously for 4 weeks. CMS procedures were performed with mild stressors such as repeated cold stress (4°C), space reduction in the homecage, changed cages within the CMS group, cage tilt, empty cage, intermittent air puff, wet bedding, white noise, overnight illumination, and social interaction with other animals of the CMS group. The particular context (stressor applied during preceding dark phase) of the water restriction intervals involved overnight illumination (Sunday-Monday), wet cage, and cage tilt (Tuesday-Wednesday and Thursday-Friday), and changed cages (Wednesday-Thursday), respectively [6]. As the following schedule:

Table 1: Procedure timeline of chronic mild stress

Day	Light phase		Dark phase	
	First half	Second half	First 2 h	Remaining 10 h
Monday	Cold stress (2×30 min)	Cold stress (30 min)	Water restriction	Space reduction
Tuesday	Changed room	Air puff (3×3 intermittent)	Wet cage	Wet cage
Wednesday	Wet cage	Social interaction	Foreign cage	Foreign cage
Thursday	Foreign cage	Social interaction	Water restriction	Cage tilt
Friday	Empty cage	Changed room	White noise	White noise
Saturday	White noise	Changed room	Changed room	Changed room
Sunday	Changed room	Changed room	Overnight illumination with 700×l	Overnight illumination with 700×l

Clinical evaluation: Forced swimming test (FST)

FST was a test conducted to assess mobility in animal model. First, the FST equipment was prepared, in the form of a cylindrical tube with a height of 60 cm and diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per mouse. Tests were conducted between 9 am and 3 pm. Each test animal was acclimatized for 10 min first, and then the mobility duration was measured in 5 min. Next, the mobility of the test animals was measured in seconds.

Animal model treatment

After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. The normal control group was not induced by CMS. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. The extract was made into a suspension preparation with 1% carboxymethyl cellulose emulgator so that the volume of suspension for every rat was 3 ml. Furthermore, the animal model was performed with perfusion so when the organ evacuation process was started, cell damage did not occur. Before perfusion was performed, the animal was anesthetized with ketamine (5 mg/kg BW) and xylazine (0.2 mg/kg BW) intraperitoneally. Perfusion was done by entering perfusor fluid, paraformaldehyde 4%, and through the left ventricle of the heart, followed by a tear in the right atrium of the heart so that blood would come out of the right atrium replaced by perfusor fluid. Perfusion continues until the fluid coming out of the right atrium became clear. Evacuation of the animal's brain was done by performing a craniotomy, through sulcus coronarius from calvaria, so that the animal's brain organ was obtained and splitting was performed to divide the right and left cerebral hemispheres. The left

cerebral hemisphere was then inserted into a fixation fluid, neutral buffer formalin 10%.

Immunohistochemistry examination

The left cerebral hemisphere that had been inserted into the fixation fluid was dehydrated using gradient alcohol and xylene, then paraffinized and cut as thick as 5 um using a rotary microtome (Leica). Next, tissue was placed on coated-object glass. Then, rehydration was carried out on the tissue using xylene and alcohol with a concentration of 96%, 90%, 80%, and 70% and rinsed with tap water. The next stage, retrieval antigen was carried out by the heat-induced epitope retrieval method, where the slides were put into a citrate buffer solution, then heated at a temperature of 95°C for 60 min. Then, tumor necrosis factor (TNF)-alpha 1:700 (cloud clone) antibody was stained, followed by overnight incubation at 4°C. The next stage was to paint with a secondary antibody, biotinylated-horseradish peroxidase, and incubation for 1 h at room temperature. Next, chromogen was added to the slide. Furthermore, the dehydration process was again carried out using concentrated alcohol and xylene. The next step was mounting and evaluating the TNF-alpha expression using ImageJ Software so that the percentage of TNF-alpha expression was obtained.

Enzyme-linked immunosorbent assay (ELISA) examination

Serotonin levels in the hippocampus were examined by human serotonin ELISA kit (cloud clone), based on the protocol found in the manufacturer's protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that had been coated with anti-serotonin and incubated at 37°C for 30 min. After the plates were washed, 100 µl of biotinylated antibody solution was added and incubated for 30 min at 37°C. After washing 3 times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 min at 37°C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 min at 37°C. Finally, 50 ul stop solution was added to stop the reaction and optical density values were measured using an ELISA reader (Bio-Rad) at wavelength 450 nm.

