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The effect of tumeric rhizome (Curcuma longa L) on radial arm maze and passive avoidance test in trimethyltin-induced rat models

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ABSTRACT

Backround: Oxidative stress can cause death of hippocampal cells associated to the deficit of learning and memory function. Turmeric rhizome contains curcuminoid which has antioxidant activity that can prevent oxidative stress, thus it was expected to prevent the deficit of learning and memory function on trimethyltin (TMT)-induced rat models.
Objectve: This study aimed to determine the effect of turmeric rhizome extract on cognitive learning and memory fuction which was tested using radial arm maze and passive avoidance tests on Wistar rats injected with TMT.
Methods: A total of 30 Wistar rats, male, 8-12 weeks of age were divided into 6 groups. The first (normal) group was given CMC-Na, the second (TMT) group was given CMC-Na, the third-the fifth groups were given turmeric rhizome extract (TRE) orally in the dosage of 120mg/kgBW (TRE120), 240 mg/kgBW (TRE240) and 480 mg/kgBW (TRE480), respectivelly. The sixth (P500) group was given piracetam in the dosage of 500 mg/kgBW. Treatments were given for 8 days orally. At day-9th, excluding the first group, all rats were injected with 12 mg/kgBW TMT solution intraperitoneally. One day after TMT injection, the cognitive learning and memory function was measured using radial maze and passive avoidance tests. The datas of cognitve function tests were analyzed statistically by ANOVA test followed by LSD test at significance level 0.05.
Results: The results of radial maze and passive avoidance tests showed that the TRE240 group was not statistically significant different with TMT group($p>0.05$). However there were significant difference between TRE120 and TMT groups, as well as between TRE480 and TMT groups ($p<0.05$). The administration of 480 mg/kgBW of TRE showed that its cognitive function was not significantly different with the administration of Piracetam ($p>0.05$).
Conclusion: It can be concluded that the administration of 480 mg/kgBW of TRE can improve cognitive learning and memory function of Wistar rats injected by TMT.

Latar Belakang: *Stres oksidatif dapat menyebabkan terjadinya kematian sel hippocampus yang dapat berakibat terhadap penurunan fungsi kognitif belajar dan mengingat. Rimpang kunyit (Curcuma longa L) mengandung kurkuminoid yang memiliki aktifitas antioksidan sehingga dapat mencegah stres oksidatif dan diharapkan dapat mencegah penurunan kemampuan belajar dan mengingat pada tikus yang diinduksi zat neurotoksik trimetiltin (TMT).*

Tujuan Penelitian: *Penelitian ini bertujuan untuk mengetahui efek ekstrak rimpang kunyit (ER) terhadap fungsi kognitif belajar dan mengingat pada tikus yang diinduksi TMT menggunakan uji radial arm maze dan passive avoidance.*

Metode: *Sebanyak 30 ekor tikus Wistar, jantan, umur 8-12 minggu dibagi menjadi 6 kelompok. Kelompok I (normal) diberi CMC-Na, kelompok II (TMT) diberi CMC-Na, kelompok III diberi ekstrak rimpang kunyit (ER) dosis 120mg/kgBB (ER120), kelompok IV diberi ER dosis 240mg/kgBB (ER240), kelompok V diberi ER dosis 480mg/kgBB (ER480), serta kelompok VI diberi Piracetam dosis 500 mg/kg BB (P500). Pemberian perlakuan dilakukan selama 8 hari secara oral. Pada hari ke 9 tikus (kecuali kelompok normal) diinjeksi TMT dosis tunggal 12 mg/kg BB. Satu hari kemudian tikus diuji fungsi kognitifnya dengan uji radial maze dan uji passive avoidance. Data yang diperoleh dianalisis statistik dengan uji Anova kemudian dilanjutkan uji LSD dengan tingkat signifikansi 0,05.*

Hasil: *Hasil uji radial maze dan passive avoidance tikus kelompok ER240 tidak berbeda bermakna dengan kelompok TMT (p>0,05), namun ada perbedaan bermakna pada kelompok ER120 dan ER480 (p<0.05) dengan kelompok TMT (p<0,05). Pemberian ER dosis 480 mg/kg BB menunjukkan fungsi kognitif yang tidak berbeda bermakna dengan piracetam (p>0,05).*

