Original Article

Red Palm Oil Prevents Cognitive Impairment in a Rat Model of D-Galactose-Induced Aging

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Abstract

Background: Age has a significant impact on neurodegenerative disease vulnerability with increasing levels of oxidative stress, causing damage to the neurons, mitochondria, protein, and DNA. Hence, brain aging has become a significant risk factor for developing neurodegenerative brain disorders such as Alzheimer's disease (AD) and Parkinson's disease. This study was carried out to determine the effectiveness of Red Palm Oil (RPO) in preventing AD induced in the rat.

Materials and Methods: Forty male Sprague-Dawley rats were divided into 5 groups (n=8) that comprised of the normal control group (saline water), negative control (D-Galactose, 100mg/kg), two treatment groups that were administered by RPO daily (200 and 400 mg/kg) and Donepezil, (0.25 mg/kg) were given as positive control for 21-days. Y-maze spontaneous alternation test was done weekly to evaluate the spatial working memory of the rats. At the end of treatment, biomarkers of oxidative stress such as GSH, SOD, and neurotransmitter biomarkers, dopamine in the blood, were measured through ELISA.

Results: Rat pre-treated RPO showed significant improvement in exploring new areas compared to untreated rats ($p < 0.05$). On the other hand, current results showed a high level of dopamine and GSH in rats treated with RPO than D-galactose-induced rats after 21 days of pre-treatment ($p < 0.05$). Meanwhile, total SOD was increased in all groups that were induced with D-galactose.

Conclusion: Overall, RPO can improve cognitive impairment in rats with brain aging. This is owing to the antioxidant properties of RPO, which play a vital role in preventing oxidative stress. In the future, RPO could appear as a novel therapeutic molecular for brain disease.

Keywords: Oxidative Stress, Brain Aging, Antioxidant, Red Palm Oil

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Introduction

The nervous system will start to become weaken and degenerate during aging. Prolong use of the brain will reduce its competency in terms of function and structure. It is because the size of the brain,

vasculature, and cognition will change during aging. The changes of the brain will take place at all levels, from molecules to morphology. Brain aging has become the major risk factor for developing neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. This happens as aging can lead to oxidative stress, increasing reactive oxygen species (ROS), and deterioration of antioxidants within the brain (1-5).

Alzheimer's disease (AD) is a chronic irreversible progressive neurodegenerative brain disorder. Oxidative stress is one of the pathophysiologies in AD that lead to the accumulation of extracellular senile plaque and intracellular neurofibrillary tangle (NFT). Hence, the cognitive function such as working memory, the speed with which information is processed, and short-term memory will gradually deteriorate over time in adults. Antioxidants can only neutralize ROS; however, these natural antioxidants, such as GSH are depleted during aging (6-10).

Oil palm (*Elaeis guineensis Jacq.*) is a tree from the Arecaceae palm family that originated from West Africa. It is also known as African oil palm, which is quite a common name to be called. Red palm oil (RPO) is obtained through the novel process of refining crude palm oil (CPO) without destroying the nutrients within it. This process is done to remove the impurities and improve the oil quality. RPO still retains more than 80% of the original vitamin E and carotenes from the CPO. Tocotrienol content found in the tocotrienol-rich fraction (TRF) of palm oil constituted about 70% to 80%. The substance has shown that its usage can reduce neurotoxicity due to Aβ exposure. TRF can help the neuronal membrane retain its integrity (11-15).

Hence, red palm oil (RPO) from *Elaeis guineensis* palm oil species known rich with antioxidant properties was used during this investigation to evaluate its effectiveness in fighting and preventing AD-induced rats.

Methods

Source of Chemicals: Red palm oil (RPO) was obtained from the market in Shah Alam, Selangor. 500 ml RPO with 100% natural palm oil was chosen with no added artificial coloring and preservative. Besides, D-galactose was purchased from ROFA Laboratory Centre in Indonesia. This chemical was used to induce brain aging for AD in rats. Next, the donepezil drug was bought from Apical Scientific Sdn. Bhd. in Seri Kembangan, Selangor, Malaysia. This drug was used in the positive control group as a commercial drug in treating AD. Glutathione (GSH), total superoxide dismutase (SOD), and dopamine ELISA kit were purchased from Elabscience.