Data analysis

All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 program. One-way ANOVA followed by *post hoc* analysis was conducted to assess the differences in the mean expression and levels of each protein as well as clinical data. $p < 0.05$ was determined as an indication that there were significant differences in the mean levels.

Results

Duration of immobility showed clinical symptoms of depression. The longer duration of immobilization indicated more depressed test animal. Figure 1 shows that CE with dose 25 mg/kg BW to 100 mg/kg BW reduced the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression.

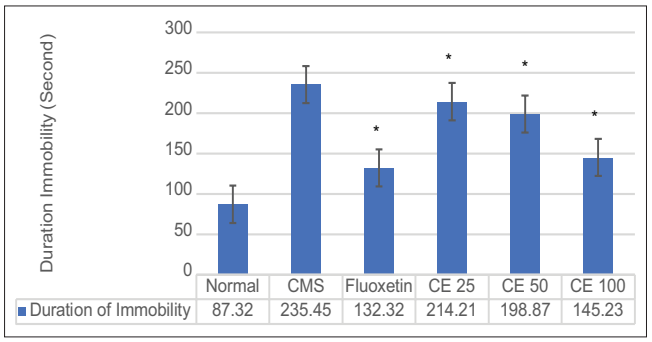

*Figure 1: Duration of immobility in rats for forced swimming test; *Versus chronic mild stress, $p < 0.05$*

Serotonin as the main neurotransmitter that plays a role in the pathophysiology of depressive disorders is an important marker in clinical depression. CE showed the potential to improve serotonin levels in the hippocampus with increasing doses (Table 1).

Table 1: Serotonin level in rats hippocampus

Group	Serotonin level (pg/mL) ± SD	p value*
Normal	178.86 ± 15.76	0.00
CMS	54.54 ± 3.12	-
Fluoxetine	112.21 ± 8.23	0.00
CE 25	65.23 ± 5.12	0.00
CE 50	87.12 ± 6.34	0.00
CE 100	98.76 ± 7.77	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

TNF-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group (Figure 2 and Table 2).

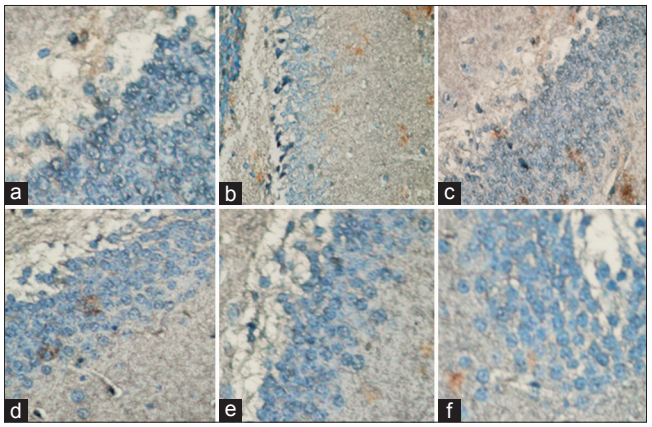

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Table 2: Tumor necrosis factor-alpha expression in rats hippocampus

