Efficacy of Cinnamon Extract (*Cinnamomum burmannii*) as Supplementation in Lir-psychotic-induced Rats through Oxidative Stress Regulation in Neuronal Cells

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**Abstract**

**BACKGROUND:** Cinnamon is a plant that is often found in Indonesia and is rich in secondary metabolites such as flavonoids, phenols, tannins, and alkaloids. Flavonoids and phenols are very potential as natural antioxidants to suppress various oxidant activities, including oxidant activity that occurs in the hippocampus, which is the underlying psychotic disorder.

**AIM:** This study was aimed to explore the potential of cinnamon extract (CE) on psychotic symptoms.

**METHODS:** Cinnamon simplicia was obtained from the Research and Testing Center for Traditional Medicine, Tawangmangu, Central Java, Indonesia. The extraction of cinnamon was carried out using the maceration method. This study involved 30 Wistar rats (12 weeks of age), including the normal control group, the lir-psychotic group with haloperidol, and the lir-psychotic group with CE supplementation (25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW). Oxidative stress in experimental animals was measured by evaluating malondialdehyde (MDA) expression in the brain tissue using immunohistochemical tests.

**RESULTS:** There were differences in clinical symptoms of psychotic disorder in the animal model between before intervention with CE supplementation and after the intervention. The higher the CE dose administered, the better the improvement in psychotic symptoms seen in the psychotic-induced rats. CE supplementation could reduce MDA expression in the hippocampus. This suggests that there was an optimal significance of cinnamon supplementation in reducing oxidative stress from the hippocampus.

**CONCLUSION:** CE was effective in improving psychotic symptoms in lir-psychotic rats through regulation of oxidative stress in neuronal cells.

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**Introduction**

Psychotic is a mental health disorder characterized by the inability to distinguish between reality and hallucinations. The inability to distinguish hallucinations from reality causes the individual to be unable to carry out physiological and social functions [1]. This disorder, of course, reduces the quality of life and decreases social and functional abilities which result in the loss of the ability to live and the socioeconomic abilities of the sufferer. This decline in socioeconomic function causes severe impacts which will become a social and economic burden for the family and the country. The prevalence of psychotic patients worldwide is estimated at almost 10% [2], [3]. In the next decade, it is predicted that this prevalence will increase to twofold. This disorder is severe to be followed up considering the large prevalence and socioeconomic burden that must be borne by the state is quite large if this disorder is not carried out with proper intervention and management.

Psychotic is a series of mental disorders that begin with a disturbance in the hippocampus area, which is rich in dopaminergic cells [3], [4]. Dopaminergic cells are neuron cells that produce the neurotransmitter dopamine. Dopamine is an important neurotransmitter that regulates differences in reality and hallucinations. Decreased dopamine secretion leads to a decrease in the individual’s ability to discern hallucinations and reality [5]. Dopaminergic cell death is essential pathophysiology behind the decreased secretion of the neurotransmitter dopamine. Several studies have shown a role for oxidative stress in the initiation of dopaminergic cell damage and death [6], [7], [8]. Oxidative stress is a condition in which oxidative reactions occur in cells, especially the cell membrane due to the large number of oxidants which are unstable compounds due to a lack of one electron. This unstable compound condition will trigger oxidative reactions by taking electrons from its surroundings, especially the cell membrane [9]. This results in activation of the pro-inflammatory cytokine and tumor necrosis factor (TNF)-α. Overproduction
of TNF-α will trigger the activation of death receptors. Activation of the death receptor will trigger the activation of the caspase protein, which in turn causes apoptosis of dopaminergic neuronal cells. Dopaminergic cell death causes ligand imbalance and dopamine receptors which cause neurosignaling system disorders, resulting in clinical symptoms in the form of positive symptoms in the form of hyperactivity and negative symptoms in the form of decreased social interactions [9], [10].

