# Steamed Watermelon (*Citrullus lanatus* Thunb.) Juice Improves Spatial Memory in Dementia Rat Model

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Abstract: Watermelon (*Citrullus lanatus* Thunb.) is one of the fruits that containing lycopene. Lycopene is able to reduce oxidative damage that causes dementia. This research was aimed to determine the activity of steamed watermelon juice in improving the spatial memory in dementia rats model induced by Electroconvulsive Shock (ECS). The animal test was divided into 6 groups, namely normal control group, negative control group, positive control group (vitamin E, 20.7 mg/kg BW), and 3 treatment groups that received steamed watermelon juice with different dose, i.e 0.92 g/kg BW,1.85 g/kg BW, and 3.7 g/kg BW. All groups were induced with ECS, except for the normal control group. The spatial memory was measured with the Radial Arm Maze (RAM) test and the observed results were analyzed with ANOVA and a post-hoc Tukey's HSD test. The ANOVA test results showed significant differences between all treatment groups. Meanwhile, the post-hoc Tukey's HSD test at 95% confidence level resulted in a significant difference between the the treatment groups with the doses of 0.92 g/kg BW and 1.85 g/kg BW and the negative control group. However, this test indicated an insignificant difference between the positive control group and the normal control group. This finding confirms that the administration of steamed watermelon juice at doses of 0.92 g/kg BW and 1.85 g/kg BW improves the spatial memory in a rat mode of dementia comparable to the positive control. The treatment groups receiving these two doses showed corresponding results, meaning that both doses of steamed watermelon juice have an equivalent spatial memory enhancement activity.



## 1 INTRODUCTION

Dementia is a syndrome of decreased intellectual function that is severe enough to interfere with social and economic activities in daily life, and is, usually followed by behavioral changes not attributable to delirium or major psychiatric disorders. The prevalence of dementia increases rapidly with life expectancy. Even though there has been no national research data regarding the prevalence of dementia the growing number of elderly population predictably increases the incidence of dementia in Indonesia. The Stroke Registry Indonesia 2013 support this assumption. It reports that stroke become more prevalent at a younger age, and 60.59% of stroke patients experience impaired cognition after returning home from hospital treatment. This registry also explains that the stroke risk factors in the country are fairly high (Perhimpunan Dokter Spesialis Saraf Indonesia, 2015).

Oxidative stress is significantly involved in the pathology of dementia even at the early stage of the disease (Mao, 2013). The brain and nerve cells are susceptible to damages by free radicals because the membrane lipids contain many side chains of polyunsaturated fatty acids. Oxidative stress also depends on other factors, namely the quantity of brain oxygen consumption, iron and other metals transition that responsible for the formation of Reactive Oxygen Species (ROS), and antioxidants in the brain. Antioxidants have been widely studied in relation to neurogenerative diseases (Rao *et al.,* 2003. Research in Parkinson's patients and vascular dementia indicates low serum lycopene levels (Foy, *et al.*, 1999), which, according to a stroke prevention study in Austria, are associated with a high risk of microangiopathy (Schmidt *et al.*, 1997). In elderly patients, a high level of lycopene is positively connected to their functional capacity, as indicated by their ability to perform self-care (Snowdon *et al.*, 1996). Given the importance of



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attention to neurogenerative diseases, studies related to the effectiveness of lycopene in the management of dementia are necessary.

Lycopene is fat-soluble antioxidant synthesized by many plants and microorganisms. It is responsible for giving the red color in various vegetables and fruits. Earlier, only tomato and its products were considered as the potential sources of lycopene, but nowadays there are scientifically proven facts that watermelon also contains an appreciable amount of *cis*-configured lycopene. Therefore, people gradually shift their preference towards watermelon and its allied products to deal with their health concerns. Nevertheless, the quantity of lycopene varies depending upon the variety and growing conditions (Fish and Davis, 2003). Overall, in the cells, lycopene is present in the form of crystalline with a range of 2.30-7.20 mg/100 g fresh weight basis (Huh *et al.*, 2008; Artes-Henandez *et al.*, 2010). In general, the human body only absorbs 10-30% of fresh lycopene in fresh lycopene in fruits or vegetables. Lycopene is more effectively absorbed when consumed from processed foods, such as sauce and juice. The increased absorption of lycopene in tomato products is due to the presence of cis-isomers of lycopene. Lycopene naturally appears as 'trans', which is thermodynamically stable, soluble in non-polar solvents, and identifiable at a wavelength of 446-506 nm. Lighting and heating can potentially change the isomer form 'trans' to 'cis' (Rao & Rao, 2007). Maulida & Zulkarnaen (2010) conclude that the optimum temperature for watermelon to release lycopene from its cell structure is $70^0$C.