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Conclusion

CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

References

1. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. https://doi.org/10.1037/e400972004-001. [Last accessed on 2019 Nov 01].
2. Du YH, Feng RZ, Li Q, Wei Q, Yin ZQ, Zhou LJ, *et al*. Anti-inflammatory activity of leaf essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao. Int J Clin Exp Med. 2014;7(12):5612-20.
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3. Partan RU, Hidayat R, Saleh MI, Parisa N, Tamzil NS. Effect of supplementation Kayu Manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy. Asian J Pharm Clin Res. 2018;11(2):363-5. https://doi.org/10.22159/ajpcr.2018.v11i2.22024
4. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, *et al*. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evid Based Complement Alternat Med. 2012;2012(429320):1-12.
5. Han X, Parker TL. Antiinflammatory activity of cinnamon (*Cinnamomum zeylanicum*) bark essential oil in a human skin disease model. Phytother Res. 2017;31(7):1034-8. https://doi.org/10.1002/ptr.5822
   PMid:28444928
6. Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: Of anhedonia, “anomalous anxiolysis” and activity. PLoS One. 2009;4(1):e4326. https://doi.org/10.1371/journal.pone.0004326
   PMid:19177164
7. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. Ups J Med Sci. 2013;118(1):3-8. https://doi.org/10.3109/03009734.2012.724118
   PMid:22970723
8. Amodeo G, Trusso MA, Fagiolini A. Depression and inflammation: Disentangling a clear yet complex and multifaceted link. Neuropsychiatry. 2017;7(4):448-57. Available from: http://www.jneuropsychiatry.org/peer-review/depression-and-inflammation-disentangling-a-clear-yet-complex-and-multifaceted-link.html. https://doi.org/10.4172/neuropsychiatry.1000236
9. Konsman JP. Inflammation and depression : A nervous plea for psychiatry to not become immune to interpretation. Pharmaceuticals (Basel). 2019;12(1):E29. https://doi.org/10.3390/ph12010029
   PMid:30769887
10. Köhler O, Krogh J, Mors O, Benrosc ME. Inflammation in depression and the potential for anti-inflammatory treatment. Curr Neuropharmacol. 2016;14(7):732-42.
    PMid:27640518
11. Abelaira HM, Réus GZ, Quevedo J. Animal models as

tools to study the pathophysiology of depression. Braz J Psychiatry. 2013;35(Suppl 2):S112-20. https://doi.org/10.1590/1516-4446-2013-1098

PMid:24271223

12. Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol. 2008;581(1-2):113-20. https://doi.org/10.1016/j.ejphar.2007.12.005

PMid:18184609

13. Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of depression: Neurotrophic and neuroinflammatory biomarkers. BioMed Res Int. 2014;2014(932757):932757. https://doi.org/10.1155/2014/932757

PMid:24999483

14. Zeldetz V, Natanel D, Boyko M, Zlotnik A, Shiyntum HN, Grinshpun J, *et al*. A new method for inducing a depression-like behavior in rats a new method for inducing a depression-like behavior in rats. J Vis Exp. 2018;132:57137. https://doi.org/10.3791/57137

PMid:29553503.

15. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34. https://doi.org/10.1038/nri.2015.5

PMid:26711676

16. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40-3. https://doi.org/10.1515/intox-2017-0006

PMid:30123035

17. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, *et al*. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200. https://doi.org/10.1186/1741-7015-11-200

18. Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars). 2004;64(4):439-48.

PMid:15586660

19. Ghaderi H, Rafieian M, Nezhad HR. Pharmacophore effect of hydroalcoholic *Cinnamomum zeylanicum* extract on reserpine-induced depression symptoms in mice. Pharmacophore. 2018;9(2):35-44. Available from: https://www.pharmacophorejournal.com/en/article/effect-of-hydroalcoholic-cinnamomum-zeylanicum-extract-on-reserpine-induced-depression-symptoms-in-mice. https://doi.org/10.4103/1735-5362.189308

20. Sharafeldin K, Raza M. Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin induced diabetic rats. J Basic Appl Zool. 2015;72:126-34. https://doi.org/10.1016/j.jobaz.2015.09.002

21. Chu X, Zhou Y, Hu Z, Lou J, Song W, Li J, *et al*. 24-hour-restraint stress induces phenotypes in mice. Sci Rep. 2016;6:32935.

22. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211-20. https://doi.org/10.1016/j.jfda.2017.03.006

Open Access Macedonian Journal of Medical Sciences

Paper Published

May 21, 2020

Dear authors,
Please note that your paper "Antidepressant effect of cinnamon (Cinnamomum burmanii) bark extract in chronic stress induced rats" was published in OAMJMS.

Thank you for your fine contributions.

