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The Efficacy of *Cinnamomum burmanii* Extract on the Protection of Neuronal Cell Death in Haloperidol Induced Male Wistar Rats

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Abstract

Background
Haloperidol is categorized as the first class antipsychotic drug. Long-term use of haloperidol may convey to increased Reactive Oxygen Species (ROS) that will yield oxidative damage which further leads to cell death. Several studies had identified the effects of cinnamon extract on cell death. This study aimed to determine the efficacy of cinnamon extract (*Cinnamomum burmanii*) on the protection of neuronal cell death in haloperidol-induced male Wistar white rats.

Methods
This study was experimental with pre and post-test design. Thirty male Wistar rats were divided into 5 groups, induced with haloperidol and followed by treatment. Caspase-3 and dopamine were assayed by ELISA sandwich method using ELISA kit. Mean difference of caspase expression and dopamine levels before and after induction were shown (p<0.05).

Results
There were mean differences of caspase-3 expression level in the positive control group, cinnamon extract of 100 and 200mg/kgBW before and after treatment (p<0.05). Whereas for dopamine levels, there were mean differences in positive control group, cinnamon extract of 50, 100 and 200mg/kgBW before and after treatment (p<0.05). With Post Hoc test, it was found that there were no mean differences of caspase-3 expression level between positive group with cinnamon extract group of 100 and 200mg/kgBW (p>0,05) and there were also no mean differences of positive group dopamine level with group of cinnamon extract of 100 and 200mg/kgBW (p>0.05).

Conclusion
Cinnamomum burmanii extract at dose of 100 and 200mg/kgBW were effective in the protection against neuronal cell death in haloperidol induced male Wistar white rats.

Keywords: Caspase-3, Dopamine, Efficacy, Cinnamon Extract, Pre and Post Test Design

Background

Psychosis is a common psychiatric disorder requiring long-term treatment. As for one of the most commonly used antipsychotic drugs for psychiatric disorders is haloperidol. Haloperidol is a first class antipsychotic drug categorized as a typical antipsychotic. Haloperidol works selectively by blocking the activity of dopamine D2 receptors, especially in the striatal and mesolimbic regions. Although this drug has been proven to be effective in suppressing psychotic symptoms, the side effects most commonly caused by this treatment are Extrapyramidal Symptoms (EPS); such as parkinsonism, akathisia, and tardive dyskinesia. One of the main mechanisms of haloperidol side effects is predicted to be induced by cell death, both apoptosis and necrosis of brain neuron cells.[1,2]

The use of haloperidol over a long period of time can trigger an increase in Reactive Oxygen Species (ROS) through various mechanisms where all mechanisms will eventually cause oxidative damage, so as the damage to DNA, proteins and mitochondrial cell lipids will occur. These damages will trigger mitochondrial dysfunction. Mitochondrial dysfunction itself will activate the release of Chitochrome C, which in turn triggers the caspase system activation cascade. Once released, cytochrome C will immediately bind to Apaf 1, where this bond will cause auto-activation of phase 9, followed by caspase-3 activation which is one of the markers playing a role in cell apoptosis.[3,4]

Given the magnitude of the impact in long-term use of haloperidol on neuronal cells, a therapeutic modality is required, not only to prevent, but also to overcome neuronal cell death due to the use of the haloperidol drug. Therefore, this study aimed to determine the efficacy of cinnamon (*Cinnamomum burmanii*) extract on its protection against neuronal cell death in Wistar male white rats induced with haloperidol.

Methods

This study was experimental with pre and post-test control group design. Negative controls determined the success of haloperidol induction and positive control were used to strengthen the test results. The study was conducted on March-April 2017 at Animal House and the Biomolecular Laboratory, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

Subjects were male white wistar (*Rattus novergicus*) strains, aged 2-3 months, body weight of 150-200 grams. The selection of male white rats subjects in this study was based on the consideration of its availability and similarity to humans. The rats were divided into 5 (five) groups randomly, namely the negative control group (Aquadest), cinnamon extract group dose of 50mg/kgBB (CE 50), 100mg/kgBW (CE 100), 200 mg/kgBW (CE 200) and positive control group (Astaxanthin 1 mg/kgBW), with each group consisted of 6 rats. Effect of haloperidol and cinnamon extract on caspase-3 expression levels and dopamine levels were analyzed using Paired T-Test. Comparison on the effect of cinnamon extract on caspase-3 expression and dopamine levels were analyzed with independent T-Test and Oneway ANOVA followed by Post Hoc test. In objective to determine the effect of haloperidol, caspase-3 and dopamine were measured on day 0 and 14. Data were then analyzed with Paired-Sample T-Test.