This research aimed to determine the activity of steamed watermelon juice in improving the spatial memory in a rat model of Electroconvulsive Shock (ECS)-induced dementia and to determine the effective dose to increas spatial memory. Steamed watermelon juice was expected to be able to improve spatial memory and function as a natural treatment, which would be potentially developed into phytopharmaceutical preparations, as well as a reference for further research to support the development of science.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The research apparatus included an electroconvulsive meter Model EC 02 from Orchid Scientific and Innovative India Pvt. Ltd. (B 59, MIDC, Ambad, Nashik-422010 India) and a radial arm maze designed with eight equidistantly spaced arm (53.5 cm x 12.5 cm) extending from a 24 cm diameter central platform elevated 11.5 of the floor.

The fresh watermelons (*Citrullus lanatus* Thunb.) were obtained from Cipanas, West Java and determined at LIPI Biological Research Center, Bogor from the H*erbarium Bogoriense* collection, Vitamin E (*tocopherol*) was acquired from PT. Cortico Mulia Sejahtera. The test animals were white male rats (*Rattus norvegicus*) strain *Sprague Dawley* aged 2-3 months and weighed ± 200 gram.

### 2.2 Methods

#### 2.2.1 Identification of Watermelon

The watermelons were taxonomically identified from the Herbarium Bogoriense collection at LIPI Biological Research Center, Bogor.

#### 2.2.2 Preparation of Steamed Watermelon Juice

Around 100 grams of watermelons were steamed for 5 minutes at a temperature of $70^0$C and the temperature was measured with a thermometer (Maulida & Zulkarnaen, 2010). The steamed watermelon was drained and the juice was extracted using a juicer machine.

#### 2.2.3 Phytochemical Screening

The steamed watermelon juice was tested for the presence of alkaloids, flavonoids, steroids, tannins, triterpenoids, and saponins.

#### 2.2.4 Treatment of Test Animal

This research is approved by the Ethics Committee of Health Research, Faculty of Medicine, University of Indonesia No. 278/UN2. F1/ETHICS/2017.

A total of 30 white male Sparague Dawley rats (*Rattus norvegicus)* aged 2-3 months old and weighed with ± 200 g was divided into six (6) groups, each consisting of five (5) rats. These six groups were: Group 1 as normal control, Group 2 as negative control, Group 3 as positive control, Group 4 as a treatment group receiving 0.92 g/kg BW of steamed watermelon juice, Group 5 as a treatment group receiving 1.85 g/kg BW of steamed watermelon juice, and Group 6 as a treatment group receiving 3.7 g/kg BW of steamed watermelon juice.

Table 1: The phytochemical screening results of steamed watermelon juice.

| Compounds | Results |
|---|---|
| Alkaloids | + |
| Flavonoids | - |
| Tannins | - |
| Steroids and Triterpenoids | + |
| Saponins | + |

The animals were acclimatized for at least five (5) days to the laboratory conditions before the behavioral experiments. They were placed in a natural light/dark cycle (12 h each) and given free access to standard food and water. From the second to the fourth day of the acclimatization, they were trained in the maze environment. After all group of rats fasted for 12 hours, they were subjected to preliminary radial arm maze test for 12 days. This test was called the Radial Arm Maze Test I (RAM I). The rats were placed on the central platform and the food (pellets) were hidden in a container at the end of the arm. The rat performance represented the ratio of the number of reward eaten pellets to the total number of arms entered in 10 minutes. There were a total of eight (8) arms in the maze.