Regards,
Prof. Dr. Mirko Spiroski
Editor in Chief

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 May 21; 8(A):273-277.
https://doi.org/10.3889/oamjms.2020.3995
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Physiology*

Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats

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Edited by: Ksenija Bogoeva-Kostovska
Citation: Parisa N, Hidayat R, Maritska Z, Prananjaya BA. Antidepressant Effect of Cinnamon (*Cinnamomum burmannii*) Bark Extract in Chronic Stress-Induced Rats. Open Access Maced J Med Sci. 2020 May 21; 8(A):273-277. https://doi.org/10.3889/oamjms.2020.3995
Keywords: Antidepressant effect; Cinnamon bark; *Cinnamomum burmannii*
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Received: 05-Nov-2019
Revised: 06-Mar-2020
Accepted: 21-Apr-2020

Funding: The authors would like to deliver gratitude to the Department of Pharmacology, Department of Biology, and Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia, for the support.
Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. An effective and commonly used drug is the selective serotonin reuptake inhibitor, namely, fluoxetine. However, this agent has so many side effects, one of them is erectile dysfunction. In order to find the better treatment, exploration and discovery of therapeutic modalities need to pursued using natural materials.

AIM: This study aimed to explore and evaluate antidepressant effects of cinnamon (*Cinnamomum burmannii*) extract (CE).

METHODS: A total of 30 male Wistar rats were obtained from Eureka Research Laboratory (Palembang, Indonesia). Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Rats were induced using chronic mild stress (CMS). CMS was a form of stress induction performed on experimental animals continuously, for 4 weeks. Forced swimming test (FST) was a test conducted to assess mobility in animal model. After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. After treatment and FST, organ evacuation was performed and followed by immunohistochemistry and enzyme-linked immunosorbent examination.

RESULTS: This study showed that CE with dose of 25 mg/kg BW to dose 100 mg/k BW could reduce the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression. Histologically, CE showed the potential to improve serotonin levels in the hippocampus with increasing doses. Tumor necrosis factor (TNF)-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group.

CONCLUSION: CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

Introduction

Depression is a mental disorder that is commonly found throughout the world. The depression could strike all ages, usually characterized by feeling of sadness, anxiety, guilty thought, insomnia, loss of interest in pleasure thing, and suicidal idea. According to the WHO, there are 300 million people in the world suffering from depression with nearly 800,000 people die due to suicide each year. Therefore, the depression is one of the mental disorders with the need of immediate proper treatment as early as possible [1].

To find the cause of depression, many studies had been conducted. It is believed that serotonin is a neurotransmitter that plays a role in the pathogenesis of depression. Inflammation is a major factor that relates in reducing the function of neurogenesis of neuronal cells in the brain. Decreased neurogenesis due to inflammation causes neuronal cell death which will lead to decreased neurotransmitter synthesis, including serotonin. Low levels of serotonin are a marker of depression, so the search for chemical compounds that can increase or maintain normal levels of serotonin is the main focus of pharmacotherapy. Today, an effective and commonly used drug is the selective serotonin reuptake inhibitor (SSRI), namely, fluoxetine. Even so, one of the side effects of this drug is erectile dysfunction, considering that depression often happen in productive age. The side effects caused by this drug cannot be ignored [2]. Therefore, exploration and discovery of therapeutic modalities need to be pursued using and utilizing natural materials.

Cinnamon (*Cinnamomum burmannii*) is one of the plants commonly found in Indonesia that is believed to have the potential to overcome various

health problems. In folk tradition, this plant was used hereditary to cure cough, fever, and joint pain. The main compound contained in this plant is cinnamaldehyde, which is a phenol group. Phenol compounds have lipophilic properties, so these compounds can penetrate the blood–brain barrier and are able to give effect to neuronal cells. Phenols have anti-inflammatory effects, which are believed to be able to inhibit the inflammatory cascade that forms the basis of the pathogenesis of depressive disorders [3], [4], [5].

This study was the first study to explore and evaluate antidepressant effects of cinnamon extract (CE) (*C. burmannii*). This study aimed to discover new modalities using and utilizing natural materials, CE (*C. burmannii*)**.**

Methods

Animal

A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 h of light/dark cycles with temperatures of 22 ± 1°C and humidity of 40–60%), fed, and drank *ad libitum*. All animal treatments and experimental procedures were approved by the Ethics and Humanities Commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No.129/kptfkunsri-rsmh/2019).

