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# Consumption of Food Sources of Antioxidant Associated with Cognitive Function and Oxidative Stress Markers 4-HNE

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**The contain of antioxidant in vegetables, fruits, spices, and tea has a protective effect from oxidative stress which can cause impaired cognitive function. This study aimed to determine the relationship between the consumption of antioxidant-rich foods such as vegetables, fruits, spices, and tea with 4-HNE plasma levels and cognitive function of elderly. The study design was cross-sectional, and was conducted in the Lima Puluh Kota district, West Sumatra in 2018.Interviewing antioxidant food intake was carried out using the Food Frequency Questioner (FFQ), cognitive function was assessed by the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina), plasma 4-HNE was measured by the ELISA method. Finally, the data was analyzed by Mann-Whitney and Chi-square statistical tests. The result showed that 83 elderly (57.2%) experience impaired cognitive function. There was no significant relationship between consumption of antioxidant foods and plasma levels of HNE. However, consumption of vegetables, fruits, spices, and tea has a significant relationship with cognitive function. This study concluded that consumption of vegetables, fruits, spices, and tea can protect the elderly from impaired cognitive function.**

**Keywords:** 4-HNE; Antioxidants food; Elderly; FFQ; MoCa-Ina.

Antioxidants in the form of phenolic compounds, found in many traditional Minangkabau dishes in West Sumatra, Indonesia which are famous for spices, vegetables, fruits and tea.[1]One of them is catechins that contain flavonoids which have the potential effect to protect against various disorders due to the aging process. So far, catechins have been widely studied as protective agents against various types of cancer, hypercholesterolemia, Parkinson's disease, and Alzheimer's disease. Catechins are known to be abundant in tea. Ejaz Ahmed *et al.*, (2013) reported that catechin hydrate compounds are effective in preventing memory loss, reducing oxidative stress and are also useful for the treatment of Alzheimer's dementia.[3] On the other hands, the antioxidant content found in vegetables, fruits, and spices also has a protective effect against oxidative stress that causes cognitive impairment.[4]

The pathological causes of cognitive function disorders are inflammation, glutaminergic neurotoxicity, increased iron and nitric oxide, depletion of endogenous antioxidants, reduced trophic factor expression, ubiquitin-proteasome system dysfunction, and proapoptotic protein expression from neurons. Several studies have shown that increasing free radicals is a major cause of neurodegenerative diseases.[5] Cell membranes are part of cells that contain a lot of polyunsaturated fatty acids (PUFAs), which produce high levels of Reactive Oxygen Species (ROS). This will cause the production of toxic lipid peroxides, which will cause oxidative stress and results in death in various types of cells. The brain cells are the cells that most easily affected by oxidative stress.[6]

Lipid peroxidation can be measured by several markers including malondialdehyde (MDA), acrolein, isoprostane and 4 Hydroxynonenal (4-HNE). 4-hydroxynonenal (4-HNE) is the most reactive, making it more damaging to the lipid membrane. Axelsen (2011) and Krumanet *al.*, (1997) found that 4 HNE caused neuronal apoptosis and this effect was not seen in other aldehydes.[7,8] McGrath *et al*., (2000) conducted a study on 29 people with Alzheimer's and found that a 4-HNE increase was associated with cognitive impairment and was not associated with malondialdehyde (MDA).[9]

Diagnosis of cognitive disorders can be measured using the Montreal Cognitive Assessment (MoCA). This method is able to detect cognitive impairments at an early stage, over time and is more sensitive than Mini-Mental State Examination (MMSE).[10] MMSE can be used to detect dementia, but it is not suitable for detecting mild cognitive impairment before the onset of dementia.[11] Consumption of various types of foods containing antioxidants has an important role for the healthy cognitive function of the elderly. This means that food consumption can be expected to improve the quality of life in the elderly. The aimed of this study was to determine the relationship between consumption of food sources of antioxidants with plasma levels of 4-HNE and cognitive function in the elderly.

