



Memory Loss induced by Aspartame in Albino Rats: Study on neurobehavioral changes

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ABSTRACT

Objective: Aspartame (ASP) consumption in various food and beverage products has generated a lot of controversy on safety. Many reports, ASP caused deterioration of health condition likes diabetes, psychiatric disorders, memory loss, etc. This study aimed to investigate the optimization duration of ASP to induce memory loss in Sprague Dawley rats. **Methods:** The ASP was administered 40 mg/kg BW orally for 28 days in rats. Analysis of memory loss by neurobehavioural changes including latency time, length of track, per cent time and frequency target quadrant using Morris Water Maze (MWM) at day 14, 21, 28, and 24 hours after the last treatment. **Results:** The administration of ASP showed the time-dependent changes for each indicator of neurobehavioural. The results demonstrated during 28 days of induction showed a significant decrease in latency time, length of track, per cent time and frequency target quadrant. **Conclusion:** From the results, it can be concluded administration of ASP during 28 days induce neurobehavioural changes related to memory loss in rats.

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Kata kunci:

Aspartam
Fungsimemori
Perilaku
Morris Water Maze

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ABSTRAK

Objektif: Penggunaan aspartame (ASP) sebagai pemanis buatan yang banyak terdapat pada makanan, dan minuman menimbulkan berbagai kontroversi dalam hal keamanan. Berbagai data menunjukkan ASP dapat menyebabkan gangguan kesehatan seperti diabetes, gangguan psikiatrik, penurunan fungsi memori, dll. Penelitian ini bertujuan untuk menentukan waktu durasi penggunaan ASP yang dapat menyebabkan gangguan fungsi memori pada tikus Sprague Dawley. **Metode:** ASP diberikan secara oral pada tikus dengan dosis 40 mg/kg BB selama 28 hari. Analisis terhadap fungsi memori dilakukan dengan adanya perubahan perilaku pada hewan, meliputi waktu latensi, Panjang lintasan, persentase waktu dan frekuensi kuadran menggunakan Morris Water Maze (MWM) setelah 24 jam setelah pemberian pada hari ke 14, 21, 28. **Hasil:** Pemberian ASP menunjukkan adanya perbedaan waktu pada setiap indikator perilaku. Pemberian selama 28 hari menunjukkan adanya perbedaan signifikan pada penurunan waktu latensi, Panjang lintasan, persentase waktu dan frekuensi kuadran. **Kesimpulan:** Berdasarkan hasil, pemberian ASP selama 28 hari dapat menyebabkan adanya perubahan perilaku yang berkaitan dengan fungsi memori pada tikus.

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INTRODUCTION

Aspartame (ASP) is an artificial sweetener which is also known as NutraTaste and NutraSweet which is used in a variety of beverage, food, pudding, etc. Aspartame was discovered in 1965 by G. D. Searle and Co. chemist James Schlatter. The ASP is odourless off the white crystalline powder with a melting point of approximately 246.5° C and stable at 30-80°C. At acidic or alkaline condition ASP undergoes hydrolysis to methanol. Interestingly, conditions during storage and ingestion may determine ASP metabolite. During storage at pH 4.3, ASP will degrade phenylalanine, aspartic acid, methanol and loss the sweetness. Meanwhile during ingestion ASP is metabolized by esterase and peptidase into 50% phenylalanine, 40% aspartic and 10% methanol (Choudhary & Pretorius, 2017).

Aspartame was first approved by the US food and drugs administration (FDA) in 1974 and marketed in 1981. Regulations regarding the safety of aspartame have been regulated by the FDA including the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the Codex Alimentarius (Food and Agriculture Organization/World Health Organization), the Scientific Committee for Food of the Commission of European Communities, the U.S. Food and Drug Administration (USFDA). JECFA recommend aspartame daily intake (ADI) of 40 mg/kg BW, while USFDA recommend 50 mg/kg BW (Nabors, 2001). In the postmarket surveillance period, ASP is associated with neurological and behavioural effects (O' Mullane, Fields, & Stanley, 2014). The toxicity effects of ASP caused by metabolite product consist of phenylalanine, aspartic acid and methanol (Humphries, Pretorius, & Naudé, 2008). Clinical studies in healthy adults, children and adolescents consumption of ASP up to 27 weeks showed accumulation of plasma aspartate, phenylalanine and methanol (Guy, 2014). This condition can lead to oxidative stress in the central nervous system (CNS), which plays a role in behaviour (Choudhary & Pretorius, 2017).