Kesimpulan: *Ekstrak rimpang kunyit dosis 480 mg/kg BB dapat meningkatkan fungsi kognitif belajar dan mengingat pada tikus Wistar yang diinjeksi TMT.*

INTRODUCTION

Memory is one example of cognitive functions that involve brain in order to store previously learned knowledge and to recall previously stored information. Memory is formed through learning process. New informations and knowledge that are received by the neural system could be observed through behavioral changes.[1,2] Hippocampus is a part of the brain that contributes in the formation of learning and navigation. Hippocampus and the cortex are the areas of the brain proned to oxidative stress. Neural cell death in hippocampus due to oxidative stress could alter the process of memory formation. [3]

Trimetiltin (TMT) is a neurotoxic organometal.[4] One of the neurons prone to TMT toxic effect are those located in the hippocampus which contributed in the process and consolidation of memories.[5] Trimetiltin could alter the cholinergic system which would disturb memory processing.[6] TMT intoxication in rat modes is coherrent with neurodegenerative models of memory disturbance, thus it would be beneficial for the study of Alzheimer dementia.[7]

A lot of herbal plants with high antioxidant content had been empirically proven to be beneficial in preventing neurodegenerative diseases. Tumeric (Curcuma longa L.) is one example of herbal plant that has high antioxidant content.[8] Tumeric contains a component called curcumin which has high potential as neuroprotecting agent.[8–10] Curcumin could also normalize the spatial memory in dementia rat models induced with intracerebroventricular streptozotocin.[9,10] The objective of this study is to determine the effect of tumeric rhizome in improving cognitive learning and memory function of TMT-induced rat models through radial arm maze and passive avoidance test. It is hoped that this research would trigger the utilization of tumeric extract in preventing memory degeneration (dementia).

METHODS

The production of tumeric rhizome ethanolic extract

The powder of tumeric rhizome was obtained from CV. Merapi Farma (Yogyakarta), The production of tumeric extract was done using maseration method. As much as 500 gram tumeric powder was maserated in 1,25 L ethanol 96% for 24 hours. Maseration was done two times. Obtained maserat was filtrated, and the filtrat was evaporated in evaporator then continued in stainless steel pan that was placed above water bath until thick extract was gained. Next, extract was suspended into CMC-Na before being used in animal models.

Standardization of tumeric rhizome ethanolic extract using TLC -Densitometry.

Extract standarization was initiated by making curcumin standard in the concentration

of 1 mg/ml. Next, it was made into standard solution in the concentration of 0.125 ; 0.25 ; 0.5; 1; 2; and 4 mg/ml, as well as sample solution by diluting 100 mg of extract in 10 ml ethanol 96%. The solutions was spotted in Thin layered chromatography (TLC) plate. Stationery phase used Silica Gel 60 F 254, while mobile phase used the mixture of chloroform and methanol in 9:1 ratio. After filtrat was eluted, the intensity of the color formed was counted in term of curcumin concentration using TLC-densitometry in 426 nm wavelength.

Intervention of animal models

The design and procedure of animal models intervention in this research has been approved by Lembaga Penelitian dan Pengujian Terpadu (LPPT) UGM with certification number 130/ KEC-LPPT/XII/2013. There were 30 adult male Wistar rats, which weigh between 180-200 gram and were divided into 6 groups. Group I (Normal) was given CMC-Na 0,5% solution orally. Group II (TMT) was given CMC-Na 0,5% solution orally. Group III, IV and V were each given tumeric rhizome ethanolic extract (TRE) in the dosage of 120 mg/kgBW (TRE120), 240 mg/kgBW (TRE240) and 480 mg/kgBW (TRE480) orally. And group VI was given piracetam solution in the dosage of 500 mg/kgBW orally. Interventions were given for 8 days. On day- 9, all rat models were injected with Trimethyltin chloride (Sigma) single dose 12 mg/kgBW intraperitoneal, except for group I (normal).

The next day, rats learning and memory capabilities were tested using passive avoidance test. The measurement consist of learning trial (LT) and retention trial (RT). The duration of retention would determine the learning and memory capability, and was counted by measuring the margin between RT and LT. After passive avoidance test was finished, it was then continued with radial maze test by measuring the duration of rat error when entering test equipment.