The current antipsychotic drugs have significant problems in terms of safety-related to side effects in the form of a higher potential for apoptosis due to the current administration of antipsychotics. Exploration of new therapeutic modalities by utilizing natural ingredients is one of the optimal strategies in solving mental problems and problems due to psychotic disorders. Cinnamon is a plant that is often found in Indonesia and has been used from generation to generation by the ancestors, especially as a cooking spice and traditional medicinal agent [11]. Cinnamon is rich in secondary metabolites such as flavonoids, phenols, tannins, and alkaloids. Flavonoids and phenols are two secondary metabolites that have potential as natural antioxidants to suppress various oxidant activities, including oxidant activity that occurs in the hippocampus, which is the underlying psychotic disorder [12].

This study is one of the first to explore in vivo the potential of cinnamon extract (CE) as an antipsychotic candidate. This study was aimed to explore the potential of CE on psychotic clinical symptoms in the form of positive symptoms (locomotor activity test [LAT]) and negative symptoms (social interactivity test). Furthermore, exploration and evaluation of the potential of CE on the expression of a marker of oxidative stress, namely malondialdehyde (MDA) protein in the hippocampus area, was carried out to ensure regulation of CE on oxidative stress.

Methods

Animal preparation

This study design was an experimental research. A total of 30 male Wistar rats (weighed 200 ± 20 g) were obtained from the Eureka Research Laboratory (Palembang, Indonesia) as a study subject. The experimental animals were housed in cages under controlled conditions (12 h light/dark cycle with temperature 22 ± 1°C and humidity 40–60%), fed and drank ad libitum. All treatments and experimental procedures had been approved by the Ethics and Humanities Commission, Faculty of Medicine, Universitas Sriwijaya (No. 213/ kptfaknisi-rsmh/2020).

Lir-psychotic animal model

The experimental animals were subjected to lir-psychotic induction by intraperitoneal injection with ketamine (30 mg/kg BW) for 5 days [12]. After 5 days of ketamine injection, the withdrawal time was carried out for 5 days. Evaluation of the psychotic induction is evaluated by assessing positive symptoms with a LAT and negative symptoms using a social interaction test (SIT). Suppose the results of the LAT evaluation had shown an improvement compared to the control group, and the results of the SIT evaluation had shown a decrease compared to control group.

Experimental animal grouping

The rats were allocated into six groups (n = 5 in each group): (a) Normal control; (b) lir-psychotic rats without treatment; (c) lir-psychotic rats with haloperidol treatment (1 mg/kg BW) and aquadest for 14 days; (d) lir-psychotic rats with haloperidol treatment (1 mg/kg BW) and CE (25 mg/kg BW) for 14 days; (e) lir-psychotic rats with haloperidol treatment (1 mg/kg BW) and CE (50 mg/kg BW) for 14 days; and (f) lir-psychotic rats with haloperidol treatment (1 mg/kg BW) and CE (100 mg/kg BW) for 14 days.

LAT

LAT is a test used to assess impulsivity that reflects positive symptoms from lir-psychotic animal models. LAT is evaluated by measuring the distance taken by the animal, where the experimental animal is placed in a box measuring 40 cm × 40 cm × 30 cm with a base of the paper. The animal's legs are tied with thread; the longer the distance travelled by the mouse will cause the line that is pulled to be longer. Measurements were assessed for 15 min. LAT is positive if there is a significant difference in the distance between the induced and the uninduced groups.

SIT

SIT is a test used to assess negative symptoms in animal models. SIT is evaluated by measuring the length of time spent by experimental animals interacting with fellow experimental animals. Measurements were evaluated for 15 min. SIT is positive if there is a significant difference between the induced and non-induced groups.

MDA examination

After the rats were sacrificed by intraperitoneal injection of 10% chlorine hydrate, then the brain was evacuated, fixed in 4% paraformaldehyde buffer for immunohistochemistry tests of brain tissue. The tissue that has been inserted into the next fixation fluid is
dehydrated using graded alcohol and xylene, then paraffinized and cut to a thickness of 5 μm using a rotary microtome (Leica, Illinois, USA). The following step, placed on a coated-object glass. Then, the tissues were rehydrated using xylene and alcohol with a gradient of 96%, 90%, 80%, and 70% concentrations and rinsed with tap water. The next step was carried out with the retrieval antigen using the heat-induced epitope retrieval method, where the slides were put in a citrate buffer solution, then heated at 95°C for 60 min. Then, MDA 1:700 (Cloud Clone, Hangzhou, PRC) antibody was stained, followed by overnight incubation at 4°C. The next step was staining with a secondary antibody, biotinylated-horseradish peroxidase, incubation for 1 h, at room temperature.