The hippocampus is located in the temporal lobe of the cerebral cortex and plays a role in learning and memory. This section can also affect the occurrence of neuropsychiatric conditions and psychiatric disorders so that this section is very susceptible to damage in the presence of stimuli (Anand & Dhikav, 2012). The hippocampus is divided into two parts, namely the right and the left. These two parts have different functions. The right part plays a role concerning spatial memory, while the left part plays a role in verbal episodic memory skills (Ezzati et al., 2017). The hippocampus plays an important role in the arrangement of spatial memory, not as a storage place for long-term memory but as an organizer so that the information obtained can be stored in long-term memory. The hippocampus also in transferring memory to be stored into long-term memory which is called the consolidation process and the memory will be in the neocortex (Wiltgen et al., 2010).

In the previous study, ASP in mice twice daily over 2 weeks can lead to oxidative stress and decreased memory (Abdel-Salam et al., 2012). In another study, using a much lower daily dose of aspartame at pregnant guinea pigs that received aspartame throughout gestation and demonstrated the aspartame-treated pups showed a disruption of odour associative learning (Magnuson et al., 2007). The rationale for this study was the need to determine changes in memory, markers of latency time, length of track, percent time and frequency target quadrant associated with repeated administration of ASP at the recommended dietary intake of 40 mg/kg using rats.

METHOD

Animals

Animals used in this study were male Sprague Dawley rats, weighing 150-250 grams and aged 2-3 months obtained from the National Food and Drugs Administration, Republic of Indonesia (BPOM RI). Animals were bred and housed at Faculty of Pharmacy, Universitas Indonesia in standard housing (12 h light/dark cycle), temperature 24-26°C, humidity 60-65%, in groups of 6 rats per cage with ad libitum access to standard water and chow. Before the study, rats were acclimatized in laboratory condition for at least 1 weeks. The experimental protocols and animal handling procedures were approved by the Ethics Committee Faculty of Medicine, Universitas Indonesia (approval number KET-1411/UN2.F1/ETIK/PPM.00.02/2020).

Equipment and apparatus

Morris water maze 50 cm diameter and height 50 cm with square platform 10 cm diameter, 1 ml syringe (Terumo, Philippines) with oral sonde, stopwatch timer, camera recorder (1/29" 2 Megapixel CMOS, 130-degree angle of view, resolution 1080P, IR distance up to 10m).

Experimental Design

The animals were randomly divided into 2 (two) groups. The first group served as a normal or vehicle group and was treated with 0.5% CMC-Na solution, the second group served as a control group and was orally administered ASP with the dose of 40 mg/kg BW based on JECFA approved aspartame daily intake (ADI) and dissolved in 0.9% saline.

Experimental Procedures

The first group was a normal group given 0.5% CMC-Na and the second group given ASP (40 mg/kg BW in 0.9% saline, orally) once daily for 28 days. The memory tests were conducted on day 14, 21, 28, and 29 (24 hours after the last treatment). The evaluation is performed using Morris Water Maze (MWM) (Vorhees & Williams, 2010). The MWM have a hidden platform and divided fourth quadrant (north, south, west and east), next MWM filled opaque water temperature 25°C. The animal was placed in one of the quadrants, as a start position in the maze, facing the tank wall. Then the animal was released into the water. A timer or computer tracking program was started when the animal is released and stopped if the animal touches the platform. A trial limit of 1 or 2 min. In case the animal not finding the platform within this time limit, the animal will be placed on the platform or guided to it. This treatment was repeated 4 times with a new location as a start point. The time latency was expressed as the time to find a platform and the length of track was expressed as distance to find a platform on day 14, 21 and 28. Then, on day 29, all animals were placed in a random position in the maze (without a platform) and facing the tank wall. The results of the experiment were expressed in the percent time and the frequency target quadrant was recorded for each animal.

