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Brief Report

Red Dragon Fruit Extract (*Hylocereus polyrhizus*) Restores Learning Ability and Memory on Test Animals Post Lead Exposure: Experimental Study on Test Animals

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The aim of this study was to analyze whether red dragon fruit (*Hylocereus polyrhizus*) administration affect learning ability and memory after lead exposure. A randomized control-group pretest-posttest was conducted with 24 mice (*Mus musculus*), which was divided through a random assignment into four groups: G1, G2, G3, and G4. Group G1 received 1.16 mg/10 g BW lead acetate. Group G2, G3, and G4 received 1.16 mg/10 g BW lead acetate and red dragon fruit extract 250 mg/kg BW (G2), 500 mg/kg BW (G3), and 1000 mg/kg BW (G4). Results revealed that some groups that has been given red dragon fruit extract (group G2, G3, and G4) showed significant improvement of latency time, frequency, and combined scoring value of latency time and frequency in posttest by using Morris Water Maze compared to the control group (p = .001; α = .05). The improvement of group G3 and G4 was better than group G2. These finding indicates that administration of red dragon fruit extract have a protective effect and improves learning ability and memory after lead exposure with 500-1000 mg/kg BW as the best dose.

Keywords: red dragon fruit extract, lead acetate, learning ability, memory

Tujuan studi ini adalah menganalisis efek pemberian ekstrak buah naga merah (*Hylocereus polyrhizus*) pada kemampuan belajar dan mengingat pasca-paparan timbal. Rancangan randomized control-group pretest-posttest dilakukan terhadap 24 ekor mencit (*Mus musculus*) yang melalui random assignment terbagi kedalam empat kelompok: kelompok K1, K2, K3, dan K4. Kelompok K1 diberikan 1.16 mg/10 g BB timbal asetat. Kelompok K2, K3, dan K4 diberikan timbal asetat 1.16 mg/10 g BB dan ekstrak buah naga merah masing-masing dengan dosis 250 mg/kgBB (K2), 500 mg/kgBB (K3), dan 1000 mg/kg BB (K4). Hasil tes ANOVA menunjukkan bahwa pada kelompok yang diberikan ekstrak buah naga merah (K2, K3, dan K4) terjadi peningkatan waktu laten, frekuensi dan angka skoring gabungan dengan menggunakan Morris Water Maze dibandingkan kelompok K1 (p = .001; α = .05). Peningkatan pada kelompok K3 dan K4 lebih baik daripada kelompok K2. Simpulan penelitian ini adalah pemberian ekstrak buah naga merah memiliki efek proteksi dan dapat memperbaiki kemampuan belajar dan memori pasca-paparan timbal dan dosis 500-1000 mg/kg BB memiliki potensi lebih baik dalam memulihkan kemampuan belajar dan memori pasca-paparan timbal.

Kata kunci: ekstrak buah naga merah, timbal asetat, kemampuan belajar, ingatan

Major developments in the mining and industry sectors have caused an increased number of exploitation of earth's resources of metal, which includes lead. As a result, humans become more susceptible to lead exposure. Lead exposure can affect humans through a number of routes such as inhalation, ingestion, and vertical transmission from mother to child through the placenta. Accumulation of lead in humans can cause oxidative stress through the production of Reactive Oxygen Species (ROS), which ultimately results in

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cell death (Gurer & Ercal, 2000). One part of the brain that could be damaged by lead is the hippocampus.

Lead disturbs the growth of new cells in hippocampal dentate gyrus area that plays an important role in the process of neurogenesis (Gilbert, Kelly, Samsam, & Goodman, 2005). Research conducted by Xu, Yan, Yang,Tong, Zou, & Tian (2009) showed disturbances in long-term and short-term memory in young rats exposed to lead before and after birth. In addition, there is also damage to the hippocampus ultrastructure (Xu et al.). Exposure to low doses of lead can have a significant effect on gene expression in the frontal cortex and hippocampus on the long-term cognitive abilities (Schneider, Mettil, & Anderson, 2013)

