RESEARCH ARTICLE

Neuroprotective Effect of Red Betel (*Piper crocatum*) Extract on Brain Monosodium Gluatame (MSG)-Induced Rat (*Rattus norvegicus*)

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Abstract

Indonesian people prefer to eat fast food which is generally rich in flavorings. The most common ingredient found in fast food is MSG (monosodium glutamate). MSG consumption that exceeds the limit will produce free glutamate which is excitotoxic in various organs, one of which is the neurological system. Red betel has a fairly high antioxidant content which can inhibit excitotoxicity by inhibiting the occurrence of free radicals. This research aimed to determine the neuroprotective effect of red betel extract (*Piper crocatum*) on the brains of rats (*Rattus norvegicus*) given monosodium glutamate (MSG). This research is a laboratory experimental study with a completely randomized design method using 5 treatment levels, namely negative control (NC), positive control (PC), treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3). The NC treatment level was given 2 mL of distilled water. The PC treatment level was given 7gr/KgBB MSG. Levels T1, T2, T3 were each given MSG 7gr/KgBW in the morning, then after 8 hours, level P1 was given 200mg/KgBW of red betel extract, P2 was given 400mg/KgBW of red betel extract, and P3 was given 600mg/KgBW of red betel extract. Examine the number of neurons in the dentate gyrus of the hippocampus using the ImageJ application and examine calcium ion levels using the RAPIDLab 348X tool. Data analysis used the One Way ANOVA test with the SPSS 24 program. The results showed that there was a significant difference ($p<0.05$) between PC and NC. The results of the calcium ion parameters showed that only the 600mg dose treatment group functioned as a protector in the occurrence of hypocalcemia, while the histopathology parameters showed that the 400mg and 600mg dose treatment groups functioned significantly as a neuroprotector.

Keywords*: antioxidant, ion calcium, MSG, neuron cells, red betel.*

INTRODUCTION

Food additives (FA) are natural ingredients or mixtures of ingredients that are not part of food raw materials but are added to food to influence the nature or shape of the food, including colorings, preservatives, flavorings, anti-caking agents, whiteners, and thickeners (Fajarini and Wahyani, 2020). FA, according to The Minister of Health of the Republic of Indonesia regulation No.772/MENKES/Per/IX/88 are materials that are not usually used as food and are not typical food ingredients, have or do not have nutritional value, which are deliberately added and mixed during food processing to produce a component or influence its unique properties and improve the quality of the food (Fajarini and Wahyani, 2020). The most common flavoring ingredient found in fast food is MSG. MSG is the sodium salt of glutamic acid, which is used to flavor food so it tastes savory, which can create the perception of sweet and salty, and can reduce bitter and sour tastes (Wahyudi, Bahar and Septinawati, 2018)

According to the Food and Drugs Administration (FDA), the recommended dose of MSG (safe limit) is around 120 mg/KgBW per day. However, continuous consumption of food flavorings can cause MSG to accumulate in the body. Continuous accumulation of MSG over a long period with small doses requires caution that it will have an effect on the body. MSG has toxic effects on humans and experimental animals. MSG can cause symptoms such as numbness, fatigue, sweating, dizziness, and headaches (Yonata and Iswara, 2016).

Lion Q et al. (2024) reported that giving 4 g/kg of MSG to mice caused a proliferation of neural cells in the hippocampus and led to local edema or neurodegenerative. This research investigated whether MSG treatment affects the offspring of adult mice. They mated male and female mice treated with MSH and obtained F1 offspring after 90 days. The result of pathological analysis of the brain tissue is found significant proliferation of hippocampus cells in the offsping of mice that received 2 g/kg MSG and cell proliferation was more obvious and necrosis in the offsping of mice that received 4 g/kg MSG (Liang *et al.*, 2024).

When MSG is consumed, some of the free glutamic acid produced will be bound in the intestines, and the rest will be released into the blood. It then spreads throughout the body, including penetrating the blood-brain barrier and binding to glutamate receptors. This free glutamic acid is excitotoxic, so it is hypothesized that it will damage brain neurons if it exceeds the brain's ability to maintain it at a low level (Martami and Holton, 2023). The mechanism of excitotoxicity can occur from various factors (Farhat *et al.*, 2021). Overstimulation of glutamate receptors can initiate various cascades that have the potential to induce cell damage and death. Activation of NMDA receptors by glutamate causes a large influx of calcium ions

(Ca2+), resulting in mitochondrial dysfunction, which causes the formation of free radicals, hypocalcemia, activation of the caspase pathway, and degradation of intracellular proteins (Jakaria *et al.*, 2018).