Statistical analysis

All data were tested for normality and homogeneity. After that, the data were analyzed using one-way ANOVA, followed by the post hoc Tukey's HSD test. All data were

analyzed using SPSS software version 23. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Results of the research consisted of descriptive statistics, test results of the assumptions and This study investigated

duration aspartame effects on neurobehavioral markers. Neurobehavioural changes were evaluated by latency time, length of track, percent time and frequency target quadrant parameter. Neurobehavioural changes are one of the indicators related to memory loss due to CNS damage. The parameters were performed by using MWM (Vorhees & Williams, 2014).

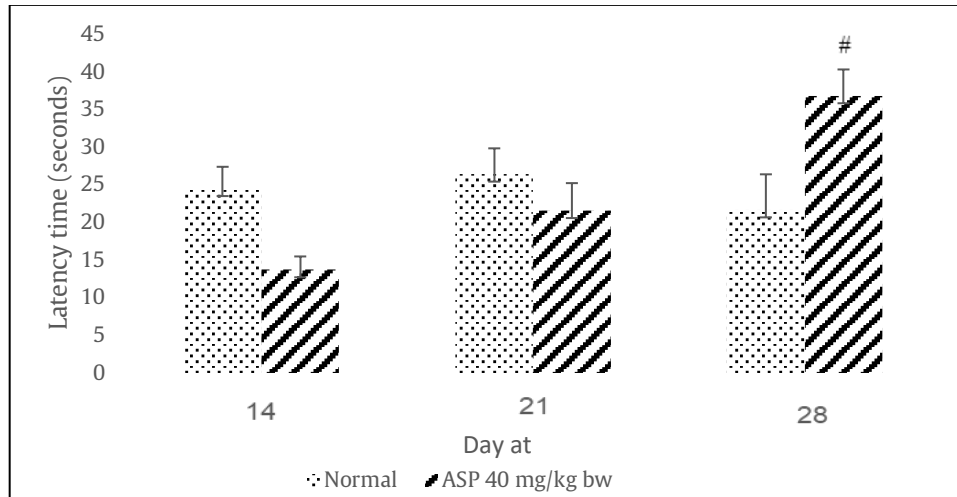


Figure 1: The latency time in the normal and ASP groups. The values are expressed as the means \pm SEM of each group ($n = 6$). # significant difference compares to day 14 and 21 ($P < 0.05$). ASP: aspartame (40 mg/kg BW, orally)

The latency time and the length of tract parameters showed the ability of the animal to find the platform in a short time related to memory function. In normal condition, the time needed was very short. Figures 1 showed the latency time increased in a time-dependent manner after administration of ASP for 28 days. The increased time was

significant compare to day 14 and 21 administration of ASP ($P < 0.05$). The length of the track showed an increase significantly on 28 days of administration of ASP ($P < 0.05$). The results mean the animal experience a decrease in memory function characterized by the longer the track is taken to reach the platform.