Experimental Chemical Solutions: D-galactose was prepared with 0.9% saline solution first. In this study, 1 mg of D-galactose was added into 10 ml of 0.9% saline solution. The appropriate amount of solution was calculated to match with 100 mg/kg of D-galactose induced in the rat from this new concentration. Donepezil drug was in the form of a tablet with a concentration of 5 mg per tablet. In this study, 5 mg of donepezil was dissolved in 100 ml 0f 0.9% saline solution. Since the concentration has changed after the dissolvent, the calculation was carried out to get the correct amount of 0.25 mg/kg of donepezil for the treatment process. Red palm oil was given to the rats in 100% of concentration according to the appropriate amount decided based on a previous study (16).

Drug Treatment and Experimental Design: Forty healthy adults Sprague Dawley rat species were used for this study. These rats were randomly divided into five groups: control group, negative control group, positive control group, treatment 1 group, and treatment 2 group. Group one was had saline solution only as the control for the saline used in other groups. Group two was induced with D-galactose only that acted as an AD group. This chemical has caused brain aging, one of the main risk factors for AD. Next, group three was induced with D-galactose after 30 minutes of being treated with the donepezil drug. Lastly, groups four and five were given 200 mg/kg and 400 mg/kg of red palm oil (RPO) respectively first before being induced with D-galactose with 30 minutes in the gap.

This pre-treatment was carried out to evaluate the effectiveness of red palm oil (RPO) in preventing oxidative stress from damaging the nerve cells. All of these treatments were given to the rats through oral gavage. The doses of the used compounds were chosen based on a previous study suited to this experimental

design (17-19). This pre-treatment study lasted for 21 days (3 weeks).

Spontaneous Alternation Y-maze Test: Y-maze test was carried out to evaluate spatial working memory, which is the short-term memory of the rats. This symptom usually appears in AD. The Y-maze was built from a corrugated board with 20 cm height, 35 cm length, and 10 cm width to form a "Y" shape. This measurement was constant for each arm, with its center was in an equilateral triangular shape. The degree for each of the arms was customized at a 120° angle. Each arm was labeled with letters A, B, and C to differentiate different arms. The design was based on published protocols with modifications to suit the rats (17). The test recorded the spontaneous alternation carried out every week on day 1, day 11, and day 21. This test was purposed to evaluate the progressive condition of AD during the experiment duration. The rat was initially placed at the center of the arm, and it was allowed to roam around the maze with all open arms.

The number and order of arm entries were recorded for four minutes. The rat was considered to enter the arm when the hind paws were entirely within the arm. The arms of the maze were sanitized with alcohol after each trial. Spontaneous alternation indicates the rats' short-term memory, which high alternation means the rat has an excellent spatial working memory. This can be noted by observing the sequence of the arm entered by the rat. It was considered alternation when the arm sequence was different (ABC, CAB, or BAC but not BAB or BBA or CBC). Percentage spontaneous alternation was calculated by using this equation: Percentage of spontaneous alternation = [(Number of alternations)/(Total arm entries − 2)] × 100 (18).

Blood Sample Collection and Sample Storage: Blood samples of the rats were collected at the end of the study duration, on day 22 after three weeks of treatment (21 days). The blood sample was drawn slowly from the heart, specifically at the ventricle, into a 5 ml syringe by using the cardiac puncture method. This blood was stored in a blood sample tube that contained EDTA as an anticoagulant to prevent the blood from clotting. It was shaken gently to mix with EDTA. After that, the blood was centrifuged for 15 minutes at 2200 rpm within 30 minutes of blood withdrawal to separate the plasma. The plasma obtained was segregated into different microtubes according to the different parameter that was carried out. It was to make sure the samples were not easily degraded. The samples were then stored properly at -20°C.

Oxidative Stress Markers – Glutathione (GSH) and Total Superoxide Dismutase (T-SOD): Oxidative stress markers in plasma samples were measured by using an ELISA kit from Elabscience. The procedure was carried out according to the manual book.

Neurotransmitter Marker; Dopamine: Neurotransmitter marker, which is dopamine, was measured through Elisa kit from Elabscience. This test was run according to the manual book.

Data Analysis: Statistical analysis was conducted by using the software Graphpad Prism version 8.0.1 for Windows. The result values for oxidative stress markers, neurotransmitter markers, and behavioral tests are expressed in mean ± SEM All results were analyzed by One-Way Analysis of Variance (ANOVA). This test was selected to evaluate the differences between different experimental groups. The analysis was further conducted by post hoc test, the Tukey test for the multiple comparisons. A p level <0.05 was taken as indicative of statistical significance for the tests used.

