ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Special issue 1, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

EFFECT OF BLACK CUMIN OIL (*NIGELLA SATIVA* L.) ON SPATIAL MEMORY OF ADULT MICE TREATED WITH TEMOZOLOMIDE

ZULFIKRI ZULFIKRI[1], URIP HARAHAP[1], MUHAMMAD ICHWAN[2]*

[1]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. [2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Email: M.ichwan@usu.ac.id

Received: 07 March 2018, Revised and Accepted:25 March 2018

ABSTRACT

Objectives: The objective of this study is to observe the preventive effect of black cumin oil (BCO) on spatial memory impairment due to temozolomide (TMZ) treatment.

Methods: Female Swiss albino mice (n=28) were divided into four groups that received aquadest per oral (p.o), TMZ intraperitoneal (i.p) and aquadest p.o, TMZ i.p and BCO 0.1 ml p.o, and TMZ i.p and BCO 0.2 ml p.o, respectively. After 9 weeks, the mice were subjected to Morris water Maze (MWM) test. The time to find the platform (latency time) was analyzed as the indicator of memory performance.

Results: We found that mice treated with TMZ (i.p) spent significantly more time to find the platform during memory acquisition phase (on day 3) than the other groups. Interestingly, the group treated with TMZ (i.p) and BCO 0.2 ml significantly had shorter latency time than the other groups, indicating the preservation of spatial memory after TMZ treatment.

Conclusion: This result indicates that BCO can ameliorate the detrimental effect of TMZ on spatial memory in adult mice.

Keywords: Temozolomide, Black cumin oil, Hippocampus, Spatial memory, Morris water maze.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11s1.26594

INTRODUCTION

Increasing evidence shows that the adult mammalian nervous system retains a capacity for self-renewal that is important for its normal functions, such as learning and memory. The existence of neuronal progenitor cells enables the generation of new neurons, so-called neurogenesis process, in the structures that are involved in the formation or integration of new memories. This process is regulated by changes in the internal and external environment to further support the normal behavior [1].

One of the important areas in the adult brain wherein neurogenesis occurs is the dentate gyrus in the hippocampus. Hippocampus is part of the limbic system that plays role in learning, remembering, and emotional setting [2].

Neurogenesis can be hampered by chemicals or drugs that inhibit cell proliferation. Temozolomide (TMZ) is an alkylating agent that is used in chemotherapy for brain cancer. It breakdowns DNA strands and thereby inhibits not only cancer cell but also neuronal progenitor cells proliferation in the hippocampus. Changes in neurocognitive attitudes and symptoms such as impairment in learning function and memory are most commonly reported in adult and pediatric cancer patients after chemotherapy [3].

Black cumin (*Nigella sativa* L.) is a plant from Rannculaceae family that has been studied for its neuroprotective effect. The beneficial effects of black cumin on learning, memory, and cognition in human and animals models have been reported [4-6]. The results of animal and human studies have shown that black cumin and thymoquinone have antiepileptic properties [7]. Thymoquinone and BCO have antioxidant effects during cerebral ischemia reperfusion injury in rat hippocampus [8]. The plants from this family grow endemically at some places in the Middle East and the Southern Mediterranean countries. Conventionally, they are often used by the local community as anti-inflammatory, anticancer, antiparasitic, antibacterial, and antioxidant [9].

METHODS

Animals

Twenty-eight female Swiss albino mice (±25 g) were used for this study. The animals were housed in the group in plastic cages and acclimatized for 1 week before experiment. The mice were divided into four groups: No TMZ, TMZ (i.p) and aquadest p.o, TMZ (i.p) and BCO 0.1 ml p.o, and TMZ (i.p) and BCO 0.2 ml p.o, respectively. Black cumin oil (BCO) was given every day for 9 weeks using oral gavage, while TMZ was administered intraperitoneally in 3 consecutive days weekly for 4 weeks starting from the 2nd week (Fig. 1). The experiment has been approved by Health Research Ethical Committee, Medical Faculty Universitas Sumatera Utara (No.238/TGL/KEPK FK USU-RSUP HAM/2017).