The red dragon fruit (*Hylocereus polyrhizus*) contains a high antioxidant content compared to other tropical fruits (Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). The antioxidant substances are vitamin C, phytoalbumin, and betalanin (Choo & Yong, 2011). Betalanin consists of *betanin, isobetanin, phyllocactin, isophyllocactin*, and *hylocerenin* (Moreno et al.). Vitamin C acts as an antioxidant because it can be oxidized by various free radicals such as ROS and non-radical reactive compounds. Vitamin C can also bind with *alpha tocopheroxyl radical* resulting from the interaction between exogenous radicals and *alpha tocopherol* in a *low-density lipoprotein* (LDL) (Padayatty et al., 2003). Betalanin contains *phenolic* and acyclic amine group, which are perfect electron donors that can stabilize free radicals (Moreno et al.).

The specific phenolic component of the red dragon fruit is *hydroxycinnamates* (Mahattanatawee et al., 2006). The role of red dragon fruit as an antioxidant was expected to reduce free radicals that can cause oxidative stress, thereby protecting and repairing memory and learning ability. The aim of this study was to analyze the effects of providing red dragon fruit (*Hylocereus polyrhizus*) extract on learning ability and memory after exposure to lead.

Method

The study was conducted with a randomized control-group pretest-posttest design. Subjects were 24 male white mice (*Mus musculus*), aged 2 months old, weighing 28±3 grams. Prior to the study, an acclimatization process was done for one week to adapt to a new environment with a 12 hours per day light-dark cycle. Food and drink were provided ad libitum.

Subjects were assigned randomly into four groups. Group G1 was induced to lead acetate with a dosage of 1.16 mg/10 g BB. Groups G2, G3, and G4 were induced to lead acetate with a dosage of 1.16 mg/10 g BB and red dragon fruit (*Hylocereus polyrhizus*) extract with a dosage of 250 mg/kg BW (G2), 500 mg/kg BW (G3), and 1000 mg/kg BW (G4).

Next, the animals attempted a memory consolidation process for five days and a memory test for one day. The consolidation and test of memory were measured using the Morris Water Maze. Afterwards, subjects received treatment according to their group for 21 days. After treatment, the animals performed memory consolidation and examination posttest. The Morris Water Maze consisted of a circular pool with a diameter of 122 cm and a height of 47 cm. A cylindrical podium with a diameter of 7 cm and height of 1cm sat underwater. The base and edge of the pool as well as the podium were colored black. The pool was filled with water until 20 cm. Three indicators were placed in the west, east, and north quadrant. The examiner observed in the south quadrant (Sharma, 2009).

Memory consolidation was done in a five day period with four times training in one day, so the animal had 20 training attempts. There was a period of 10-15 seconds break between trainings. The animal was placed at the starting point in the pool. The animal was expected to find a podium beneath the water surface in one minute. If it failed, it will be assisted and guided to find the podium. Then, the animal will be placed on the podium for 15 seconds. The starting point varies with each training. The pattern of each starting point was described as follows (Sharma, 2009).

The result of memory consolidation indicated latency period. Latency period is the time needed by the animal to find the podium. One day after the last memory consolidation training, the animal attempts the memory test. The podium was taken out of the pool. The animal was placed in the water and was let free to swim for 60 seconds. The animal's activity during the memory test was recorded with a video recorder. The recording showed how many times the

Table 1
Starting Point Pattern on Memory Consolidation and Test

Day	Training 1	Training 2	Training 3	Training 4
1	N	E	SE	NW
2	SE	N	NW	E
3	NW	SE	E	N
4	E	NW	N	SE
5	N	SE	E	NW
Memory test	NE			

Note. N=North, E=East, S=South, W=West, SE=South East, NW=North West, NE= North East animal tried to go across the podium area.

The red dragon fruit extract was made with a research procedure done by Swarup et al. (2010). The pulp of the fruit was extracted with water on a 1:2 ratio (1 kg of dragon fruit pulp with 2 L water). Next, the pulp of the dragon fruit was extracted until an extract in the form of a gel was produced (Swarup et al).