Furthermore, chromogen was administered. Next, the dehydration process was again carried out using a concentration of alcohol and xylene. After that, mount and assess MDA expression using ImageJ software so that the percentage of MDA expression will be obtained.

**CE preparation**

Cinnamon simplicia was obtained from the Research and Testing Center for Traditional Medicine, Tawangmangu, Central Java, Indonesia. Furthermore, the extraction of cinnamon was carried out using the maceration method. A total of 500 g of simplicia were macerated with 96% ethanol for 72 h. Then do the separation between the pulp and macerate. The macerate was then evaporated using a rotary evaporator (Heidolph), to obtain CE.

**Phytochemical test for cinnamon**

**Test for phenols**

The test was performed using the method of Sofowora [13]. Two milliliter extract was taken in a beaker glass. Then, 2 ml of ferric chloride solution was added. A deep bluish-green solution indicated the presence of phenols.

**Test for terpenoids**

Salkowski test was performed using the method of Edeoga et al. [14]. Five milliliters of aqueous extract were mixed in 2 ml of chloroform. Then, 3 ml of concentrated sulfuric acid was poured to form a layer. A reddish-brown coloration of interface indicated the presence of terpenoids.

**Test for saponins**

The test was performed using the method of Edeoga et al. [14]. Two grams of the powdered sample boiled in 20 ml of distilled water in a water bath and filtered the solution. Then, 10 ml of the filtrate was mixed with 5 ml of distilled water and shake vigorously for stable, persistent foam. The foam was mixed with three drops of olive oil and shakes vigorously, which leads to the formation of the emulsion; indicated the presence of saponins.

**Test for flavonoids**

The test was performed using the method of Harborne [15]. One gram powdered sample was heated with 10 ml ethyl acetate over a steam bath (40–50°C) for 5 min. The filtrate was treated with 1 ml dilute ammonia. A yellow color demonstrated positive test for flavonoids.

**Test for alkaloids**

The test was performed using the method of Harborne [15]. One gram powdered sample was extracted with 5 ml methanol and 5 ml of 2N hydrochloric acid. Then, the filtrate was tested with Meyer’s and Wagner’s reagents. The samples were scored positive, based on turbidity.

**Data analysis**

Data were analyzed using SPSS 25.0 (SPSS, Inc., Armonk, NY, United States). One-way ANOVA followed by post hoc analysis (Bonferroni test) was carried out to assess differences in mean levels of MDA, dopamine, serotonin, and SIT-LAT expression. p < 0.05 was determined as an indication that there was a significant difference in mean levels.

**Results**

Table 1 shows that there is a clinical improvement in the study subjects, namely an improvement in the LAT test value which describes positive symptoms of schizophrenia and an improvement in the SIT test score which represents the negative symptoms of schizophrenia. Statistically, there are differences in LAT and SIT values between before intervention with CE supplementation and after the intervention. The higher the CE dose administered, the better the improvement in psychotic symptoms (SIT and LAT) seen in the psychotic-induced rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>LAT Value</th>
<th>SIT Value</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>A</td>
<td>43.1 ± 0.7</td>
<td>43.52 ± 0.4</td>
<td>0.321</td>
</tr>
<tr>
<td>B</td>
<td>43.2 ± 0.6</td>
<td>43.26 ± 0.3</td>
<td>0.011</td>
</tr>
<tr>
<td>C</td>
<td>44.0 ± 0.1</td>
<td>44.96 ± 0.7</td>
<td>0.087</td>
</tr>
<tr>
<td>D</td>
<td>43.3 ± 0.8</td>
<td>46.3 ± 0.5</td>
<td>0.013</td>
</tr>
<tr>
<td>E</td>
<td>44.0 ± 0.4</td>
<td>40.58 ± 0.9</td>
<td>0.016</td>
</tr>
<tr>
<td>F</td>
<td>43.7 ± 0.9</td>
<td>38.28 ± 0.5</td>
<td>0.014</td>
</tr>
</tbody>
</table>

* p ≤ 0.05, Student’s t-test; † p ≤ 0.01, t-test dependent, ANOVA; ‡ Others are used for post hoc analysis.
Figure 1 shows the oxidant expression in the form of peroxidase lipid, MDA in the hippocampus rats. Figure 1 shows that CE supplementation could reduce MDA protein expression in the hippocampus, where the higher the cinnamon dose, the lower expression of MDA protein. This suggests that there was an optimal significance of cinnamon supplementation in reducing oxidative stress from hippocampus.