Results

Table 1: Effect of Haloperidol Induction on Caspase-3 Expression and Dopamine Levels

Variable	Day-0 (before induction)	Day-21 (after induction)	Changes	*p*
Caspase-3 Level	0.494± 0,015	0,547± 0,037	0,053± 0,039	0,020
Dopamine Level	21,98± 1,83	19,53± 0,267	-2,45± 1,81	0,021

Paired-Sample T-Test

As shown in Table 1, the obtained value of all the probabilities of both variables were <0.05. This indicated that there were mean differences of caspase-3 expression levels (p=0.020) and dopamine levels (p=0.021) before and after induction, where there was an increase in caspase-3 expression levels and a decrease in dopamine levels 21 days after haloperidol induction.

Table 2: Effect of Cinnamon Extract Administration on Caspase-3 Expression Level

Group	Caspase-3 Level Day-21	Caspase-3 Level Day-35	Changes	*p*
Negative Control	0.547± 0,037	0,545± 0,035	-0,0018± 0,0027	0,159
CE 50 mg/kgBW	0.547± 0,037	0,545± 0,036	-0,0017± 0,0007	0,054
CE 100 mg/kgBW	0.547± 0,037	0,523± 0,029	-0,0240± 0,0092	0,001
CE 200 mg/kgBW	0.547± 0,037	0,509± 0,022	-0,0370± 0,0157	0,002
Positive Control	0.547± 0,037	0,478± 0,031	-0,0690± 0,0523	0,023

Paired-Sample T-Test

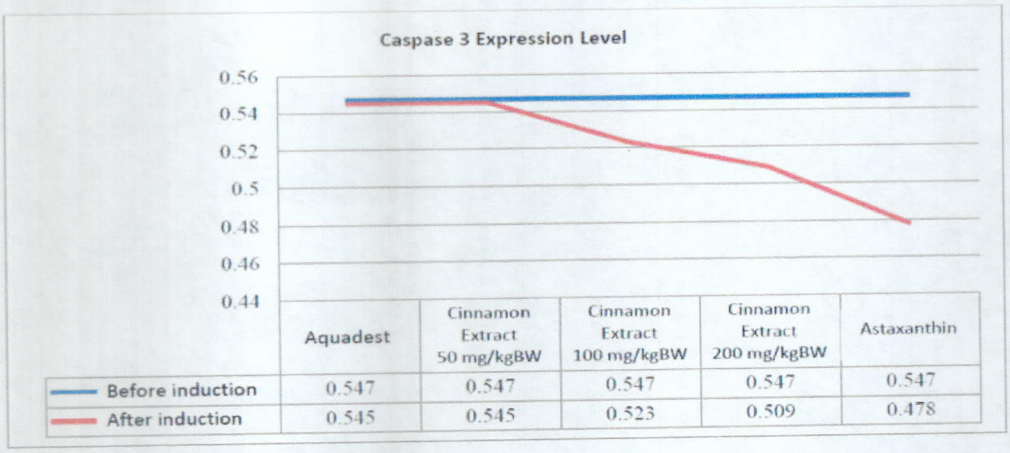


Figure 1: Effect of Cinnamon Extract Administration on Caspase-3 Expression Level

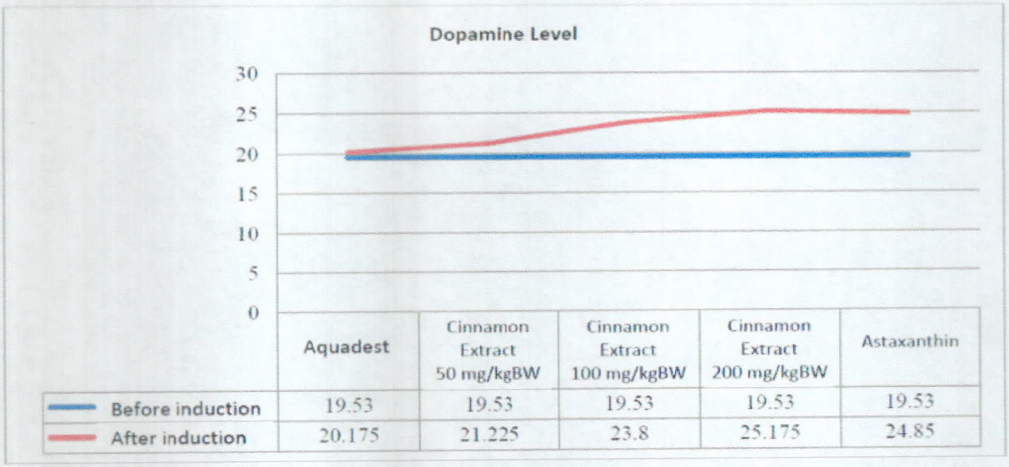
As exhibited in Table 2, the probability value of the negative control group and cinnamon extract of 50 mg/kgBW were 0.159 and 0.054, respectively ($p > 0.05$). This showed the absence of mean difference of caspase-3 expression after administration of aquadest or

cinnamon extract of 50 mg/kgBW. In addition, the probability values of the positive control group, cinnamon extract of 100 mg/kgBW and 200 mg/kgBW were obtained at 0.001, 0.002 and 0.023 (p<0.05) respectively. This showed that there was a difference of mean level of caspase-3 expression in the three groups, where there was a decrease in caspase-3 expression level day 21 compared to day 35 after administration of astaxanthin and cinnamon extract of 100mg/kgBW and 200mg/kgBW.