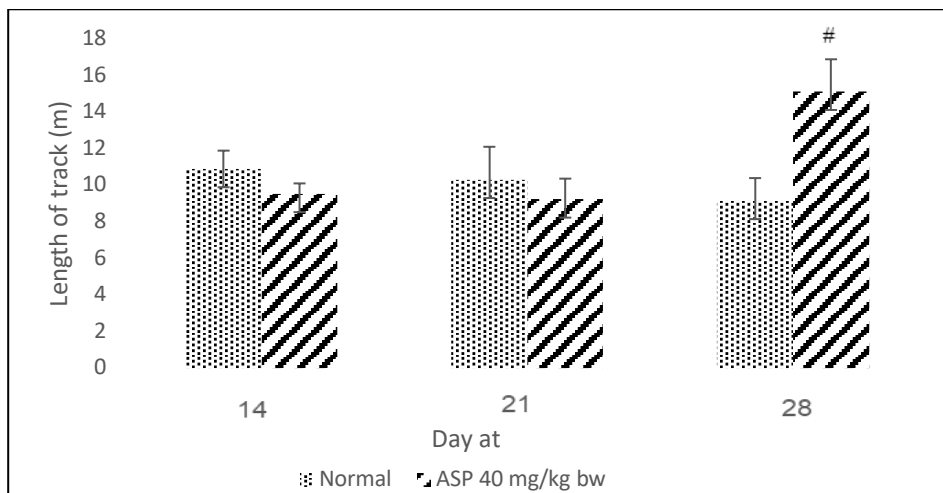


Figure 2: The length of the track in the normal and ASP groups. The values are expressed as the means \pm SEM of each group ($n = 6$). # significant difference compares to day 14 and 21 ($P < 0.05$). ASP: aspartame (40 mg/kg BW, orally)

The nervous system is structured to produce or modulate behaviour. Neurobehaviour is the phenotypic expression of the vector of neurotransmitter functions. Several studies have shown aspartame exhibiting mixed neurobehavioral effects (Lindseth, Coolahan, Petros, & Lindseth, 2014). Results

of this study showed that administration of ASP for 28 days resulted in memory loss. Aspartame has been shown to have adverse effects on memory when administered at high doses (75 mg/kg BW) and chronically in albino rats (Iyyaswamy & Rathinasamy, 2012). But, in this study, the dose of ASP was a

recommended dose dietary (40 mg/kg BW) and also have adverse effects on memory in long term administration. Memory is a process of being able to remember and then build or reconstruct every detail of an event(Kandel, Dudai, & Mayford, 2014). In the brain, there are two types of memory, namely explicit memory (declarative) and implicit memory (non-declarative). Explicit memory is used and linked to an object. This memory involves part of the hippocampus and

cortex. Implicit memory is a memory that is used in motor activity and a perception involving the cerebellum, striatum, and amygdala[14, 15]. The previous study showed in Japanese quail, spatial memory was used in the learning process [16], while in rodents spatial memory functions as explicit memory in remembering an object (Vorhees & Williams, 2014).

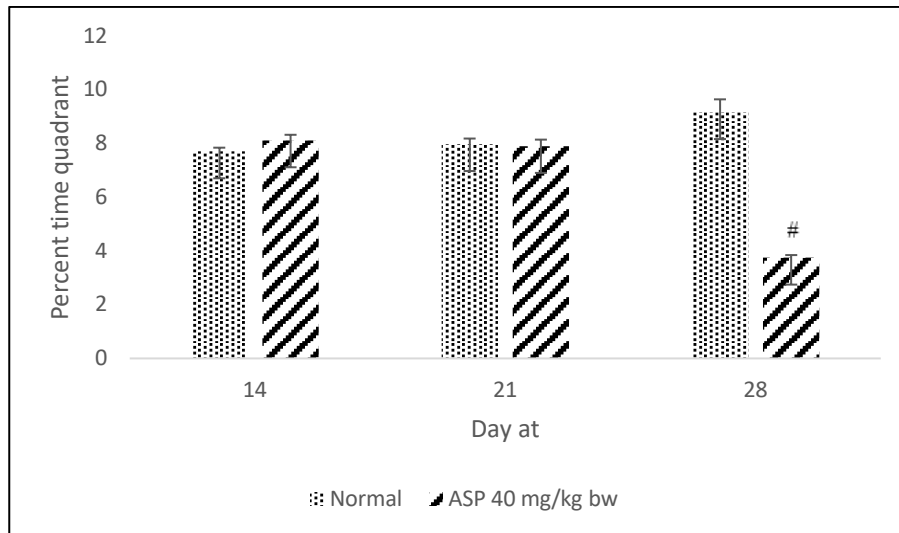


Figure 3: The per-cent time quadrant in the normal and ASP groups. The values are expressed as the means ± SEM of each group (n = 6). # significant difference compares to day 14 and 21 (P< 0.05). ASP: aspartame (40 mg/kg BW, orally)