Table 2 shows that CE is rich in flavonoids and phenolics with very high antioxidant potential. Flavonoids and phenolics are the main secondary metabolites that are believed to play a role in inhibiting oxidative stress due to the accumulation of oxidants in the hippocampus rats.

Table 2: Phytochemical test of CE

<table>
<thead>
<tr>
<th>Test material</th>
<th>Saponin</th>
<th>Alkaloid</th>
<th>Triterpenoid</th>
<th>Phenolic</th>
<th>Flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>
| CE: Cinnamon extract.

Discussion

Various hypotheses have been proposed regarding the pathophysiological models and causes of psychotic disorders [16], [17]. One of them is the role of free radicals related to the pathology of psychotic which was put forward about 50 years ago. The brain is a tissue that is susceptible to oxidative stress due to low levels of antioxidants, high levels of saturated fatty acids, and increased oxygen demand [18], [19]. Ketamine-induced rats displayed symptoms that resemble those of psychotic in humans [20]. The symptoms shown are positive symptoms as measured by the LAT; and negative symptoms as measured by the SIT [21]. When the N-Methyl D-Aspartate membrane receptor is blocked by ketamine on the GABAergic interneurons, there is a failure to interpret the degree of excitation of the pyramidal neurons; which results in an excessive increase in glutamate action, in turn, induces overstimulation of the ventral tegmental area, ultimately leading to the hyperdopaminergic state characteristic of psychotic [22], [23], [24]. This is what produces both positive and negative symptoms in psychotic [24], [25], [26]. Cinnamon supplementation had been shown to reduce MDA expression in the hippocampus area (Figure 1).

Besides, this study found that there is a clinical improvement in the lir-psychotic rats. Statistically, there was significant improvement of LAT and SIT values after the treatment with CE. Cinnamon which is rich in antioxidants, in the form of cinnamaldehyde, can block the oxidant activity that occurs in psychotic conditions to maintain the survival of dopaminergic and serotonergic neuronal cells, which leads to optimization of the neurotransmitters dopamine and serotonin [27], [28], [29], [30].

The increase in oxidants found in psychotic disorder causes oxidative stress to neuronal cells, especially in the basal ganglia, which is the center of mental and mood regulation [25], [31]. The oxidative...
stress will trigger a series of inflammatory cascades and a series of apoptotic cascades of neuronal cells [26], [27]. Activation of the death receptor by oxidants causes activation of caspase in neuronal cells, which will lead to the activation of caspase three and followed by neuronal cell death [27], [28]. Hippocampus is rich in a variety of neuronal cells, including dopaminergic and serotoninergic neuronal cells [23], [24].

Cinnamon was useful as a supplement to the lir-psychotic rat. This is mainly due to the phytochemical content in cinnamon which functions as an antioxidant. Based on the results of phytochemical tests, it was found that the content of flavonoids and phenolics was higher than saponins, alkaloids, and terpenoids. Cinnamon is rich in antioxidants with the main compound, namely cinnamaldehyde. Cinnamaldehyde is the main compound which is believed to be beneficial for health. Cinnamaldehyde is one of the secondary metabolites of cinnamon, a phenol group, with very potent antioxidant abilities [32], [33].

**Conclusion**

CE is effective as supplementation to improve psychotic symptoms in lir-psychotic rats through regulation of oxidative stress in neuronal cells. Further studies need to be done to explore the safety and effectiveness of cinnamon as supplementation in psychotic disorders.

**References**

PMid:29922188

PMid:29649252

PMid:22131939

PMid:26681495

PMid:17804133

PMid:16777663


PMid:19771301

PMid:18299157


PMid:21449915


PMid:25592751


PMid:18654878

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