Result of the consolidation and memory test were shown as latency period and frequency of going across the podium. Data was displayed in score values. For latency period, scores are as follows. There were three different scoring values which are latency period scoring, scoring value of the frequency of going across podium, and combined scoring value of latency period and frequency. Data was analysed using a one way ANOVA and LS (SPSS 17 inc.) to determine whether there are any significant differences among the scores.

Results

Test Animals

The weight of the test animals was measured each week during treatment. There were a total of four weight measurements (in grams) during the study. Data were processed to determine the variant homogeneity among the groups. Results showed that the weight of test animals were homogenous. Data were further processed using a one-way ANOVA. The results were displayed in Figure 1.

Figure 1 showed that there was no significant difference among animals in group G1, G2, G3, and G4 on each measurement from week 1 until week 4 ($p > .05$). Comparison of Combined Scoring Values of Pre-test and Post-test Among Groups G1, G2, G3, and G4

Table 2
Scoring Value of Latency Period on Memory Consolidation

Latency period	Scoring value	Latency period	Scoring value
58-60	1	28-30	11
55-57	2	25-27	12
52-54	3	22-24	13
49-51	4	19-21	14
46-48	5	16-18	15
43-45	6	13-15	16
40-42	7	10-12	17
37-39	8	7-9	18
34-36	9	4-6	19
31-33	10	1-3	20

Combined scoring value is a combination of two scoring values, which were scoring values of latency period and frequency. On pre-test data, combined scoring value on each group was compared to ensure that there are no significant differences among each group. Results were shown in Figure 2.

Figure 2 showed that there were no significant differences among the combined pretest scoring values of G1, G2, G3, and G4 ($p > .05$). In the post-test data, combined scoring value of groups G1, G2, G, and G4 were compared with a control group. The control group showed pre-test data before treatment was given. Results were shown in Figure 3.

Figure 3 showed that the combined score of group G1 decreased significantly compared to control, G2, G3, and G4 groups ($p < .05$). Combined post-test scores of groups G2 and G3 showed an increase compared to the control group, but not significant ($p > .05$). Combined post-test score of G4 was found to have a significant increase compared to the control group ($p < .05$).

Comparison of Combined Score Values among G2, G3, and G4 groups. To investigate the effects of providing red dragon fruit extracts with differing dosages towards learning ability and memory, combined scores of G2, G3, and G4 were compared. Group G2 was induced with 250 mg/kg BW red dragon fruit extract, group G3 was induced with 500 mg/kg BW, and group G4 with 1000 mg/kg BW dosages. Results are shown on Figure 4.

Figure 4 showed that there were no significant difference among groups G2 and G3, as well as groups G3 and G4 ($p > .05$). Combined post-test score between G2 and G4, however, showed a significant difference ($p < .05$).

Discussion

Measurement of learning ability and memory using Morris Water Maze were influenced by numerous factors that could interrupt measurement results. Such factors may originate from the test animal such as sex, species, strain, age, nutrition, and stress. Those factors may influence test results (D’Hooge & De Deyn, 2001). In this study, the weight of test animals were homogenous. This is in accordance with the prerequisite of using the Morris Water Maze as a measurement tool. Heavier animals are slower to reach the podium and are often easily fatigued. A homogenous increase in test animals’ weight will thus maintain the validity and reliability of test results.