Results

The spontaneous alternation y-maze assessed short-term spatial working memory by utilizing mice's natural exploring behavior. This test was carried out weekly on day 1, day 11, and day 21. The figure below shows the percentage of spontaneous alternation on day 1. During week 1, each experimental group did not show any significant effect as the treatment merely began ($p > 0.05$).

Table 1: Experimental groups for the 'Effectiveness of RPO in Preventing Alzheimer's Disease' study design.

NO	GROUP (n=8)	TREATMENT
1	Normal Control Group	Saline solution
2	Negative Control	D-galactose (D-gal) (100 mg/kg)
3	Positive Control	Donepezil (DPZ) (0.25 mg/kg) + D-Galactose (D-gal) (100 mg/kg)
4	Treatment 1 (200 mg/kg)	Red palm oil (RPO) 200 mg/kg + D-galactose (D-gal) 100 mg/kg
5	Treatment 2 (400 mg/kg)	Red palm oil (RPO) 400 mg/kg + D-galactose (D-gal) 100 mg/kg

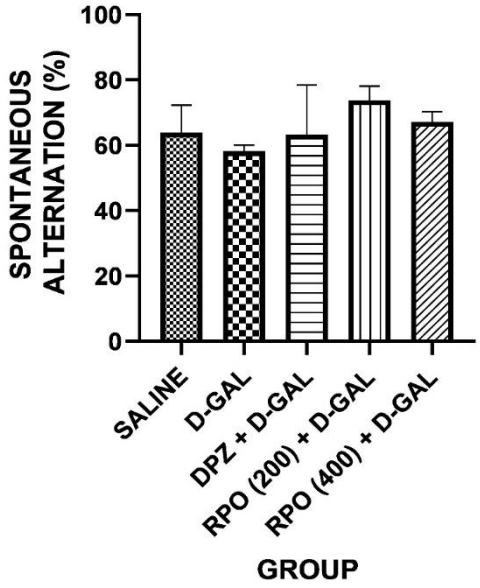

Figure 1. Effect of red palm oil (RPO) on percentage of spontaneous alternation in Alzheimer's disease induced rat in week 1. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). The mice were put in the Y-maze for 4 minutes one by one. Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the groups. The values are mean ± S.E.M (n = eight animals per group).

During week 2, group pre-treated with donepezil and red palm oil, positive control group (DPZ + D-gal), treatment 1 [RPO (200) + D-gal], treatment 2 [RPO (400) + D-gal] started to have high spontaneous alternation compared to D-galactose alone treated group (D-gal). One-way ANOVA test showed a significant effect of these three groups against the D-Galactose group ($p<0.005$). However, D-galactose alone treated group (D-gal) showed no significant effect of D-galactose consumption compared to the normal control group (saline) ($p>0.05$). Besides, the pre-treatment in preventing AD also showed no significant result against the normal control group ($p>0.05$).

During week 3, D-Galactose alone treated group had a low significant spontaneous alternation percentage compared to the normal control group (saline), which may indicate brain aging progressively taking place ($p < 0.005$). Besides, both groups treated with RPO keep giving good spontaneous alternation percentage compared to the D-Galactose group ($p < 0.05$). Moreover, pre-treatment of donepezil did not show a significant effect against D-gal, which was against with previous study ($p > 0.05$) (19).

Glutathione (GSH) Level: This test showed that pre-treatment of red palm oil significantly increases anti-oxidant in the group of treatment 1 (RPO 200 mg/kg + D-gal) and treatment 2 (RPO 400 mg/kg + D-gal) compared to D-galactose alone treated group ($p<0.05$).