Data Analysis

The data of latent period results of passive avoidance test were statistically analyzed with Kruskal-Wallis test, and continued with Mann-Whitney test. While the data of numerical error in radial maze test was statistically analyzed with One way ANOVA test continued with Post Hoc LSD test. Significancy was determined when p value <0,05.

RESULTS

Tumeric rhizome extract

From 1000 gram tumeric rhizome powder, 297,7 gram extract was obtained with 29,77% yield. The results of TLC showed that tumeric extract produced three different chromatogram, which were curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Standard curcumin had Rf 0,75, which was the same as tumeric extract Rf 0,75. The result of TLC-desitometry showed that the concentration of curcumin in the extract was 27,3 %. The result of TLC test could be seen in Figure 1.

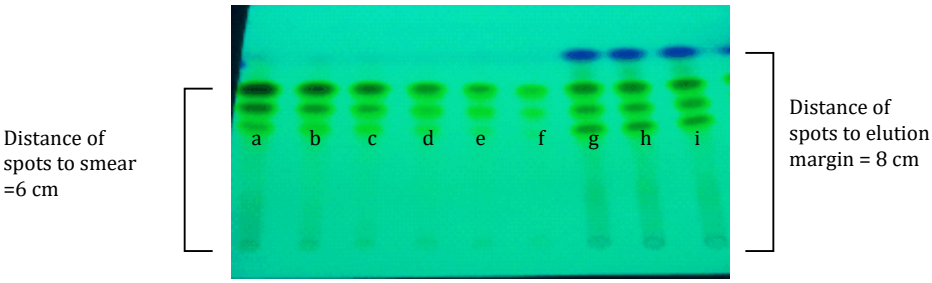

Figure 1. The results of thin layer chromatography (a = standard curcumin, b – f = standard curve with concentration variation, g – i = ethanolic extract of tumeric rhizome samples) stationery phase used silica gel 60 F254 and mobile phase used chloroform : methanol (9 : 1), showed the measurement of Rf = the distance of spots to smear/the distance of spots to elution margin. Rf standard curcumin: 6cm/8 cm = 0,725; Rf extract : 6cm/8 cm = 0,725

The results of learning and memory test in passive avoidance and radial maze test Passive avoidance and radial maze test was done to measure the learning and memory capabilities of animal models. The results of retetion trial in passive avoidance test could be seen in Figure 2, while the error of entering the arm in radial maze test could be seen in Figure 3.

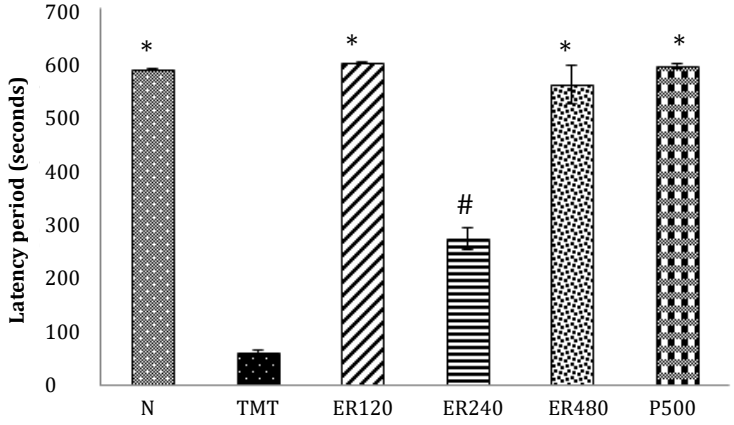

Figure 2. The length of retention period of learning and memory capability in passive avoidance test in all intervention groups. **Note**: N, normal; TMT, trimetiltin; ER, tumeric rhizome extract; *p<0,05 compare to TMT; #p<0,05 compare to N.

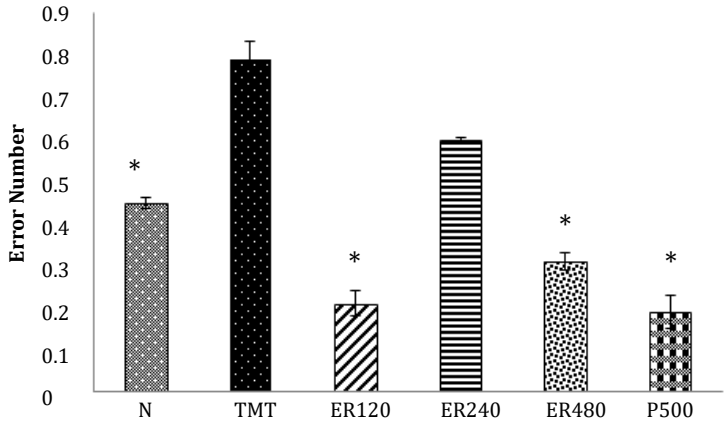

Figure 3. The number of error in radial maze test of all intervention groups. Note.: N, normal; TMT, trimetiltin; ER,tumeric rhizome extract;*p<0,05 compare to TMT group