CE preparation

Cinnamon simplisia was obtained from the Institute for Research and Testing of Traditional Medicine, Tawangmangu, Central Java, Indonesia. Furthermore, extraction from cinnamon was done by maceration method. A total of 500 g of simplisia were macerated with 96% ethanol for 72 h. Then, it was separated to obtain the pulp and macerate. Macerate was then evaporated with a rotary evaporator (Heidolph) so that CE was obtained.

Animal model depression

Animal model depression was induced using chronic mild stress (CMS). CMS was a form of stress induction performed on the experimental animals continuously for 4 weeks. CMS procedures were performed with mild stressors such as repeated cold stress (4°C), space reduction in the homecage, changed cages within the CMS group, cage tilt, empty cage, intermittent air puff, wet bedding, white noise, overnight illumination, and social interaction with other animals of the CMS group. The particular context (stressor applied during preceding dark phase) of the water restriction intervals involved overnight illumination (Sunday-Monday), wet cage, and cage tilt (Tuesday-Wednesday and Thursday-Friday), and changed cages (Wednesday-Thursday), respectively [6]. As the following schedule:

Table 1: Procedure timeline of chronic mild stress

Day	Light phase		Dark phase	
	First half	Second half	First 2 h	Remaining 10 h
Monday	Cold stress (2×30 min)	Cold stress (30 min)	Water restriction	Space reduction
Tuesday	Changed room	Air puff (3×3 intermittent)	Wet cage	Wet cage
Wednesday	Wet cage	Social interaction	Foreign cage	Foreign cage
Thursday	Foreign cage	Social interaction	Water restriction	Cage tilt
Friday	Empty cage	Changed room	White noise	White noise
Saturday	White noise	Changed room	Changed room	Changed room
Sunday	Changed room	Changed room	Overnight illumination with 700×l	Overnight illumination with 700×l

Clinical evaluation: Forced swimming test (FST)

FST was a test conducted to assess mobility in animal model. First, the FST equipment was prepared, in the form of a cylindrical tube with a height of 60 cm and diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per mouse. Tests were conducted between 9 am and 3 pm. Each test animal was acclimatized for 10 min first, and then the mobility duration was measured in 5 min. Next, the mobility of the test animals was measured in seconds.

Animal model treatment

After induction for 4 weeks, rats were randomly divided into six groups which each contained five rats: Normal control group, CMS group (negative control), CMS + fluoxetin (Fluox 1: mg/kg), CMS + CE 25 mg/kg, the CMS + CE 50 mg/kg, and the CMS + CE 100 mg/kg. The normal control group was not induced by CMS. Treatment with fluoxetine or CE was given for 14 days intragastrically using gastric sonde. The extract was made into a suspension preparation with 1% carboxymethyl cellulose emulgator so that the volume of suspension for every rat was 3 ml. Furthermore, the animal model was performed with perfusion so when the organ evacuation process was started, cell damage did not occur. Before perfusion was performed, the animal was anesthetized with ketamine (5 mg/kg BW) and xylazine (0.2 mg/kg BW) intraperitoneally. Perfusion was done by entering perfusor fluid, paraformaldehyde 4%, and through the left ventricle of the heart, followed by a tear in the right atrium of the heart so that blood would come out of the right atrium replaced by perfusor fluid. Perfusion continues until the fluid coming out of the right atrium became clear. Evacuation of the animal's brain was done by performing a craniotomy, through sulcus coronarius from calvaria, so that the animal's brain organ was obtained and splitting was performed to divide the right and left cerebral hemispheres. The left

cerebral hemisphere was then inserted into a fixation fluid, neutral buffer formalin 10%.