TMZ

TMZ at the concentration of 25 mg/kg body weight was reported effectively to suppress proliferation of neuronal progenitor cells by more than 90% after 4 cycles of treatment [3].

The mice received injections of TMZ at 25 mg/kg (intraperitoneal., 2.5 mg/ml in 0.9% NaCl), whereas the control group received a saline injection. This regimen was given on the first 3 days of a week for 4 weeks to resemble paradigms used for glioma treatment in humans. Behavioral testing was performed 4 weeks after the final TMZ injection with Morris water maze (MWZ) test [3].

BCO

BCO was purchased from PT. Habbatussauda International, Bandung, with the brand name Habbat's Blackseed Oil. The dosage was 0.1 and 0.2 ml/days by oral gavage.

MWZ task

1 day after the last administration of BCO (on week 9), mice were tested in the reversal version of the MWZ task [10] to locate a hidden escape platform in a circular pool (1.8 m diameter). Water was made opaque by non-toxic white paint and kept at a temperature of 19–20°C. Each mice

was subjected to 6 trials a day for 5 consecutive days with an intertrial interval of maximum 30 min. Platform position was changed after day 3 to the opposite quadrant (Fig. 2). Mice were dropped into the water from different starting points each day and allowed to search for the platform maximum up to 120 s. Mice were removed from the pool after finding the hidden platform or were guided to the platform and allowed to sit there for at least 2s before being placed into a lamp-heated box. The time needed by the each mouse to find the platform (latency time) was recorded [3].

Data analysis

The latency time for each day was obtained by calculating the mean of latency time from all mice in each group per day. The mean comparison among groups was analyzed by ANOVA with Tukey's *post hoc* test in Graphpad Prism® version 7 software (GraphPad Software Inc., USA). The data were presented as mean ± standard error of mean.

RESULTS

Learning capacity assessment

To assess the spatial learning capacity, the latency time of the mice to find the submerged platform was compared among groups (Fig. 3).