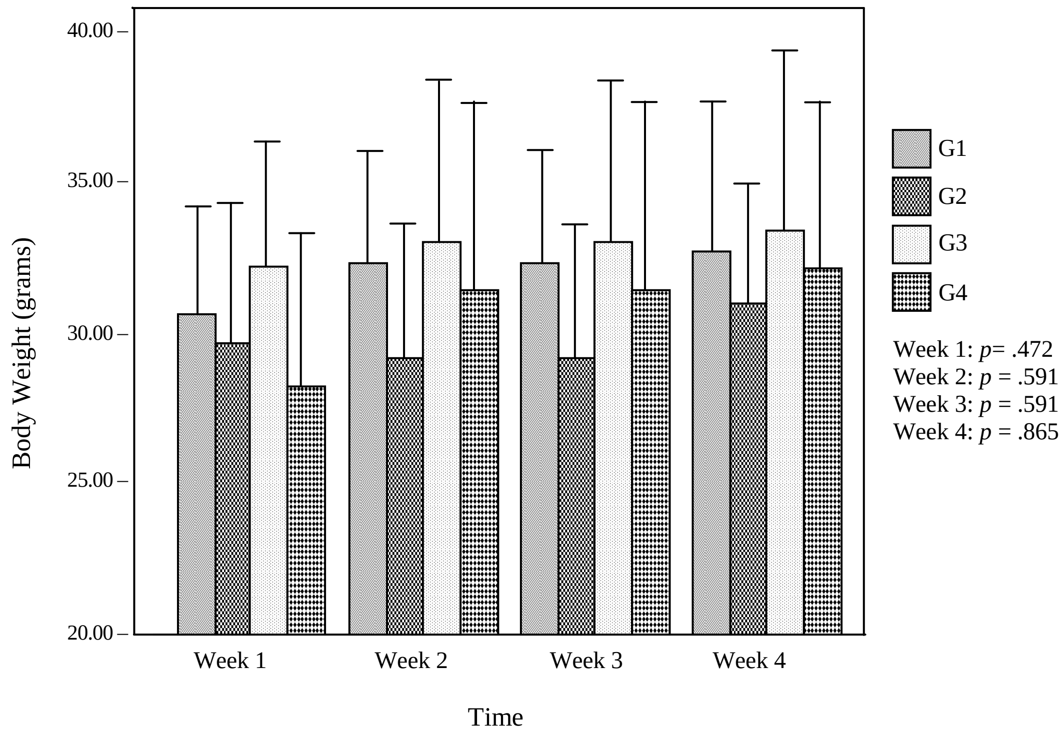

Figure 1. Weight of test animals.

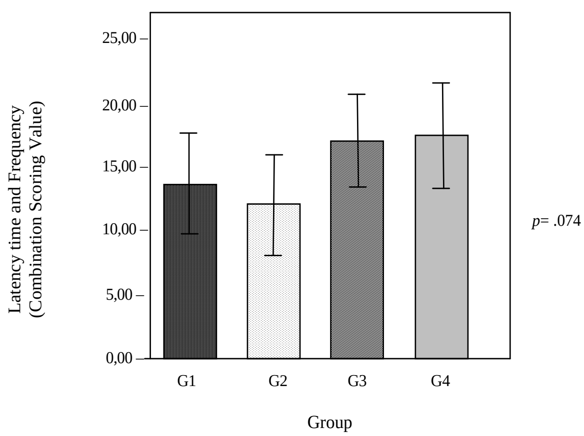

Figure 2. Comparison of combined pre-test and post-test scoring values of G1, G2, G3, and G4.

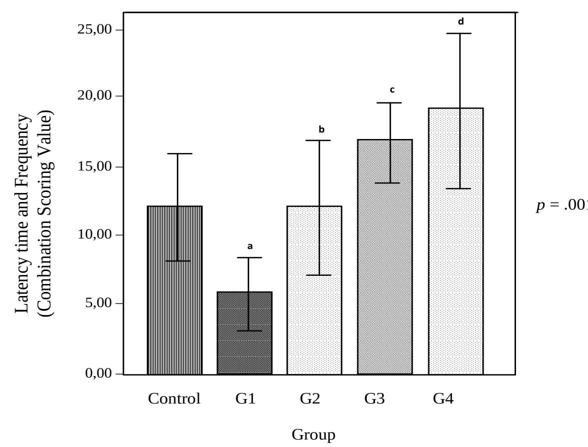

Figure 3. Comparison of combined post-test scores among G1, G2, G3, G4, and the control.