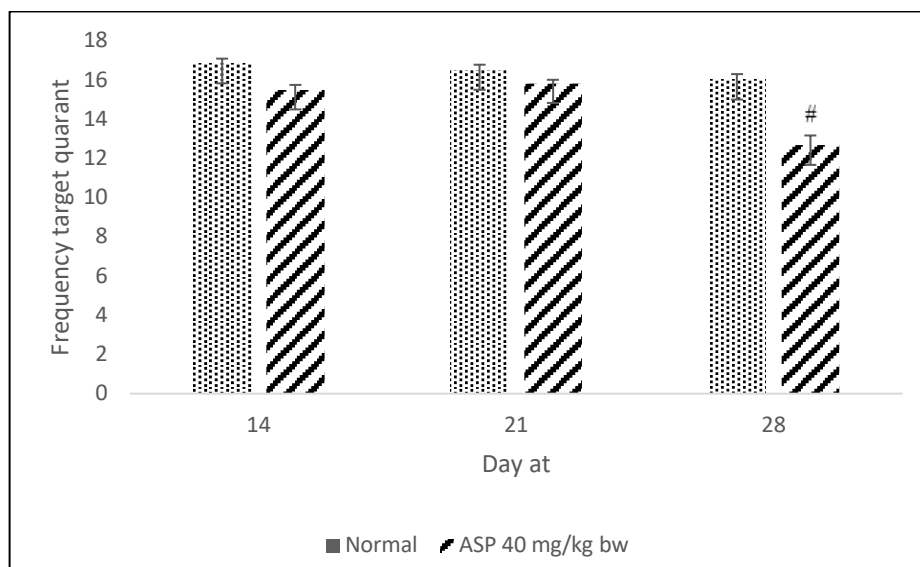


Figure 4: The frequency target quadrant in the normal and ASP groups. The values are expressed as the means ± SEM of each group (n = 6). # significant difference compares to day 14 and 21 (P< 0.05). ASP: aspartame (40 mg/kg BW, orally)

The memory loss induced by ASP caused by increased free radicals, then can lead to oxidative stress. Furthermore, it will cause lipid peroxidation, protein peroxidation, DNA damage and degeneration of cells(Peña-Bautista, Baquero, Vento, & Cháfer-Pericás, 2019). Besides, ASP can cause an imbalance of neurotransmitter as a result of aspartic acid metabolism. Aspartic acid makes up approximately 40% of aspartame's breakdown products(Humphries et al., 2008) and constant consumption of products containing the sweetener increases its concentration in blood and brain. The

presence of excess aspartic acid in the brain will have impact excitotoxicity of brain neurons. Aspartic acid is believed to play an active role as an excitatory neurotransmitter in the central nervous system(Onalapo, Onalapo, & Nwoha, 2016). Acetylcholine (ACh) is a neurotransmitter that is used by all cholinergic neurons with the cholinergic receptor, which plays a role in the peripheral and central nervous system (H. Ferreira-Vieira, M. Guimaraes, R. Silva, & M. Ribeiro, 2016). Nicotinic receptors play a role in learning and memory functions, located in the striatum, cortex, superior

colliculus, lateral geniculate nucleus and cerebellum. Muscarinic receptors play a role in cognitive function, located hippocampus, cortex and thalamus (Kumar, Kumar, Keegan, & Deshmukh, 2018).

CONCLUSIONS AND SUGGESTIONS

The administration of ASP for 28 days with a daily dose of 40 mg/kg BW can lead to memory loss in male rats Sprague Dawley.

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Conflict of Interest Statement

There is no conflict of interest in this study.

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