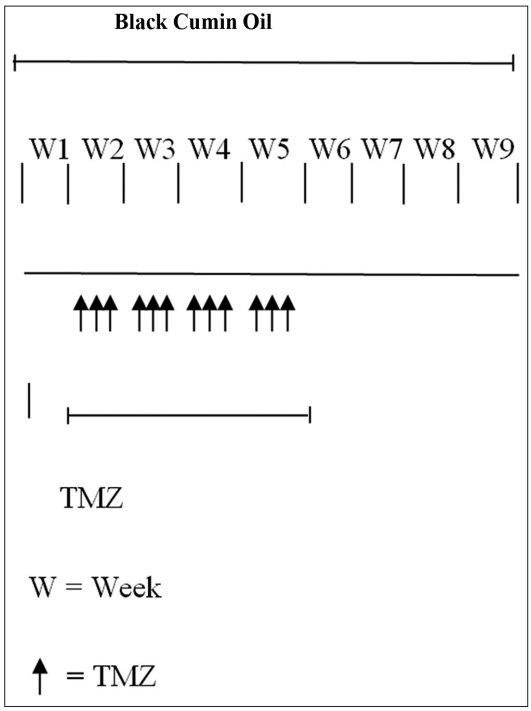

Fig. 1: Experiment scheme

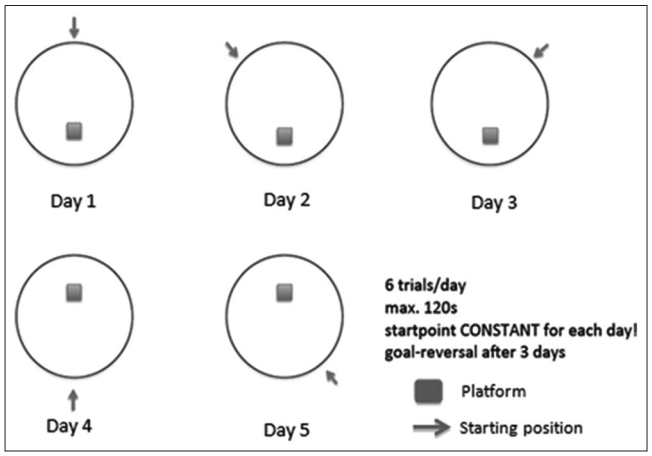

Fig. 2: Platform position in Morris water maze test

Table 1: Comparison of latency time at MWZ test

Group	Latency time (s)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control versus TMZ	86.02±6.83 versus 91.57±5.95	54.05±6.6 versus 64.83±7.32	57.79±6.51 versus 82.48±6.54*	86.07±6.5 versus 83.29±6.1	76.9±6.95 versus 68.21±7.19
Control versus TMZ-BCO 0.1	86.02±6.83 versus 74.5±6.82	54.05±6.6 versus 62.24±7.21	57.79±6.51 versus 52. 19±5.99	86.07±6.5 versus 62.17±7.26	76.9±6.95 versus 52.9±6.3*
Control versus TMZ-BCO 0.2	86.02±6.83 versus 67.93±7.12	54.05±6.6 versus 44. 88±6.9	57.79±6.51 versus 38.36±5.21	86.07±6.5 versus 47.5±6.24**	76.9±6.95 versus 37.95±5.23**
TMZ versus TMZ-BCO 0.1	91.57±5.95 versus 74.5±6.82	64.83±7.32 versus 62.24±7.21	82.48±6.54 versus 52.19±5.99**	83.29±6.1 versus 62.17±7.26	68.21±7.19 versus 52.9±6.3
TMZ versus TMZ-BCO 0.2	91.57±5.95 versus 67. 93±7.12	64.83±7.32 versus 44. 88±6.9	82.48±6.54 versus 38.36±5.21**	83.29±6.1 versus 47.5±6.24**	68.21±7.19 versus 37.95±5.23**
TMZ-BCO 0.1 versus TMZ-BCO 0.2	74.5±6.82 versus 67.93±7.12	62.24±7.21 versus 44. 88±6.9	52.19±5.99 versus 38.36±5.21	62.17±7.26 versus 47.5±6.24	52.9±6.3 versus 37.95±5.23

MWZ: Morris water Maze, TMZ: Temozolomide, BCO: Black cumin oil

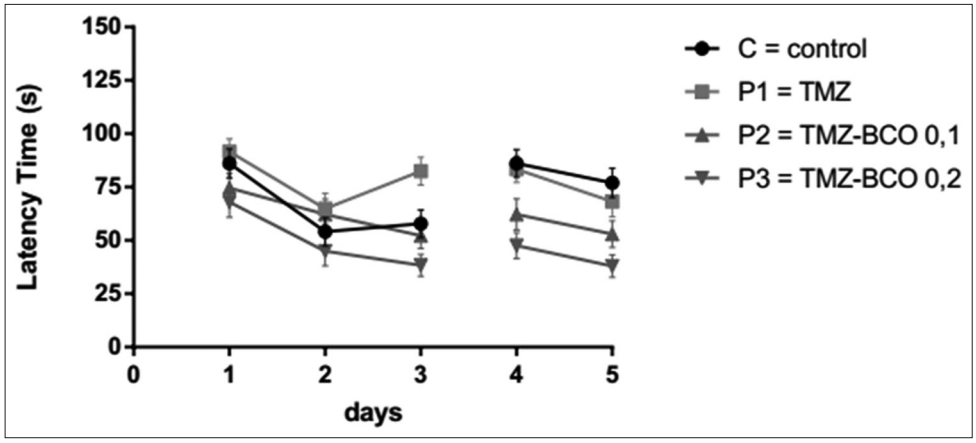

Fig. 3: Latency time during spatial acquisition and spatial reversal in Morris water maze test

Each group showed decreased latency time on day 2, indicating that the mice learned to find the platform. However, on day 3, the latency time of the TMZ-treated Group (P1) was increased again and higher than the other group. This indicated that TMZ impaired the learning capacity. Interestingly, BCO-treated Groups (P2 and P3) showed improvement in their time to find the hidden platform on days 2 and 3, dose dependently.