Superscript a indicated a significant difference ($p < .05$) compared to control G2, G3, and G4 groups
Superscript b indicated a significant difference ($p < .05$) compared to G1 and G4 groups
Superscript c indicated a significant difference ($p < .05$) compared to G1 group
Superscript d indicated a significant difference ($p < .05$) compared to G1 and G2 groups

Test results showed a significant difference between combined post-test score of group G1 and groups G2, G3, and G4. Group G1 showed a significant decrease in combined post-test scores compared to groups G2, G3, and G4. Memory of group G1 that was only induced with lead acetate without being given red dragon fruit extract.

Lead is an element found naturally in small numbers in the earth's crust. Lead is a free element. In nature, lead is found in the form of lead acetate (EPA, 2013). Humans can be exposed to lead through inhalation, ingestion, and vertical transmission from mother to child through the placenta (Tait, Vora, James, Fitzgerald, & Pester, 2002). After being absorbed, lead will enter the blood flow and will be lodged in the bone. The

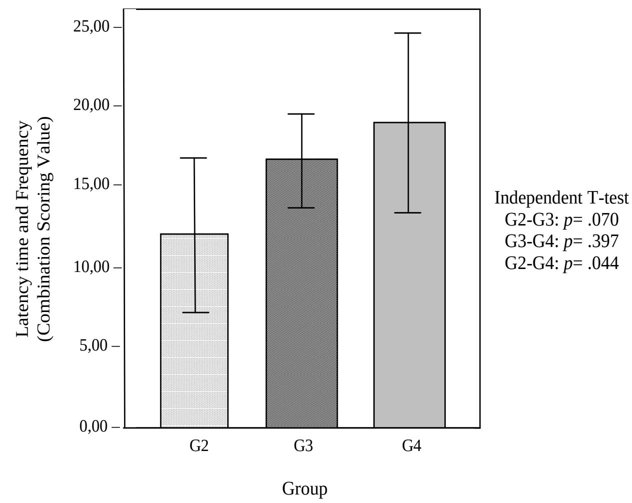

Figure 4. Comparison of combined post-test score values of groups G2, G3, and G4.

Superscript a indicated a significant difference ($p < .05$) compared to group G4

human bone is a dynamic reservoir for lead. Lead will form a bone structure called hydroxyapatite crystal like calcium, and will be transferred to the bone matrix creating hydroxyapatite. More than 90% of lead in an adult human body is stored in the bone. The storage of blood in the bone will be distributed back to the blood during the process of bone turnover. This turnover process increases in pregnancy and breastfeeding period (CDC, 2010).

Lead can reduce learning ability and memory through an oxidative stress proces. Oxidative stress caused by lead targets the brain, particularly neuron cells that are actively developing to form synapses between neurons. The newly developed cells are susceptible to the negative effects of lead. The negative effect of lead to neuron is cell death through apoptosis, disrupted intraneuron regulation, disrupted neurotransmission, and deposit on glia (Lidsky, 2003).

The brain is one of the targets of neurotoxicity caused by lead. The brain is protected by the blood brain barrier consisting of lipid. The movement of impulse in the brain involves a protein component in the form of synapses and enzymes particularly in the hippocampus area where Long Term Potentiation (LTP) occurs, which is influential in changing short term memory into long term memory. DNA expression will cause neurogenesis and synaptogenesis. The lipid, protein, and DNA components will experience destruction as a consequence of oxidative stress caused by lead (CDC, 2010).

In the learning process and memory, impulse propagate through three pathways, which are the Performant Pathway, Mossy Fiber Pathway, and Schaffer Collateral or Associational Commisural Pathway that needs optimal synaptic function especially in the Girus Dentata area, CA1, CA2, and CA3 (MRC, 2010). The negative effect of lead on protein causes the destruction of a protein component in the presynaptic area which is the brain-derived neurotrophic factor (BDNF) and the decrease of cAMP response element binding protein (CREB) expression in the post-synapse. The two components are essential in the process of forming new synapses (Vaynman, Ying, & Gomez-Pinilla, 2004)

Lead can cause oxidative stress through two mechanisms. The first is by stimulating ferro ion that will initiate lipid peroxidation of membrane and inhibiting the performance of delta-aminolevulinic acid dehydratase enzyme (δ-ALAD). Lipid peroxidation can cause Reactive Oxygen Species (ROS). The inhibition of δ-ALAD enzyme will cause the accumulation of delta-aminolevulenic acid (δ-ALA). δ-ALA that binds with oxy-haemoglobin will form δ-ALA-OxyHb complex. δ-ALA and δ-ALA-OxyHb complex can experience auto-oxidation and create ROS. ROS created from the process of lipid peroxidation, accumulation of δ-ALA and δ-ALA-OxyHb complex will create oxidative stress on lipid, protein, and DNA (Ahamed & Siddiqui, 2007).