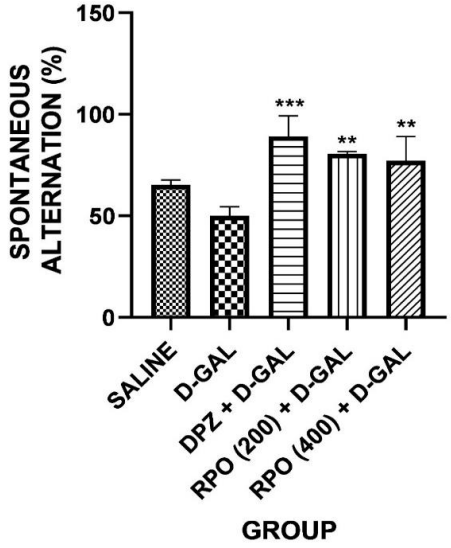

Figure 2. Effect of red palm oil (RPO) on percentage of spontaneous alternation in Alzheimer's disease induced rat in week 2. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). The mice were put in the Y-maze for 4 minutes one by one. Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the groups. The values are mean ± S.E.M (n = eight animals per group). Star above bars indicate a significant difference, $^{**}p < 0.005$, $^{***}p > 0.0005$ compared to D-galactose alone treated group (D-gal).

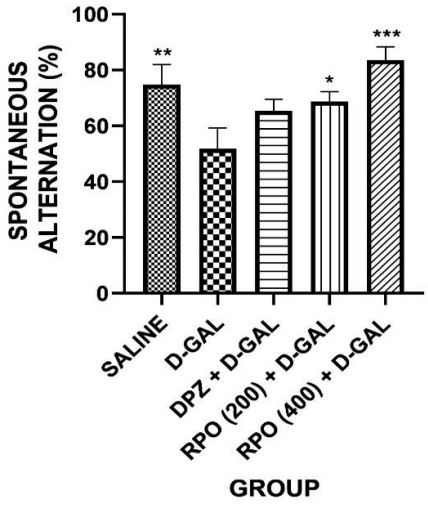

Figure 3. Effect of red palm oil (RPO) on percentage of spontaneous alternation in Alzheimer's disease induced rat in week 3. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). The mice were put in the Y-maze for 4 minutes one by one. Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the groups. The values are mean ± S.E.M (n = eight animals per group). Star above bars indicate a significant difference, $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p > 0.0005$ compared to D-galactose alone treated group (D-gal).

Pre-treatment with 200 mg/kg of red palm oil (RPO) was showing a better effect compared to 400 mg/kg of red palm oil (RPO) group as the glutathione (GSH) level increased slightly higher than another dose of

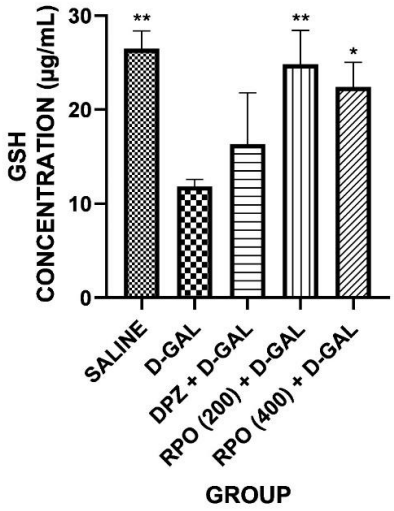

Figure 4. Effect of red palm oil (RPO) on glutathione (GSH) activity in Alzheimer's disease induced rat. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the group. After that, Tukey *post hoc* test was used for multiple comparisons. The values are mean ± S.E.M (n = eight animals per group). Star above bars indicate a significant difference, $*p < 0.05$, $**p < 0.005$ compared to D-galactose alone treated group (D-gal).

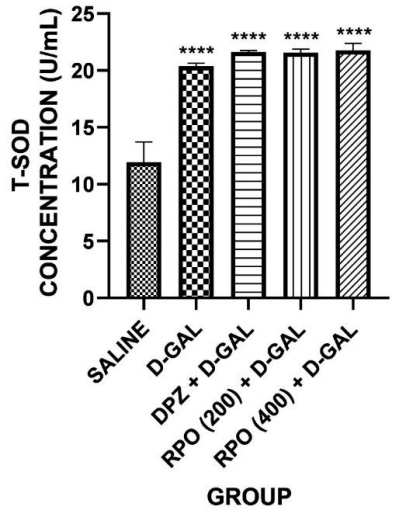

Figure 5. Effect of red palm oil (RPO) on total superoxide dismutase (T-SOD) activity in Alzheimer's disease induced rat. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the group. After that, Tukey *post hoc* test was used for multiple comparisons. The values are mean ± S.E.M (n = eight animals per group). Star above bars indicate a significant difference, $****p < 0.0001$ compared to normal control group (saline).