Immunohistochemistry examination

The left cerebral hemisphere that had been inserted into the fixation fluid was dehydrated using gradient alcohol and xylene, then paraffinized and cut as thick as 5 um using a rotary microtome (Leica). Next, tissue was placed on coated-object glass. Then, rehydration was carried out on the tissue using xylene and alcohol with a concentration of 96%, 90%, 80%, and 70% and rinsed with tap water. The next stage, retrieval antigen was carried out by the heat-induced epitope retrieval method, where the slides were put into a citrate buffer solution, then heated at a temperature of 95°C for 60 min. Then, tumor necrosis factor (TNF)-alpha 1:700 (cloud clone) antibody was stained, followed by overnight incubation at 4°C. The next stage was to paint with a secondary antibody, biotinylated-horseradish peroxidase, and incubation for 1 h at room temperature. Next, chromogen was added to the slide. Furthermore, the dehydration process was again carried out using concentrated alcohol and xylene. The next step was mounting and evaluating the TNF-alpha expression using ImageJ Software so that the percentage of TNF-alpha expression was obtained.

Enzyme-linked immunosorbent assay (ELISA) examination

Serotonin levels in the hippocampus were examined by human serotonin ELISA kit (cloud clone), based on the protocol found in the manufacturer's protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that had been coated with anti-serotonin and incubated at 37°C for 30 min. After the plates were washed, 100 µl of biotinylated antibody solution was added and incubated for 30 min at 37°C. After washing 3 times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 min at 37°C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 min at 37°C. Finally, 50 ul stop solution was added to stop the reaction and optical density values were measured using an ELISA reader (Bio-Rad) at wavelength 450 nm.

Data analysis

All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 program. One-way ANOVA followed by *post hoc* analysis was conducted to assess the differences in the mean expression and levels of each protein as well as clinical data. $p < 0.05$ was determined as an indication that there were significant differences in the mean levels.

Results

Duration of immobility showed clinical symptoms of depression. The longer duration of immobilization indicated more depressed test animal. Figure 1 shows that CE with dose 25 mg/kg BW to 100 mg/kg BW reduced the duration of immobility when compared to the CMS group. Clinically, CE possessed the potential to reduce the duration of immobility and potentially reduce symptoms of depression.

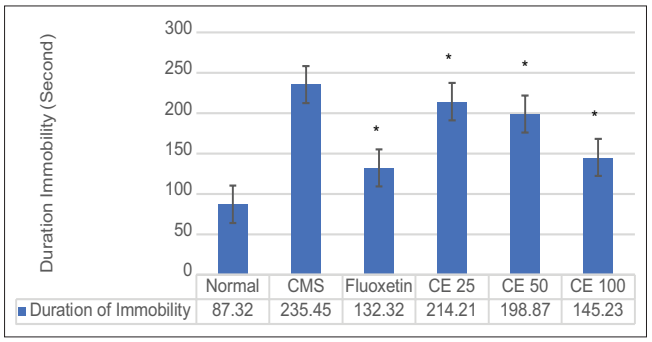

*Figure 1: Duration of immobility in rats for forced swimming test; *Versus chronic mild stress, $p < 0.05$*

Serotonin as the main neurotransmitter that plays a role in the pathophysiology of depressive disorders is an important marker in clinical depression. CE showed the potential to improve serotonin levels in the hippocampus with increasing doses (Table 1).

Table 1: Serotonin level in rats hippocampus

Group	Serotonin level (pg/mL) ± SD	p value*
Normal	178.86 ± 15.76	0.00
CMS	54.54 ± 3.12	-
Fluoxetine	112.21 ± 8.23	0.00
CE 25	65.23 ± 5.12	0.00
CE 50	87.12 ± 6.34	0.00
CE 100	98.76 ± 7.77	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

TNF-alpha expression in the hippocampus as a marker of inflammation had increased in the CMS group. CE was able to reduce the expression of TNF-alpha compared to the CMS group (Figure 2 and Table 2).