After the RAM I was comleted, Groups 2, 3, 4, 5, and 6 received ECS with a magnitude of 112 mA/150 V for 1 sec/day. Afterward, the experimental animals were rested for 10 minutes. Then, they were placed in the maze again and re-tested to prove that the ECS induced a dementia model. The ECS was performoed for eight (8) days.

The positive control group (Group 3) received Vitamin E at a dose of 20.7 mg/kg BW, Groups 4, 5, and 6 were given steamed watermelon juice at different doses, namely 0.92 g/kg BW, 1.85 g/kg BW and 3.7 g/kg BW, respectively. The steamed watermelon juice and vitamin E were administered orally once a day every morning for 14 days.

The next stage was a post-test, i.e.,a radial arm maze test for 12 consecutive days, referred to as RAM II. Before the post-test the rats fasted for 12 hours.The performance of the test rats in the RAM assay was calculated with the formula (1) (Sari & Ar Rochman, 2015):

$$\text{Performance (\%)} = \frac{\text{(Number= of eaten reward (food))/}}{\text{(Number of arms entered by the rats)}} \text{x}100\% \qquad (1)$$

Table 2: The observation and assessment results of the performance (%) of the experimental rat groups in RAM I.

| Day | The Performance of the Groups (in %) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 23.3 | 5 | 3.3 | 20 | 0 | 10.6 |
| 2 | 0 | 26.6 | 23.3 | 32.5 | 9.8 | 5.3 |
| 3 | 68 | 23.1 | 57.6 | 58.6 | 43.3 | 76 |
| 4 | 20 | 53.8 | 28.3 | 37.5 | 34.4 | 39.2 |
| 5 | 25 | 73.8 | 74.2 | 68 | 61.1 | 100 |
| 6 | 20 | 64.2 | 35 | 63.6 | 73 | 37.5 |
| 7 | 7.3 | 46.2 | 74.2 | 60 | 55 | 57.5 |
| 8 | 20 | 56.6 | 40 | 46 | 65.8 | 57.1 |
| 9 | 20 | 56.6 | 40 | 46 | 65.8 | 53.1 |
| 10 | 79.6 | 33.3 | 89.2 | 36.6 | 100 | 68 |
| 11 | 100 | 97.1 | 100 | 42 | 87.1 | 76.6 |
| 12 | 100 | 80 | 96 | 40 | 90 | 80 |

### 2.2.5 Data Analysis

The mean percentage of the performance of the experimental groups in RAM II was tested for normality using the Shapiro-Wilk test and homogeneity using the Levene's test. Afterward, the data were analyzed with one way ANOVA at 95 % significance level ($\alpha = 0.05$). If the results showed a statistically significant difference, then the data analysis was continued with the Tukey test (Priyatno, 2012).

## 3 RESULTS AND DISCUSSION

The phytochemical screening showed that steamed watermelon juice was positive for alkaloids, terpenoids, steroids and saponins (Table 1).

Before the induction of dementia with ECS, all groups of experimental animals were subjected to the radial arm maze test I; henceforth referred to as RAM I, this test identified the basic memory of each rat and the whole six groups, and affirmed that all of them had the same condition before the treatment. The results of RAM I are described in Table 2. The entire groups showed remarkable spatial memory every day during the 12 days observation. Spatial learning and memory help animals find locations that provide, among other things, food and safety, and are thereby crucial for survival (Dogru *et al.*, 2003).

The performance of each test group in selecting the arms and feeding in RAM I showed an increase every day. Even though Group 1 had lowest average score

Table 3: The performance (%) of the experimental rat groups in the RAM test after the induction of ECS.

| Day | The Performance of the Groups (in%) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 50.3 | 24 | 0 | 0 | 59.3 | 20 |
| 2 | 54 | 40 | 20 | 0 | 30 | 55 |
| 3 | 60.3 | 37.3 | 0 | 0 | 46.3 | 53.3 |
| 4 | 81.5 | 52 | 0 | 0 | 20 | 18.3 |
| 5 | 94 | 14 | 16.6 | 10 | 6.6 | 40 |
| 6 | 85.6 | 20 | 6.6 | 0 | 26.6 | 0 |
| 7 | 87 | 80 | 12 | 0 | 50 | 10 |
| 8 | 86.6 | 55 | 35 | 0 | 40.6 | 20 |

of performance among all the test groups (38.3%), it scored 100% on Day 11 and 12. Meanwhile, Group 5 had the highest average (57.1%), followed by Group 3 and 6 (55%), Group 2 (51.4%), and Group 4 (45.8%).