DISCUSSION

Statistical analysis showed significant difference between latency period and error of entering the test equipment in radial maze test between TMT and normal group. Rats that was induced with only TMT showed significantly smaller retention period and error number (p<0,05) compared to normal group. This showed that the administration of TMT could affect learning process and memory of animal models. Trimetiltin injection would cause brain cells degeneration in rat models through oxidative stress mechanism. Neurons located in the hippocampus, piriformis cortex, entorinal cortex, amygdala, neocortex, and olfactory tubercle are sensitive to the toxic effect of TMT.[5] TMT could alter the cholinergic system, thus causing disturbance in memory processing.[6] TMT could increase the formation of reactive oxigen species (ROS) and increase the formation of free radicals like hidroxyl, malondialdehide and carbonil protein in the hippocampus.[11,12]

Hippocampus is the selective target of TMT toxicity, thus when neuron cell death occurs in the hippocampus, memory capability would decrease.[13]

The administration of tumeric rhizome extract in the dosage of 120 and 480 mg/kg BW showed significantly prolonged latency period and smaller error numbers ($p<0,05$) compared to TMT group. This result was different when compared to the administration of tumeric rhizome extract in the dosage of 240 mg/kg BW which showed no statistically significant difference of retention period of learning and memory in passive avoidance test and number of error in radial maze test ($p>0,05$). This indicates a non-proportional response to dosage increase. Tumeric contains curcumin, demethoxycurcumin and bisdemethoxycurcumin which is potential as antioxidant and neuroprotective agent.[8,14,15] Its highly lipophylic character would increase curcumin disposition in the brain.[16] In vitro and in vivo studies showed that curcumin could prevent neurodegenerative process caused by oxidative stress.[17,18] Curcumin in vitro could inhibit lipid peroxidation process in the brain.[19] Other studies also showed that in vivo curcumin could inhibit the increase of malondialdehide formation which is a side product of lipid peroxidation, it could also improve glutathion decrease in oxidative stress-induced rat models with kainat acid.[20] Curcumin could also normalize spatial memory of dementia rat models induced with intracerebroventricular streptozotocin.[9,10] However, there are other studies who found oxidant activity of curcumin in certain dosage which could contradict its antioxidant activity. This might be what causes the effect differences between the administration of TRE/dose 240 mg/kg BW with dose 120 mg/kg BW and 480 mg/kg BW. Nonetheless, further research is needed to determine the appropriate dose of tumeric extract in order to achieve its pro-oxidant activity.

This research used piracetam as comparison. Piracetam is a drug that commonly use as standard medicine in the management of cognitive disorder such as cognitive impairment or dementia. In this study, the administration of piracetam in the dosage of 500 mg/kgBW showed no statistically significant diference in learning and memory capability when compare to normal group. Piracetam (2-oxo-1-pyrrolidine-acetamide) is a gamma-aminobutyric acid analog that has been used as standard drug in order to improve memory function of dementia patients. The administration of piracetam has been proven to decrease malondialdehide concentration and increase superoxidedismutase (SOD) and glutathion peroxydase (GPx), thus it could prevent oxidative stress.[21]

The limitation of this research is the absence of passive avoidance and radial maze test prior to intervention, hence the initial data of leaning and memory capability of rat models could not be determined.

CONCLUSION

Ethanolic extract of tumeric rhizome could enhance cognitive learning and memory function of trimetiltin-induced Wistar rats (as dementia model) and the dosage of the extract that can improve cognitive learning and memory function in this study are 120 mg/kgBW and 480 mg/kg BW.

Suggestion

Further research about the effect of ethanolic extract of tumeric rhizome using other learning and memory function test method is needed to be done. Other tests could include Morris water maze test. Furthermore, further research on the hystopathological figure of hippocampus is needed.

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