Lead also decreases antioxidant defence system that causes oxidative stress (Lee, Lim, Song, Boo, & Jacobs, 2005). Reduction of short and long term memory occurred because lead is able to bind glutamate receptor, *N-methyl-D-aspartate receptors* (NMDARs). Glutamate plays an important role in brain development, and as such the binding of lead on glutamate receptor will disrupt the expression of the receptor even though affinity-wise it is not affected (Xu et al., 2009). This mechanism causes a decrease in learning ability and memory due to lead exposure.

Research results showed an increase in learning ability and memory in groups G2, G3, and G4. Combined post-test scores of G2, G3, and G4 increased significantly compared to G1. The three groups (G2, G3, and G4) received lead acetate induction and red dragon fruit extract whereas G1 only received lead acetate without red dragon fruit extract. The red dragon fruit extract can increase learning ability and memory of test animals following lead exposure due to the high concentration of antioxidants.

The antioxidant content of red dragon fruit extract which are vitamin C, phytoalbumin, and betalanin can increase antioxidant defense mechanism towards oxidative stress (Choo & Yong, 2011). Betalanin consists of betasianin, *betanin*, *isobetanin*, *phyllocactin*, *isophyllocactin*, and *hylocerenin*. Betalanin has phenolic and acyclic amine group which are the perfect electron donor to stabilize free radicals (Moreno et al., 2008). The specific phenolic component of red dragon fruit is *hydroxycinnamates* (Mahattanatawee et al., 2006). Only lipophyllic antioxidants can prevent lipid peroxidetions such as tocopherol (TocH) found in cell membrane. Vitamin C cannot directly prevent lipid peroxidation. Vitamin C inactivates tocopheryl radical (TocH) which is a free radical produced from a reaction between TocH, L· (a lipid radical produced by the removal of a hydrogen atom/H from a lipid molecule), and LOO (a peroxy radical formed by the reaction of the lipid radical with molecular oxygen/O_2) (Murray et al., 2003).

Research results showed that there is no significant difference between combined scores of group G2 that received an induction of 250 mg/kg BB red dragon fruit extract and scores of group G3 that received an induction of 500 mg/kg BB red dragon fruit extract. These results are in line with the findings by Swarup et al. (2010). Their study showed that there is no meaningful difference between dosages of red dragon fruit extracts 250 mg/kg BB and 500 mg/kg BB with an antioxidant mechanism similar to the present study (Swarup et al).

The best improvement in learning ability and memory was found in group G4. However, the increase was not significantly different compared to group G3. This is because the effective dosage of red dragon fruit to increase learning ability and memory is 500-1000 mg/kg BB. The largest concentration of antioxidant in red dragon fruit extract is vitamin C, with an amount of 32.65 ± 1.59 mg/100 gram extract of red dragon fruit (Choo & Yong, 2011). Vitamin C is a vitamin that dissolves in water (Murray, 2003). Thus, it is possible that when the human body does not need vitamin C, it will be excreted through urine.

A difference of 500-1000 mg/kg BB of red dragon fruit extract will not give a significantly different effect. However, the present study did not directly examine oxidative stress markers and red dragon fruit extract levels. It is concluded that the provision of red dragon fruit extract offers protective and restorative effects on learning ability and memory following lead exposure and a dosage of 500-1000 mg/kg BB is best to restore learning ability and memory following lead exposure. Further research is needed on the cellular changes of the hippocampus and impulse pathways that are negatively affected by lead to further investigate the mechanisms underlying the process of destruction and reconstruction.

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