Antioxidants are molecules or compounds that are quite stable for donating electrons or hydrogen to radical molecules or compounds free and neutralize it, so reduces his ability to carry out a free radical chain reaction. These antioxidants delay or inhibit cell damage, especially through their free radical scavenger properties (Ibroham, Jamilatun and Ika, 2022). Types of antioxidants such as favonoids, isothiocyanate, and putative polyphenols play a neuroprotective role (Vaiserman, Lushchak and Koliada, 2016). One source of antioxidants is red betel leaves because of the number of active compounds they contain, including flavonoids, alkaloids, polyphenolades, tannins, and essential oils (Naufalin and Yanto, 2009). Flavonoids are good reducing compounds, inhibiting many oxidation reactions, both enzymatic and non-enzymatic. Flavonoids act as good scavengers of hydroxyl and superoxide radicals, thereby protecting membrane lipids against damaging reactions. Based on their antioxidant activity, flavonoids can be active components of plants traditionally used to treat liver dysfunction (Frandsen and Narayanasamy, 2018). Flavonoids are known as antioxidants and have attracted some researchers to research flavonoids as drugs that have the potential to treat diseases caused by free radicals. Most flavonoids have antioxidant activity due to the presence of phenolic hydroxy groups in their molecular structure. When these compounds react with free radicals, they will form new radicals that are stabilized by the aromatic nuclear resonance effect (Shen *et al.*, 2022). Based on this background, we are interested in doing this research. The parameter observed was the number of rat neurons in the area hippocampus dentate gyrus, as well as calcium ion testing in rat serum. Formulation The problem in this research is whether there is an influence from red betel extract in inhibiting the occurrence of degenerative neuron cells up to the death of neuron cells. This research aims to determine the effect of deep red betel extract inhibits degenerative and death of rat brain neuron cells.

METHODS

The rats were randomly divided into 5 groups (negative control group, positive control group, treatment 1, treatment 2, and treatment 3). The red bettel extracts that have been made are then sent to the Research Institute for Spices and Medicinal Plants (BALITRO) to carry out a phytochemical screening test for red betel leaves using a qualitative method aimed at finding out what content is contained in red betel leaves.

Negative control (NC) that were given 3 mL of distilled water with a sonde probe. Positive control (PC) that were given MSG at 7g/KgBW/day with a sonde probe. Treatment level 1 (T1), that were given 7g/KgBB/day of MSG after which they were given 200mg/KgBB/day of red betel with a sonde probe. Treatment level 2 (T2) that were given MSG at 7g/KgBW/day after which they were given red betel at 400mg/KgBW/day with a sonde probe. Treatment level 3 (T3), namely a group of mice that were given MSG at 7g/KgBW/day, after which they were given red betel at 600mg/KgBW/day with a sonde probe. The calcium ion examination procedure use serum, where the blood is first centrifuged at a speed of 3000 rpm for 15 minutes. Then, the serum was examined using a Siemens RAPIDLab 348X instrument, the principle of which is selective ion. Histology preparations of rat brains were made at the anatomical pathology laboratory at Dharmais Cancer Hospital. The brain tissue that was obtained was fixed by immersing the brain tissue in 10% neutral buffer formalin for 24 hours. Hematoxylin-eosin staining is performed to view bone tissue visually. Neuron cells in the brain's hippocampus were counted using the ImageJ application. The results of the number of neurons were analyzed using statistics with an independent T-test.

RESULTS AND DISCUSSION

Phytochemical Screening and Antioxidant Levels

Testing of red betel extract was carried out using 2 methods, quantitative to measure levels of antioxidants, flavonoids, and tannins. Meanwhile, the qualitative method is to determine whether there are phytochemicals in the extract. The screening results on red betel extract are as follows:

Table 1. Results of testing antioxidant levels and phytochemical screening of red betel extract

Test Parameters	Result	Method
- Antioxidant IC 50% (ppm)	196,10	
- Flavonoid as Quarsetin (%)	0,50	DPPH/ spectrophotometry
- Tannin (%)	1,85	
Phytochemical		Qualitative
- Alkaloid	+	
- Saponin	+	
- Tannin	+	
- Fenolic	+	
- Flavonoid	+	
- Triterpenoid	+	
- Steroid	+	
- Glycosida	+	

Ps : (+) = the presence of phytochemicals

Calcium Ion Levels

Measurement of calcium ion levels uses the ion selective electrode method. An ion-selective electrode (ISE) is a type of electrode (half-cell) used in potentiometry to measure the number of dissolved ions in a solution. The following are the results of calcium ions at various levels of treatment:

Table 2. Effect of red betel extract on calcium ion levels

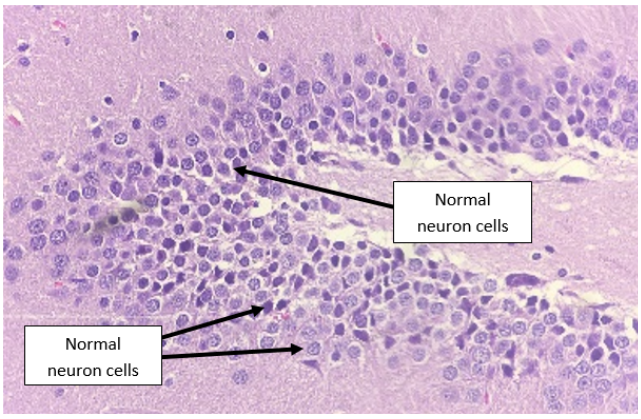
Group	Calcium ion (mmol/L)
NC	1,22 ± 0,0632[b]
PC	0,94 ± 0,0358[a]
T1	1,05 ± 0,0614[a]
T2	1,06 ± 0,0179[a]
T3	1,26 ± 0,2863[b]

Note: Data are presented as mean ± SD (n=5). Different letters [a, b] indicate significant differences ($p<0.05$) between each other. The same letters indicate no significant differences ($p>0.05$) between each other.

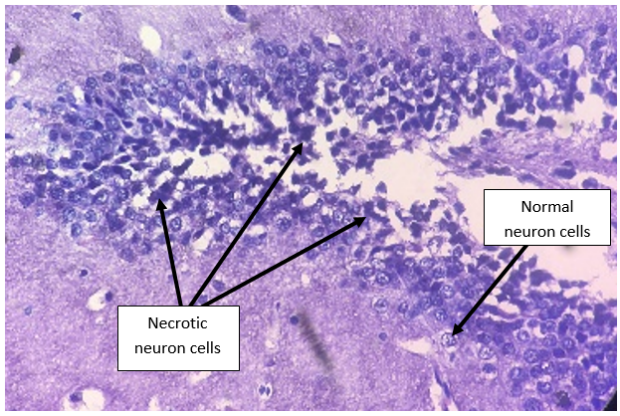
Based on the International Federation of Clinical Chemistry (IFCC), the threshold value for calcium ions ranges from 1.20-1.40 mmol/L. Based on Table 3, it can be seen that the NC and T3 (600mg) groups have the highest (normal) calcium ion levels compared to PC, T1 (200mg), and T2 (400mg). Meanwhile, the PC group had the lowest calcium ion levels (hypocalcemia) compared to NC, T3, T1, and T2. The following is a bar diagram of the results of rat serum calcium ion levels:

Histopathology Results of Brain Organs

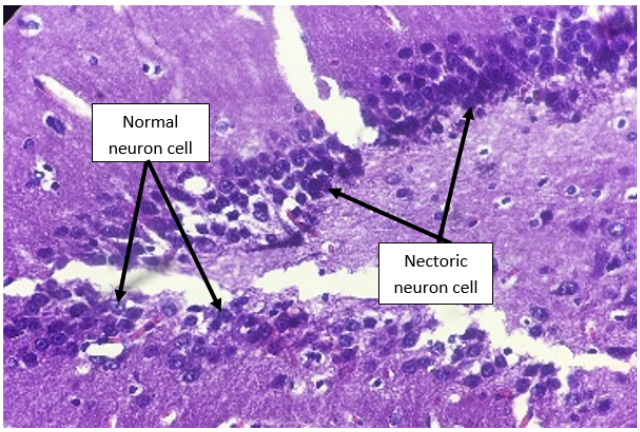
In brain histopathology, the part observed is the dentate gyrus of the hippocampus. The hippocampus is one of the most studied structures in the human brain. The hippocampus is one part of the limbic system. The limbic system is a system that regulates emotions, habits, motivation, long-term memory, and the sense of smell. Histopathology results showed the presence of normal neuron cells and necrotic neuron cells. Neuronal cells are in normal condition, characterized by clear cytoplasm and nuclei (Anand and Dhikav, 2012). Clearly, round cell walls and nuclei can be observed. Meanwhile, in Figures 2B, 2C, 2D, and 2E, apart from normal neuron cells, there are also necrotic neuron cells. Necrotic neuron cells are characterized by cell swelling, dense nuclei, cell rupture, irregularity of cell structure, and loss of cell nuclei (Miller and Zachary, 2017). All these characteristics indicate necrotic characteristics of the cells. The following histopathology results of brain organs in the hippocampus area can be seen in Figures 2a, 2b, 2c, 2d, and 2e:



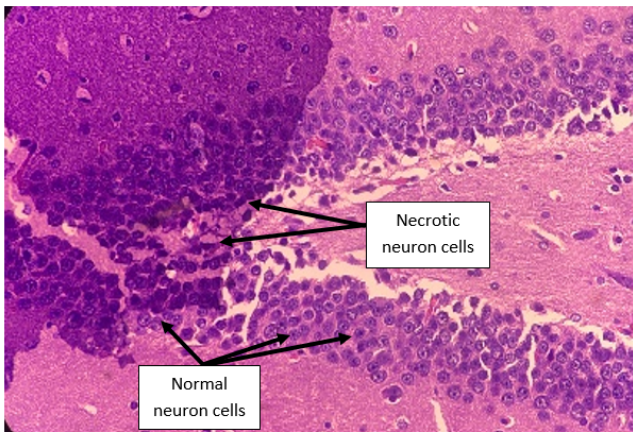
(2a)