After the 12-day observation in RAM I, the animal model of dementia was prepared. Except for the normal control group, all of the experimental groups were given ECS with an intensity of 150 V/112mA for 1 sec/day for eight (8) days. Each rat was electrocuted at the top of the ear and jammed until the electricity caused muscle spasms and stiffness. After rested for 10 minutes, the rats were tested in the maze to prove that the group had already developed dementia. The observation results are summarized in Table 3.

The results of the RAM assays after the ECS showed a decrease in the percentage of feeding and arm entries. When compared with normal controls (no ECS), all of the treatment groups showed low performances. Groups 4 even failed seven times (0%) and it was the lowest performance of all test groups. These results are the effect of ECS-induced memory degradation. They indicate poor working and reference memory capabilities, as well as low learning abilities in rats. During the maze test, the rats behaved as if in confusion and remained silent in the maze. They moved slowly and did not recognize the food placed at the end of the arm. The ECS decreased their memory because it involved the formation of free radicals that attacked the walls and nerve cells of their brains. These cells are composed of unsaturated fatty acids. Therefore, ECS would lead to peroxidation of fatty acids and neurotoxicity, causing neurotransmitter death and, subsequently a decline in memory (Luo e*t al.*, 2011).

Groups 4, 5, and 6 received steamed watermelon juice at different doses namely 0.92 g/kg BW, 1.85 g/ kg BW, and 3.7 g/kg BW respectively, for 14 days.

Table 4: The Performance (%) of the Experimental Rat Groups in RAM II.

| Day | The Performance of the Groups (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 39.3 | 13.3 | 22.1 | 18.7 | 0 | 0 |
| 2 | 40 | 0 | 29.1 | 50 | 37.5 | 0 |
| 3 | 51.6 | 13.3 | 50 | 62.5 | 33.5 | 27 |
| 4 | 94.4 | 21 | 87.5 | 100 | 83.3 | 46.6 |
| 5 | 66.6 | 10 | 45.8 | 55.3 | 100 | 66.6 |
| 6 | 50 | 20 | 26.1 | 80 | 72.2 | 56.2 |
| 7 | 66.6 | 20 | 66.6 | 87.5 | 91.6 | 75 |
| 8 | 66.6 | 13.2 | 61.1 | 83.3 | 44.4 | 66.6 |
| 9 | 50 | 20 | 91.6 | 100 | 66.6 | 66.6 |
| 10 | 66.6 | 20 | 100 | 100 | 88.8 | 33.3 |
| 11 | 66.6 | 20 | 100 | 83.3 | 100 | 33.3 |
| 12 | 66.6 | 0 | 100 | 75 | 100 | 33.3 |

The steamed watermelon juice was freshly made each day. The watermelon was prepared in small pieces, weighed using an analytical scale according to the needs of the three (3) treatment groups steamed at 70°C for 5 minutes and rested until the temperature decreased. Afterward, the steamed watermelon was drained and ready for juice extraction. The positive control received Vitamin E at a dose of 20.7 mg/kg BW dissolved in Na CMC. After these treatments, the rats were subjected to the second performance test, i.e., RAM II to identify any increases in spatial memory. The observation results are presented in Table 4.

Table 4 shows an increase in spatial memory during the 12 day observation in RAM II. The spatial memory of the treatment groups began to increase on Day 4 and reached its peak on the last day of the observation. Group 4 showed the highest performance with an average of 74.6%. The spatial memories of Groups 3, 5, and 6 increased by 65%, 68.1% and 42% respectively. The normal control (Group 1) had a different performance with negative control (Group 2). Furthermore, the performance of the negative control in RAM II was significantly different from its performance in RAM I. This group showed the lowest performance demonstrating the poor working and reference memory capabilities and the low learning ability of rats.