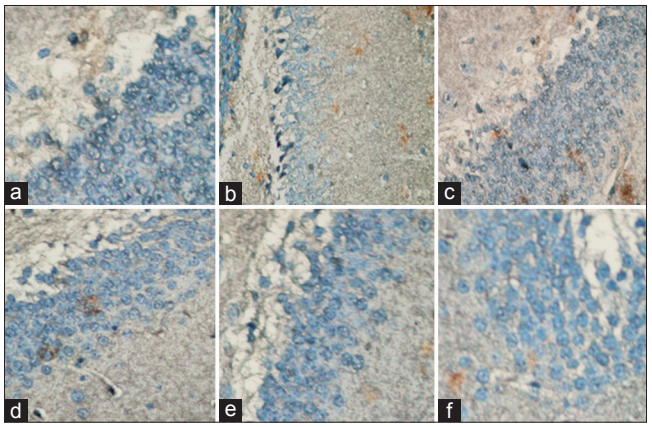

Figure 2: Tumor necrosis factor (TNF)-alpha expression in rats hippocampus. Black arrows: TNF-alpha expression. ×400. (a) Normal, (b) chronic mild stress, (c) fluoxetine, (d) cinnamon (CE) 25, (e) CE 50, (f) CE 100

Table 2: Tumor necrosis factor-alpha expression in rats hippocampus

Group	Tumor necrosis factor-alpha expression (%) Mean ± SD	p value*
Normal	2.86 ± 0.76	0.00
CMS	53.87 ± 3.62	-
Fluoxetine	19.11 ± 1.12	0.00
CE 25	45.56 ± 3.11	0.00
CE 50	37.31 ± 1.34	0.00
CE 100	28.76 ± 1.87	0.00

*Versus CMS, p<0.05; ANOVA, *post hoc* (Bonferroni), CMS: Chronic mild stress, CE: Cinnamon extract.

Discussion

Depression is a psychiatric disorder that has become a serious health problem in the past decade. This disorder is characterized by prolonged dysphoric mood, and in more severe condition would result in decreased self-care and even life-threatening action. Serotonin is believed to play a role in the regulation of mood elation in depressive disorders. Decreased levels of serotonin in the hippocampus will cause an increased dopamine in mesolimbic dopamine neuronal cells. Increased mesolimbic dopamine will cause a decrease in dopamine in the mesocortical area so that it will cause the sufferer to experience a decrease in mood which results in depressive symptoms [7], [8], [9], [10].

Current management related to depressive disorders is aimed at maintaining optimum levels of serotonin in the synaptic cleft. Drugs, such as SSRI group and fluoxetine, play a role in inhibiting the uptake of serotonin in the synaptic cleft so that serotonin remains optimum and is able to improve depressive symptoms. Recent study was more focused on maintaining the viability of neuronal serotonin cells. Decreased neuronal serotonin cell viability will reduce serotonin levels so that it will cause symptoms of depression [11], [12], [13], [14], [15], [16].

Oxidative stress due to oxidants plays an important role as an initiator of inflammation. Inflammation is an important pathogenesis that plays a role in the initiation of cell death. Chronic inflammation will cause activation of the inflammatory cascade, which in turn activates the death receptor. Death receptor activation will then activate caspase which will cause apoptosis of neuronal serotonin cells [17], [18]. Therefore, it is important to make efforts to inhibit inflammation in neuronal cells. Phenol is a metabolite that is quite common in CE. Phenol has the potential to penetrate the blood–brain barrier, so it has the potential to have an effect on neuronal cells. Various studies show that phenol contained in cinnamon possesses antioxidant potential. Antioxidants are believed to be important compounds capable of neutralizing oxidants, to reduce the activation of inflammatory cascade and optimize the viability of serotonin neuronal cells [19], [20], [21], [22]. This study showed that CE improved clinical depression by inhibiting the expression of TNF-alpha in the hippocampus so that the viability of serotonin neuronal cells remained optimal and serotonin levels would increase compared to the group not administered with CE.

Conclusion

CE possessed antidepressant efficacy by inhibiting the inflammatory process in the hippocampus so it was able to optimally increase serotonin levels in the hippocampus.