The reversal learning on day 4 indicates whether the animals can extinguish their initial learning. All group except Group P1 showed an increase in latency time on day 4 compared to day 3. This indicated that relearning process occurred in these groups. On day 5, we observed that the latency time in all groups was lower than the previous day. Interestingly, we found that BCO-treated mice had shorter latency time dose dependently compared to control and TMZ only group.

Comparison of latency time

Latency time of each group per day is shown in Table 1. Latency time on the 1st day of MWZ test in each group did not differ significantly ($p>0.05$) because the animals were still doing the observation to found the hidden platform and collecting. On the 2nd day, there was a decrease in latency time but not significantly different in all groups ($p>0.05$). In this situation, the ability to remember the position of the platform began to form based on the spatial clues around the pool (spatial memory). On the 3rd day, there was a significant difference in latency time between Group C versus P1 ($p<0.05$), P1 versus P2 (<0.01), and also P1 versus P3 ($p<0.01$). On the 4th day, a significant latency time difference was found in Group C versus P3 ($p<0.01$) and in Group P1 versus P3 ($p<0.01$). On the 5th day, there was also a significant difference on latency between Group C versus P3 ($p<0.01$), C versus P2 ($p<0.01$), and P1 versus P3 ($p<0.01$).

DISCUSSION

Based on Table 1, it was found that groups that were given TMZ significantly took longer time to find a hidden platform compared to other groups, especially in the memory acquisition phase (day 3rd). This suggests that there is an effect of TMZ with spatial memory function in mice. While the TMZ group and BCO significantly showed faster time in finding the platform, even mice given 0.2 ml BCO showed better memory functionality than either group either in the acquisition phase or in the relearning phase (the 4th day). The time difference of latency to find a platform in the TMZ group with TMZ and BCO showed an effect of BCO compared with non-cumin oil.

Cell proliferation is an early part in the formation of neurons and then migrates and survives to become mature, integrated, and functioning as new neurons (adult neurogenesis) [11]. Neurogenesis occurring in the hippocampus of the adult brain plays a role in the process of memory storage and neuronal repair processes due to disease as well as anxiety in the brain [12].

The hippocampus is the part of the brain quickly. Decreased hippocampal function may be due to increased age and exposure to chemical compounds. Newly formed memory must pass through the hippocampus before it is permanently stored. Damage to the hippocampus results in the inability of the brain to form new memories [13].

The effects of BCO in preventing the decline in spatial memory ability due to TMZ can be mediated by the efficacy of some substances contained in BCO, including thymoquinone, phenolic compounds, and fatty acids. Purwanto [14] mentioned that the content of phenolic compounds epigallocatechin gallate in green tea can increase alanine-glyoxylate aminotransferase (AGT) enzyme that functioned to transfer the alkyl group attached to O6-guanin-DNA due to the carcinogenic substance to the internal cysteine group on AGT enzyme so that DNA becomes normal again. Thus, DNA damage due to alkylation process can be avoided DNA damage due to alkylation process can be avoided. The same mechanism can also occur in the administration of TMZ (alkylating agent group) that works to alkylate the DNA at the position of O6 guanine. Polyphenols contained in BCO may also have the same properties as polyphenols in green tea that is able to increase AGT enzymes in preventing DNA alkylation. Further research is required to prove this hypothesis.

Thymoquinone is a major element of essential oil obtained from *Nigella sativa* seeds known to have strong antioxidant properties [15]. Thymoquinone serves to protect the organs against oxidative damage caused by various free radical-producing agents including effectively protecting the damage of neurons in the hippocampus by lowering high levels of malondialdehyde and elevated levels of glutathione, catalase, and superoxide dismutase activity to normal levels [16]. In addition, the fatty acids present in BCO may also activate and induce enzymes in the peroxysomal β oxidation pathway which is a catalyst in the process of cutting the acetyl CoA chains and resulting in acyl CoA, acetyl CoA, and propionyl-CoA [17]. Acetyl CoA plays a full role in the synaptoplasmic replication of cholinergic nerve terminals governing the synthesis of acetylcholine [18]. As known acetylcholine is a neurotransmitter that plays a role in learning and memory by increasing the rate of long-term potentiation process in some parts of the brain including the hippocampus [19].