treatment. However, there is no significant difference between these two pre-treatments ($p>0.05$). Besides, administration of D-galactose (D-gal) had significantly caused brain aging as it showed a low concentration of GSH compared to the normal control group (saline) ($p<0.005$). Next, pre-treatment of donepezil (DPZ + D-

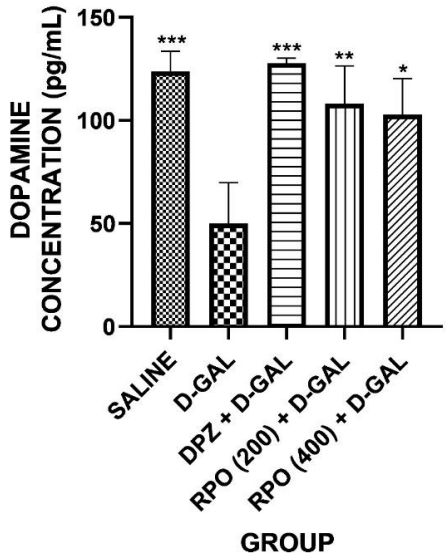

Figure 6. Effect of red palm oil (RPO) on dopamine activity in Alzheimer's disease induced rat. Three pre-treatments were given to prevent the disease which donepezil (DPZ + D-gal), 200 mg/kg of red palm oil [RPO (200) + D-gal] and 400 mg/ kg of red palm oil [RPO (400) + D-gal]. Another two groups acted as normal control group (saline) and negative control group (D-gal). Results were analyzed using Graphpad Prism software for Windows version 8.0.1. One-Way Analysis of Variance (ANOVA) test was used to evaluated the differences between the groups. After that, Tukey *post hoc* test was used for multiple comparisons. The values are mean ± S.E.M (n = eight animals per group). Star above bars indicate a significant difference, *$p < 0.01$, **$p < 0.005$, ***$p > 0.001$ compared to D-galactose alone treated group (D-gal).

gal) was not showing a significant result compared to the group of AD-induced rats (D-gal) ($p>0.05$).

Total Superoxide Dismutase (T-SOD) Level: Total superoxide dismutase (T-SOD) test showed a significant high level of SOD in all groups induced with AD, which included negative control group (D-gal), positive control group (DPZ + D-gal), treatment 1 [RPO (200) + D-gal] and treatment 2 [RPO (400) + D-gal] compared to the normal control group (saline), ($p < 0.0001$). Donepezil (DPZ + D-gal) and RPO pre-treated group have a slightly higher T-SOD concentration than D-Galactose alone treated group (D-gal). However, there is no significant difference in all AD-induced groups ($p>0.05$).

Dopamine: Figure 6 showed that dopamine level significantly depleted in the group treated with D-galactose (D-gal) only compared to the normal control group (saline) ($p<0.001$). The concentration of dopamine in pre-treatment of AD are not showing any significant difference with the normal control group (saline) ($p > 0.01$). Moreover, all three pre-treatments in preventing AD, positive control group (DPZ + D-gal), treatment 1 [RPO (200) + D-gal], and treatment 2 [RPO (400) + D-gal], significantly increase the dopamine level in the rats that had exposed to oxidative stress by consuming D-galactose ($p<0.01$). It also showed no significant difference between red palm oil (RPO) and donepezil treatment ($p >0.05$).

Discussion

Spontaneous Alternation Behavior Y-maze Test: AD can cause cognitive defects due to oxidative stress, senile plaque, and tau protein. These components are progressively interfering with the function of neurons and lead them to neuronal death. One of the Alzheimer's diseases symptoms is the decline in short-term spatial working memory. Hence, a Y-maze test was conducted to evaluate the spatial working memory of the rats after being treated with red palm oil.

Results showed no significant difference among the experimental group during the first week as the treatment merely began. During week 2, the results indicated that pre-treatment of donepezil and red palm oil (RPO) were giving effect and the inducement of AD. Meanwhile, D-galactose alone treated group (D-

gal) was significantly low compared to other pre-treatment groups. This means that the rats had the effect of reactive oxygen species (ROS) on the neurons. Thus, the percentage of spontaneous alternation was impaired. However, there is no significant difference between the negative control group (D-gal) and the normal control group (saline), which means that AD was still not being induced yet.