References

1. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. https://doi.org/10.1037/e400972004-001. [Last accessed on 2019 Nov 01].
2. Du YH, Feng RZ, Li Q, Wei Q, Yin ZQ, Zhou LJ, *et al*. Anti-inflammatory activity of leaf essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao. Int J Clin Exp Med. 2014;7(12):5612-20.
   PMid:25664080
3. Partan RU, Hidayat R, Saleh MI, Parisa N, Tamzil NS. Effect of supplementation Kayu Manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy. Asian J Pharm Clin Res. 2018;11(2):363-5. https://doi.org/10.22159/ajpcr.2018.v11i2.22024
4. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, *et al*. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evid Based Complement Alternat Med. 2012;2012(429320):1-12.
5. Han X, Parker TL. Antiinflammatory activity of cinnamon (*Cinnamomum zeylanicum*) bark essential oil in a human skin disease model. Phytother Res. 2017;31(7):1034-8. https://doi.org/10.1002/ptr.5822
   PMid:28444928
6. Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: Of anhedonia, “anomalous anxiolysis” and activity. PLoS One. 2009;4(1):e4326. https://doi.org/10.1371/journal.pone.0004326
   PMid:19177164
7. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. Ups J Med Sci. 2013;118(1):3-8. https://doi.org/10.3109/03009734.2012.724118
   PMid:22970723
8. Amodeo G, Trusso MA, Fagiolini A. Depression and inflammation: Disentangling a clear yet complex and multifaceted link. Neuropsychiatry. 2017;7(4):448-57. Available from: http://www.jneuropsychiatry.org/peer-review/depression-and-inflammation-disentangling-a-clear-yet-complex-and-multifaceted-link.html. https://doi.org/10.4172/neuropsychiatry.1000236
9. Konsman JP. Inflammation and depression : A nervous plea for psychiatry to not become immune to interpretation. Pharmaceuticals (Basel). 2019;12(1):E29. https://doi.org/10.3390/ph12010029
   PMid:30769887
10. Köhler O, Krogh J, Mors O, Benrosc ME. Inflammation in depression and the potential for anti-inflammatory treatment. Curr Neuropharmacol. 2016;14(7):732-42.
    PMid:27640518
11. Abelaira HM, Réus GZ, Quevedo J. Animal models as

tools to study the pathophysiology of depression. Braz J Psychiatry. 2013;35(Suppl 2):S112-20. https://doi.org/10.1590/1516-4446-2013-1098

PMid:24271223

12. Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol. 2008;581(1-2):113-20. https://doi.org/10.1016/j.ejphar.2007.12.005

PMid:18184609

13. Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of depression: Neurotrophic and neuroinflammatory biomarkers. BioMed Res Int. 2014;2014(932757):932757. https://doi.org/10.1155/2014/932757

PMid:24999483

14. Zeldetz V, Natanel D, Boyko M, Zlotnik A, Shiyntum HN, Grinshpun J, *et al*. A new method for inducing a depression-like behavior in rats a new method for inducing a depression-like behavior in rats. J Vis Exp. 2018;132:57137. https://doi.org/10.3791/57137

PMid:29553503.

15. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34. https://doi.org/10.1038/nri.2015.5

PMid:26711676

16. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40-3. https://doi.org/10.1515/intox-2017-0006

PMid:30123035

17. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, *et al*. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200. https://doi.org/10.1186/1741-7015-11-200

18. Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars). 2004;64(4):439-48.

PMid:15586660

19. Ghaderi H, Rafieian M, Nezhad HR. Pharmacophore effect of hydroalcoholic *Cinnamomum zeylanicum* extract on reserpine-induced depression symptoms in mice. Pharmacophore. 2018;9(2):35-44. Available from: https://www.pharmacophorejournal.com/en/article/effect-of-hydroalcoholic-cinnamomum-zeylanicum-extract-on-reserpine-induced-depression-symptoms-in-mice. https://doi.org/10.4103/1735-5362.189308

20. Sharafeldin K, Raza M. Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin induced diabetic rats. J Basic Appl Zool. 2015;72:126-34. https://doi.org/10.1016/j.jobaz.2015.09.002

21. Chu X, Zhou Y, Hu Z, Lou J, Song W, Li J, *et al*. 24-hour-restraint stress induces phenotypes in mice. Sci Rep. 2016;6:32935.

22. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211-20. https://doi.org/10.1016/j.jfda.2017.03.006