In addition to thymoquinone, Nigella sativa oil is also a rich source of phenols that builds up its antioxidant capacity [20]. Therefore Nigella sativa oil is suggested to exert its effect through neuroprotective mechanism by its antioxidant properties and modulates molecular pathway through interfering cellular enzymatic reactions.

CONCLUSION

We found that mice treated with TMZ had worse spatial learning and relearning capacity compared to control. The TMZ-treated mice those

were supplemented with BCO significantly performed better in MWZ test compared to the TMZ only group. Our result showed that BCO can ameliorate the detrimental effect of TMZ on spatial memory in mice.

ACKNOWLEDGMENT

This research was partially supported by Indonesia Ministry of Health through PPSDMK Scholarship Program. We thank our colleagues Cut Azwanidar and Putri for their assistance in animal care and data collection

REFERENCES

1. Gage FH. Mammalian neural stem cells. Science 2000;287:1433-8.
2. Duvernoy HM, Cattin F, Risold PY, Vannson JL, Gaudron M. The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI. 4th ed. Berlin: Springer; 2013.
3. Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. PLoS One 2009;4:e5464.
4. Jain SK, Parihar S, Pandey N. Medicinal plants with neuropharmacological properties from Indian origin. Int J Pharm Pharm Sci 2014;6(10):36-40.
5. Bin Sayeed MS, Asaduzzaman M, Morshed H, Hossain MM, Kadir MF, Rahman MR, *et al*. The effect of *Nigella sativa* Linn. Seed on memory, attention and cognition in healthy human volunteers. J Ethnopharmacol 2013;148:780-6.
6. Sahak MK, Mohamed AM, Hashim NH, Hasan Adli DS. *Nigella sativa* oil enhances the spatial working memory performance of rats on a radial arm maze. Evid Based Complement Altern Med 2013;2013:180598.
7. Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. Phytomedicine 2004;11:56-64.
8. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 2007;14:621-7.
9. Ping NC, Hashim NH, Hasan Adli DS. Effects of *Nigella sativa* (*Habbatus sauda*) oil and nicotine chronic treatments on sperm parameters and testis histological features of rats. Evid Based Complement Altern Med 2014;2014:59-65.
10. Morris R. Developments of a water-maze procedure for studying spatial leaning in the rat. J Neurosci Methods 1984;11:47-60.
11. Emsley JG, Mitchell BD, Kempermann G, Macklis JD. Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells. Prog Neurobiol 2005;75:321-41.
12. Lazarov O, Mattson MP, Peterson DA, Pimplikar SW, van Praag H. When neurogenesis encounters aging and disease. Trends Neurosci 2010;33:569-79.
13. Anderson BJ, Rapp DN, Baek DH, McCloskey DP, Coburn-Litvak PS, Robinson JK, *et al.* Exercise influences spatial learning in the radial arm maze. Physiol Behav 2000;70:425-9.
14. Purwanto DA. Analysis of O6-[H3]Mehyilguanine-DNA in rat liver cell culture by liquid scintillation counter after (-)-epigallocatechin gallate exposure from green tea. J Kedokt Indones 2011;2:5-10.
15. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 1995;61:33-6.
16. Al-Majed AA, Al-Omar FA, Nagi MN. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol 2006;543:40-7.
17. Poirier Y, Antonenkov VD, Glumoff T, Hiltunen JK. Peroxisomal beta-oxidation--a metabolic pathway with multiple functions. Biochim Biophys Acta 2006;1763:1413-26.
18. Szutowicz A, Tomaszewicz M, Bielarczyk H. Disturbances of acetyl-CoA, energy and acetylcholine metabolism in some encephalopathies. Acta Neurobiol Exp (Wars) 1996;56:323-39.
19. Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 2006;16:710-5.
20. Mosbah A, Sobhi W, Benboubetra M. Antioxidant and antihemolitic activities of *Algerian Nigella* sativa total oil and its neutral lipid fraction. Int J Pharm Pharm Sci 2015;7(11):50-54.