Table 3: Effect of Cinnamon Extract Administration on Dopamine Levels

Group	Dopamine Level Day-21	Dopamine Level Day-35	Changes	*p*
Negative Control	19,53± 0,267	20,175± 0,758	0,65± 0,85	0,121
CE 50 mg/kgBW	19,53± 0,267	21,225± 1,278	1,70± 1,21	0,018
CE 100 mg/kgBW	19,53± 0,267	23,800± 2,473	4,28± 2,62	0,010
CE 200 mg/kgBW	19,53± 0,267	25,175± 1,648	5,65± 1,62	0,000
Positive Control	19,53± 0,267	24,850± 3,113	5,33± 3,29	0,011

Paired-Sample T Test



	Aquadest	Cinnamon Extract 50 mg/kgBW	Cinnamon Extract 100 mg/kgBW	Cinnamon Extract 200 mg/kgBW	Astaxanthin
Before induction	19.53	19.53	19.53	19.53	19.53
After induction	20.175	21.225	23.8	25.175	24.85

Figure 2: Effect of Cinnamon Extract Administration on Dopamine Levels

From the statistical analysis, the probability value of the negative control group was 0.121 (p>0.05), interpreted as the absence of mean difference in dopamine levels after administration of aquadest. In addition, the probability values of the positive control group, cinnamon extract of 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW were 0.018; 0.010; 0,000 and 0,011 respectively (p<0,05). This showed mean differences in dopamine levels of the four groups, where there was an increase in dopamine levels on day 35 compared to day 21 after administration of astaxanthin, and cinnamon extract dose of 50mg/kgBW, 100mg/kgBW and 200mg/kgBW.

Table 4: Comparison of the Efficacy of Cinnamon Extracts on Caspase-3 Expression Levels

	Aquadest	CE 50	CE 100	CE 200	Astaxanthin
Aquadest		1,000	0,733	0,315	0,008
CE 50	1,000		0,727	0,311	0,008
CE 100	0,733	0,727		0,949	0,122
CE 200	0,315	0,311	0,949		0,407
Astaxanthin	0,008	0,008	0,122	0,407	

Post Hoc Test (*Tukey*)

Table 5: Comparison of the Efficacy of Cinnamon Extracts on Dopamine Levels

	Aquadest	CE 50	CE 100	CE 200	Astaxanthin
Aquadest		0,897	0,036	0,002	0,004
CE 50	0,897		0,216	0,019	0,036
CE 100	0,036	0,216		0,768	0,897
CE 200	0,002	0,019	0,768		0,999
Astaxanthin	0,004	0,036	0,897	0,999	

Post Hoc Test (*Tukey*)

The Post Hoc test as seen in Table 4 obtained probability values between negative control with positive control group as <0.05. This showed mean differences in caspase-3 expression level in the negative control with the positive control group. In addition, the probability values between positive groups with cinnamon extract group dose of 100mg/kgBW and 200mg/kgBW were >0.05, interpreted as the absence of mean differences of caspase-3

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expression level in the positive group with cinnamon extract groups of 100mg/kgBW and 200mg/kgBW.

The Post Hoc test as exhibited in Table 5 obtained probability values between negative control with positive control groups, and cinnamon extract groups of 100 mg/kgBW and 200 mg/kgBW were <0.05. There were mean differences in dopamine levels of those groups. In addition, the obtained probability value between positive groups with cinnamon extract groups of 100mg/kgBW and 200mg/kgBW were >0.05 so there were no mean differences of dopamine levels in the positive control group with cinnamon extract of 100mg/kgBW and 200mg/kgBW.