## MATERIAL AND METHODS

The study design was cross-sectional, and was conducted in the Lima Puluh Kota district, West Sumatra in 2018. The study sample of 150 elderly people aged over 60 years was selected by multi stage random sampling. Other supporting factors such as age, level of education, and habits were also asked. 10 people were excluded due to blood sample errors and analysis of food consumption. Most subjects are under 75 years old. Most of them are women with less than 6 years of education (elementary school). We found that subjects with impaired cognitive function were 57.1%.

### Data collection

Data on antioxidant food consumption was obtained using a food frequent questionnaire (FFQ) which was developed by Lipoeto*et al.,* (2004).[12] Cognitive function was measured by the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina) to assess executive function, visuospatial ability, attention and concentration, memory, language, thought concepts. Interpretation of results is a normal examination (26-30 points) and impaired (<26 points). Blood samples were taken to observese plasma 4-HNE using the ELISA method. The ELISA kit used is a kit from Elabscience with a sensitivity of 0.38 ng/ml.

### Examination of 4-HNE

Add the standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 ul for each well). Add the samples to the other wells (50 ul for each well). Immediately add 50 ul of biotinylated detection 4-HNE working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37 $^{0}$C. Aspirate or decant the solution from each to well, add 350 ul of wash buffer to each well. Soak for 102 min and aspirate or decant the solution from each well and pat it dry againts clean absorbent paper. Repeat this wash step 3 times. Add 100 ul of HRP Conjugate working solution to each well. Cover with the plate sealer. Incubate for 30 min at 37 $^{0}$C. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2. Add 90 ul of substrate reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37 $^{0}$C. Protect the plate from light. Add 50 ul of stop solution to each well. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

### Ethical approval

This research was conducted in accordance with human ethical standards. Before the study began, informed concentrations were requested from all respondents in accordance with the code of ethics established and approved by the Research Research Ethics Committee of the Faculty of Medicine, Andalas University, Padang.

**Data processing and analysis**

Statistical analysis was performed using SPSS software ver. 22. Each variable is collected and presented using tables. The Spearman correlation test was used to see the correlation between consumption of food sources of antioxidants with 4-HNE results. The Mann-Whitney test was used to see the degree of association of biological markers with cognitive function (MoCA-INA). Analysis was also carried out to see the relationship between consumption of functional antioxidants and cognitive function. The total energy intake of less than 500 calories and more than 3500 calories was excluded from the study.

**Table 1.** Characteristics of respondents

| Characteristics | | N | % |
|---|---|---|---|
| Age | 60-74 year | 124 | 88.60% |
| | 75-86 year | 16 | 11.40% |
| Gender | Man | 30 | 21.40% |
| | Woman | 110 | 78.60% |
| Education | Primary school | 104 | 74.30% |
| | Junior high school | 26 | 18.60% |
| | Senior high school | 4 | 2.90% |
| | College | 6 | 4.30% |
| Cognitive function | Normal | 60 | 42.90% |
| | Disturbed | 80 | 57.10% |

## RESULTS AND DISCUSSION

Most of the respondents were women and under 75 years old with education level is elementary school. From the table it can be seen that the difference in the number of respondents with normal and impaired cognitive function is not too significant.

**Table 2.** Table consumption of food sources of antioxidants

| Variables | Mean ± SD | Median | Min-Max |
|---|---|---|---|
| Total intake of vegetables (g) | 184.27 ± 91.27 | 176.54 | 28-465 |
| Total intake of fruit (g) | 129.68 ± 84.2 | 120.98 | 11-559 |
| The intake of spices (g) | 17.72 ± 15.0 | 10.67 | 2 - 59 |
| The intake of tea (g) | 107 ± 118 | 57.14 | 0-800 |

**Table 3.** The relationship between consumption food sources of antioxidants with cognitive functions

| Intake of Average (gr/person/ days) | Interrupted (n = 80) | Normal (n = 60) | p |
|---|---|---|---|
| Intake of vegetables (g) | 164.67 ± 81.63 | 210± 97.4 | 0.03 |
| Intake of fruit (g) | 109.5 | 139.5 | 0.017 |
| Intake of spices (g) | 14.28 | 15.84 | 0.018 |
| Intake of tea (g) | 28.57 | 100 | 0.033 |