Group 4 and 5 each receiving steamed watermelon juice at the doses of 0.92 g/kg BW and 1.85 g/kg BW, respectively, scored 100% three times and ranked as the highest performance groups. This result suggest that these doses are effective to increase the spatial memory of rats with ECS induced dementia. The memory improvement may caused by an increase in the synaptic strength between the neurons in rat's hippocampus or referred to as neuronal plasticity, especially in the *gyrus dentatus* and CA1 hippocampus that are responsible for spatial information (Poirer *et al.*, 2008).

Interestingly, unlike the rest of the treatment groups, Group 6 that recieved steamed watermelon juice at a dose of 3.7 g/kg BW showed a decreased performance, even lower than normal control by 42%. This group failed two times (i.e., 0% performance) on the first and second day of observation. Its second lowest constant rate (33.3%) ocurred on Day 10, or one day after the increased performance of the negative control group, until the end of the observation. The poor working memory may be behind this result. This memory is responsible only for the information received from one session of RAM test. It is erased shortly before the next session of RAM test, and therefore the rats try to habituate themselves to the maze environment again and form a new working memory, while making mistake (i.e., incorrect arm entries). According to Crusio & Schwegler (2005), the working memory is closely related to spatial memory. The RAM test also observed reference memory that, for example, encoded and retained information about the food at the end of some arms. This memory is always useful in any maze test because it has already faded befor 24 hours. In this case, the rats must form new memory again from scratch allowing for a 24-hou maze test (Rao *et al.,* 2003).

The average percentage of the group performance in RAM II was subjected to homogeneity and normality tests. The normality test yielded $p$-value 0.142 ($p>0.05$), while the homogeneity test resulted in $p$-value 0.1 ($p>0.05$). The data analysis was continued with a one-way ANOVA to determine any statistically significant difference between the treatment groups in completing the maze test after the induction of ECS. The ANOVA test produced $p$-value 0.000 ($p<0.05$), representing a significant difference between the groups in RAM II.

The subsequent Tukey test proved that in the positive control group (Group 3), as well as the treatment groups receiving 0.92 g/kg BW (Group 4) and 1.85 g/kg BW (Group 5) of steamed watermelon juice, the spatial memory in the rat model of dementia improved. These three groups had a similar spatial memory improvement to the normal control group. The two treatment groups i.e., Group 4 and 5 showed comparable results as well, meaning, that the groups receiving 0.92 g/kg BW (Group 4) and 1.85 g/kg BW (Group 5) experience an equivalent spatial memory enhancement activity. Meanwhile, receiving steamed watermelon juice at a dose of 3.7 g/kg BW, Group 6 exhibited significantly different activities with the normal and positive control groups, but it was comparable to the negative control. In other words, the treatment in Group 6 was not effective in increasing the spatial memory of the experimental animals. The effective doses of steamed watermelon juice in this study were 0.92 g/kg BW and 1.85 g/kg BW. The average percentage of spatial memory improvement in these doses exceeded the positive control group (Vitamin E, 20.7 mg/kg BW).

Theoretical oxidative damage is preventable if free radicals and antioxidants in the body are in balance. In this condition antioxidants will not exhibit any effects (Asri, 2014). The antioxidative properties of lycopene contained in steamed watermelon juice are allegedly one of the factors that prevent ECS-induced oxidative damage. Lycopene is a highly unsaturated straigh-chain hydrocarbon with a total of 13 double bonds, eleven (11) of which are conjugated. This unique nature makes lycopene molecule a very potent antioxidant. An in vitro study affirms that lycopene is twice as potent as β-carotene and its singlet oxygen quenching ability is ten times greater than α tocopherol (Rao *et al.,* 2003).

## 4 CONCLUSIONS

This study concludes that the administration of steamed watermelon juice at the doses of 0.92 g/kg BW and 1.85 g/kg BW can improve the spatial memory in a rat model of dementia, which is comparable to the positive control. Treatment groups that recieved these doses had corresponding spatial memory enhancement activities.

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