During week 3, which was on day 21, the group of D-galactose only (D-gal) had significantly low spontaneous alternation, indicating that AD was progressively taking place. A previous study mentioned that D-galactose consumption had decreased the spontaneous alternation percentage (20). Moreover, pre-treatment that contains an antioxidant effect can reverse the effect of D-galactose and increase the spontaneous alternation test (18). It can be concluded that antioxidants in red palm oil (RPO) can affect short-term working memory by lowering down the oxidative stress in the rats. Donepezil is a proven treatment for AD and it can improve cognitive defects in this disease. We hypothesized that this may happen due to a low dose of donepezil being given to the rat. Donepezil consumption can cause extreme weight loss, and the rats cannot withstand the treatment when their weight reaches about 85 g (21). Hence, an alternative decision was made by changing the initial dose of donepezil from 1 mg/kg to 0.25 mg/kg to ensure adequate study duration was conducted to induce the AD model.

Glutathione (GSH) Level: Glutathione (GSH) is an endogenous antioxidant that is naturally produced in the cytosol. It is one of the prime anti-oxidants that protect the brain against reactive oxygen species (ROS) and maintain the cellular membrane. The brain is the most susceptible organ towards reactive oxygen species (ROS) than other organs because of high oxygen consumption in the processing, analysis, coordination, and execution of electrical signals (22). AD is a common neurodegenerative disorder that happens worldwide, with ten million new cases every year—aging people especially in the range of 70 years old and above, easily affected with this disease due to depletion of antioxidants including glutathione (GSH) thus, leaving the cell membrane exposed to the attack of reactive oxygen species (ROS) (23).

The result of the glutathione (GSH) ELISA kit showed that red palm oil consumption as pre-treatment is capable of increasing the amount of glutathione (GSH) within the brain of the AD rat model. This high level of glutathione (GSH) is competent enough to counterattack oxidative stress by detoxifying them into a stable molecule (24). Hence, the neurons are protected against damage that causes learning and memory loss in AD. Symptom of AD, which includes impaired short-term memory, was evaluated through a Y-maze test. The result showed that treatment of red palm oil had reversed the reduction of spontaneous alternation, which correlates with this high elevation of glutathione (GSH) in both groups treated with red palm oil (RPO).

The Control group has a slightly high glutathione (GSH) concentration compared to the pre-treatment groups. This result matched other research that treatment could reverse neuronal damage but is not comparable to a healthy rat (18). D-galactose (D-gal) had induced brain aging by increasing reactive oxygen species (ROS) and thus, led to reduced glutathione (GSH) concentration. Hence there was a low amount of GSH in D-galactose alone treated group. This result was the same as a previous study that usage of D-galactose in inducing AD would result in a low amount of glutathione (GSH) (19).

Moreover, the result of donepezil pre-treatment was against other previous studies as donepezil can increase the level of glutathione in brain aging-induced rats (25). Besides, donepezil is one of the approved treatments for AD in treating the symptoms relating to impaired memory, thinking, and judgment. Thus, it can reduce the damage from reactive oxygen species (ROS) and maintain the production of glutathione (GSH) from the body. We hypothesized that this might happen due to the low dose of donepezil was given to the rat. Donepezil consumption can cause extreme weight loss, and the rats cannot withstand the treatment when their weight reaches about 85 g (21). Hence, an alternative decision was made by changing the initial dose of donepezil from 1 mg/kg to 0.25 mg/kg to ensure adequate study duration was conducted to induce the AD model.

Total Superoxide Dismutase (T-SOD) Level: Superoxide dismutase (SOD) is another type of powerful antioxidant, and it is one of the enzyme-type

of antioxidants. This antioxidant neutralizes most of the reactive oxygen species (ROS) in the tissue. When getting older, the production of antioxidants will be reduced by about 10% to 15%. Thus, aging can increase the body's susceptibility to neurodegenerative diseases such as AD.