**Table 4.** Relationship between consumption of antioxidant food sources with 4-HNE Plasma levels

| Food sources of antioxidants | 4-HNE plasma levels Correlation Coefficient | p |
|---|---|---|
| Consumption of vegetables | 0.026 | 0.763 |
| Consumption of fruit | 0.028 | 0.321 |
| Consumption of spices | 0.036 | 0.679 |
| Consumption of tea | 0.006 | 0.941 |

The average consumption of vegetables is quite high compared to fruit,spices, and tea. However, the range is quite varied in all types of food sources of antioxidant, seen from the minimum and maximum values.

The relationship between vegetable, fruit, spices, and tea intake with cognitive function was seen using the T-Test. The average antioxidant food intake of subjects with impaired cognitive function was lower than those with normal cognitive function.The results show a statistically significant relationship between cognitive function and intake of vegetables, fruits, spices, and tea but there is no significant difference between the food sources (p> 0.05).

The results of the p value showed that the relationship between consumption of food sources of antioxidants with plasma levels 4-HNE did not reach statistical significance.

## CONCLUSION

In this study, the average vegetable intake was 184.27 ± 91.27 g/person/day, fruit intake was 129.68 ± 84.2 g/person/day, spices 17.72 ± 10.67 g/person/day, Meanwhile according to FAO, the average intake of vegetables and fruits in the world today is 200 grams/person/day and vegetable intake in Indonesia in 201 is 88.52 g/person/day, average intake fruits are 56.86 g/person/day and spices 7, 88 grams/person/day.[13] The results of this study are higher than the national consumption average, because the study sites are located in rural areas where consumption of fruits and vegetables is available in their own gardens. High spices intake is caused by traditional cuisine of people in West Sumatra (Minangkabau) which is famous for spices in the form of herbs, vegetables and fruit (4). The average tea intake is 107 g/person/day. The results showed that respondents with low education had a significant relationship with cognitive function p <0.05 (p = 0.01) where respondents with low education tended to experience impaired cognitive function, so it can be concluded that education is an important component affecting cognitive function in the elderly. There were statistically significant differences from the average intake of vegetables, fruits, spices and tea between subjects with impaired cognitive function compared to those with normal cognitive function.

Nine cohort studies reviewed by Loef*et al*., (2012) concluded that increased vegetable intake was associated with a lower risk of dementia and slower decline in cognitive function at older ages, this also applies to high fruit consumption.[14] The review results Crichton *et al*., (2013) reported an association between antioxidant intake and cognitive function, in 8 cross-sectional and 13 longitudinal studies included in the review. There are different results depending on the relationship between antioxidant intake and cognitive function, the risk of dementia and Alzheimer's disease is caused by differences in study design, differences in control and confounding factors, difficulty measuring cognitive decline, difficulty seeing relationships with results of diet assessment is not always permanent. Overall these findings are not consistent in showing the intake of dietary antioxidants with better cognitive function.[3]

The association of antioxidant food sources intake with 4-HNE plasma levels showed a weak correlation (correlation coefficient between 0 and 0.25) in the intake of vegetables, fruits, spices, tea, which means that the higher intake of food sources of antioxidants then the lower plasma levels of 4-HNE. But there is no statistically significant relationship where p > 0.05. In the elderly, cognitive impairment is caused by chronic oxidative stress.[15] 4-HNE is a component for determining oxidative stress, where 4-HNE is formed when the body experiences oxidative stress.[16,17]The elderly need to increase food consumption such as vegetables, fruits, spices, and tea to reduce oxidative stress which causes cognitive function impairment. The results of this study can be used as a basis for further investigation into how the mechanism of antioxidants food effects on the survival of neurons can prevent deterioration of cognitive function.

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**Conflict of interest**

There is no conflict of interest in this research

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