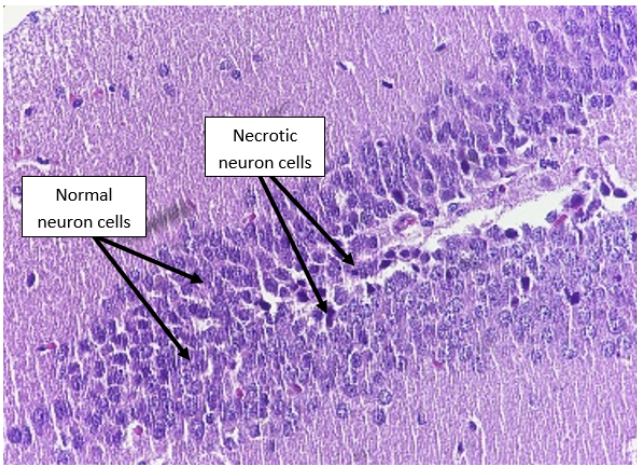
(2b)



(2c)



(2d)



(2e)

Figure 2. HE Staining Results of the hippocampal region of the brain with 40X magnification from NC preparation that every neuron cell appears normal *(a);* PC preparation, neuronal cells are dominated by necrotic cells (b); T1 (200mg), there are few normal cells and predominately necrotic cells (c); T2 (400mg), there are normal cells that dominate and necrotic cells that are starting to be few (d); T3 (600mg), there are normal cells that dominate with a few necrotic cells (e).

Based on the histopathology results of the mouse brain organ above, data on the percentage of necrotic cells was obtained by counting total neuron cells and necrotic cells using the ImageJ application. The percentage of necrotic neuron cells in each treatment group can be seen in Table 4.

Table 3. Effect of red betel extract on the number of necrotic cells in neurons

Group	Necrotic cells
NC	0,000 ± 0,000[a]
PC	75.56 ± 5.162[c]
T1 (200mg)	70.52 ±4.087[c]
T2 (400mg)	26.36 ± 8.346[b]
T3 (600mg)	7.82 ± 3.134[a]

Note: Data are presented as the mean ± SD of the percentage of necrotic cells (n=5). Different letters, a, b, c, d, indicate significant differences ($p<0.05$) between each other. The same letters indicate no significant differences ($p>0.05$) between each other.

Consuming MSG for a long time can cause an imbalance between antioxidants and reactive oxygen species (ROS), which causes oxidative stress. Excessive ROS causes neurodegeneration. Neurodegeneration disorder is the term used for the progressive loss of neuronal cell structure or function, including neuronal cell death. Following the mechanism by which glutamate can damage the brain by opening calcium channels excessively, calcium ions can be used as an indicator of disturbances in brain cells. A calcium imbalance in brain cells indicates a disorder in the brain cells. Free glutamate (free acid from MSG) is thought to cause the opening of calcium channels so that more calcium enters the nerve cells. The large amount of calcium that enters causes excessive stimulation, resulting in the death of nerve cells and hypocalcemia in the extra cells. The activation of NMDA receptors by glutamate causes a large influx of calcium ions (Ca2+), resulting in mitochondrial dysfunction, which causes the formation of free radicals(Eimerl and Schramm, 1994).

Overstimulation of glutamate receptors will initiate various cascades that have the potential to induce cell damage and death. Activation of NMDA (N-Methyl-D-Aspartate) receptors by glutamate causes a large influx of calcium ions (Ca2+), resulting in mitochondrial dysfunction, which causes the formation of free radicals(Rama and García, 2016). Based on research conducted by Vanzour 2008, flavonoids can protect the brain from injury and suppress neuroinflammation and are able to inhibit nerve cell damage induced by oxidative stress and

neurotoxins and suppress neuroinflammation. Inhibition of caspase activation causes flavonoids to be able to inhibit nerve cell damage induced by oxidative stress. In preventing neuroinflammation, flavonoids suppress COX-2 and iNOS expression, NO production, cytokine release, NADPH oxidase activation, and ROS formation (Vauzour *et al.*, 2008).

CONCLUSION

The red betel leaf extract has a significant effect in inhibiting damage to neuron cells in the hippocampal dentate gyrus area in rat brains and inhibiting hypocalcemia in the brain of rats induced by MSG. The doses of 400mg and 600mg of red betel extract are quite effective in inhibiting neuronal cell damage. Meanwhile, at a dose of 600 mg, it is effective in preventing hypocalcemia.

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