Discussions

Cell death come to pass due to the exposure of toxic materials (radiation, free radicals, toxins, etc.) in massive amount and/or long periods of time where this process is one of the normal mechanisms of protection and regulation of cell numbers, one of which regulates cell growth so as not to excessive cancer does not form. There are two types of cell death, namely apoptosis and necrosis. For apoptotic cell death, there are three pathways of apoptosis mechanism in which these three pathways have the same expression pathway, caspase-3. Both intrinsic, extrinsic and additional pathways result in activation of caspase, which subsequently results in cell apoptosis.[5,6]

In this study, caspase-3 expression levels and dopamine levels were examined on day 0, 21 and 35. Day 1 to day 21, the study subjects were administered with pretreatment of haloperidol, and obtained an increase in caspase-3 expression level by 0.053±0.039 (10.73%) and a decrease in dopamine levels by 2.45 ± 1.81 (11.15%) in day 21. These results were in accordance with the theory that haloperidol administration lead to an increase in caspase-3 expression levels and a decrease in dopamine levels which, if this condition persists, would yield oxidative stress, that in turn would increase ROS so that mitochondria experience dysfunction and stimulate apoptosis.

On day 22 to 35, various doses of cinnamon extract and astaxanthin were administered. All groups experienced a decrease in caspase-3 expression levels where in negative control, there was a significant decrease in caspase-3 expression level by 0.0018 (0.3%), cinnamon extract group of 50mg/kgBW had a decreased caspase-3 expression level which was not significant, by 0.0017 (0.3%), cinnamon extract group of 100mg/kgBW experienced a significant decrease in caspase-3 expression level by 0.024 (4.39%), cinnamon extract group of

200mg/kgBW had a decreased caspase-3 expression level which was significant, by 0.037 (6.76%) and the astaxanthin group at 1 mg/kgBW had the most significant decrease in caspase-3 expression level by 0.069 (12.61%). It was concluded that the greater the dose of cinnamon extract, the greater the effect of decreasing caspase-3 expression level although it was not as impactful as the astaxanthin.

From the bivariate independent T-test statistical analysis between groups, it was obtained the absence of mean difference of caspase-3 expression level between the cinnamon extract group of 200mg/kgBW with astaxanthin 1mg/kgBW. Whereas, based on Post Hoc multivariate statistical analysis, there were mean differences in caspase-3 expression levels between the cinnamon extract groups dose of 100mg/kgBW and 200mg/kgBW with 1mg/kgBW astaxanthin. Hence it was concluded based on the suitability test, cinnamon extract of 100 mg/kgBW and 200 mg/kgBW were equivalent to astaxanthin 1 mg/kgBW.

The results on day 35, all groups experienced an increase in dopamine levels, where in positive control group there was an insignificant increase in dopamine level by 0.65 (3.33%), cinnamon extract group of 50mg/kgBW experienced an increase in dopamine level by 1.7 (8.7%), cinnamon extract group 100mg/kgBW experienced a significant increase in dopamine level by 4.28 (21.92%), cinnamon extract group of 200mg/kgBW experienced the largest significant increase in dopamine levels amounting to 5.65 (28.93%) and astaxanthin group 1 mg/kgBW had a significant increase in dopamine level by 5.33 (27.29%). It was concluded that the greater the dose of cinnamon extract administered, the greater the effect of increased dopamine levels. The cinnamon extract at dose of 200mg/kgBW showed a greater change in dopamine levels compared to astaxanthin.

From statistical analysis both independent T-test and Post Hoc obtained no mean differences of dopamine levels between cinnamon extract groups of 100mg/kgBW and 200mg/kgBW with 1mg/kgBW astaxanthin. Hence it was concluded based on the suitability test, cinnamon extract of 100 mg/kgBW and 200 mg/kgBW were equivalent to astaxanthin 1 mg/kgBW.

The results of this study were in accordance with several prior studies. Cinnamon (*Cinnamon cassia*) and its metabolite, sodium benzoate (NaB), could penetrate the blood brain barrier (BBB) in mice. With 200 mg/kgBW/day of cinnamon in C57/BL6 mice for 10 days, it yielded neuroprotective effects that elevated neurotrophic factors (BDNF and NT 3). The study showed the role of proantocyanidin compounds from cinnamon extracts were useful in

inhibiting the activity of astrocyte and neuron cells apoptosis in ischemic conditions in vitro. According to study conducted by Said and Husein (2009), administration of 100 mg/kgBW/day of cinnamon extract for 7 days reduced oxidative stress (MDA) markers in rat liver tissue induced by oxidative stress with carbon tetrachloride (CC14).[7-10]

The bioactive components of cinnamon are phenolic compounds and flavonoids which include cinnamic acid, cinnamaldehyde, cinnamon and proanthocyanidin, with good antioxidant and anti-inflammatory properties. Proantocyanidin is a compound that is widely contained in cinnamon extract, with a strong antioxidant effect.[11-13]

Conclussion

There were no mean differences of caspase-3 expression level and dopamine after administration of astaxanthin 1mg/kgBW with cinnamon extract of 100mg/kgBW and 200mg/kgBW. The extract of *Cinnamomum burmanii* at dose of 100mg/kgBW and 200mg/kgBW were effective in protecting against neuronal cell death in haloperidol induced Wistar male white rats.

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