In a recent study regarding AD, it is mentioned that there is increasing in SOD activity, which means there is an elevation of superoxide production associated with the development of AD (26). Different types of SOD enzymes will show various concentrations depending on age and gender in brain aging. In the present study, this happens due to the difference in redox states between the central nervous system and peripheral in the early stage of AD. Besides, the level of total SOD will mirror the stages of AD, which will rise during the early phase of AD and start to decrease in later stages of the disease (27). It can be concluded that the elevation of superoxide dismutase (SOD) in all three groups induced with D-galactose, which were negative control group (D-gal), positive control group (DPZ + D-gal), treatment 1 group [RPO (200) + D-gal] and treatment 2 group [RPO (400) + D-gal] happened due to early stage of AD ($p < 0.0001$). This chemical had been induced for only three weeks, and a high level of superoxide dismutase is a way of the body fighting the excessive reactive oxygen species (ROS). Moreover, a previous study showed that total superoxide dismutase increased along with neurofibrillary tangle (NFT) (28). We hypothesized that the D-galactose consumption had slowly caused the formation of Alzheimer's pathological hallmark in this study models.

D-galactose alone treated group still managed to have signs and symptoms of AD since there was a significant depletion of glutathione (GSH). This can be observed in the spontaneous alternation Y-maze test, showing significantly low spontaneous activity than the regular control group ($p < 0.005$). Donepezil and red palm oil (RPO) pre-treated groups have a slightly high concentration of T-SOD compared to the D-galactose group only. However, there is no significant difference in these Alzheimer's induced groups ($p > 0.05$). We hypothesized that this difference in superoxide dismutase (SOD) elevation might occur due to pre-treatment of red palm oil (RPO). Because of its antioxidant properties, it is capable of reducing reactive oxygen species (ROS) and increasing the production of endogenous antioxidants (Loganathan et al., n.d.). Furthermore, no recent study proves that donepezil contains any antioxidant properties; however, Superoxide dismutase (SOD) may increase as donepezil had acted to prevent the damage with D-galactose consumption (26, 27).

Dopamine: A recent study has shown that AD is associated with an impaired dopaminergic system. This system undergoes several neuropathological changes during aging, such as impairment of neurotransmission, progressive synaptic disarrangement, and cell loss. These changes have led to the formation of extracellular amyloid protein and intracellular fibrillary tangles. Dopamine mainly functions in synaptic plasticity mechanisms; thus, impairment of the dopaminergic system can cause a decline in cognitive functioning (15, 29).

A previous study had stated that D-galactose (D-gal) could deteriorate the hippocampus and diminish dopamine concentration in the brain (3, 30). Since dopamine plays a role in brain function, this reduction has impaired cognitive function and led to AD progression. The result on spontaneous alternation Y-maze test for the negative control group (D-gal) also corresponds with depletion of dopamine concentration ($p < 0.005$).

Donepezil is believed can increase the level of dopamine hippocampus area (19). Hence, normal function in transmitting the synapse can occur and prevent the development of cognitive impairment. Besides, both doses of red palm oil are capable to increase dopamine in the brain due to their antioxidant properties. This exogenous antioxidant detoxified the reactive oxygen species (ROS) supplied by the induction of D-galactose and prevented damage to the dopaminergic system.

High concentration of dopamine in treatment 1 group [RPO (200) + D-gal] and treatment 2 group [RPO (400) + D-gal] had shown good short-term spatial working memory through the Y-maze evaluation test. These results were correlated with each other and explain the effect of red palm oil in maintaining the cognitive function of the rats ($p < 0.05$). However, the Tukey *post hoc* test showed no significant difference between these two treatments ($p > 0.05$). Besides, this result showed that red palm oil's

effect is nearly the same as a clinically approved treatment for AD.

Conclusion

In conclusion, this study reaffirmed the neuroprotective agents of red palm oil (RPO) from *Elaeis guineensis* species on oxidative stress in preventing AD-induced rats. The results showed that it could elevate the concentration of antioxidants, glutathione (GSH), and superoxide dismutase (SOD) when consumed along with D-galactose. This increased antioxidant level reversed D-galactose's effect and showed no significant difference with the healthy rats without AD inducement. Besides, pre-treatment of red palm oil (RPO) also has maintained the production of donepezil in AD rats' model. Hence, the cognitive function that normally declines in AD can be sustained. Spontaneous alternation Y-maze test also showed better spatial working memory in rats pre-treated with red palm oil (RPO) compared to AD rats' model. Hence, it is proven that red palm oil effectively attenuates the oxidative stress in rats induced with AD. This is due to red palm oil's antioxidant properties (RPO), which play a vital role in preventing oxidative stress. Further investigation needs to be done for clinical studies to enhance the understanding of red palm oil (RPO) properties in humans as a neuroprotective agent.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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