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THE COMBINED EFFECT OF OMEGA-3 AND VITAMIN E AS NEUROPROTECTIVE AGENT IN RATS INDUCED DIAZEPAM: RANDOM POST CONTROL STUDY USING MORRIS MAZE WATER

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In Indonesia, 2.3 billion benzodiazepines were consumed in a year and about 81% were diazepam used. They used them for treating sleep lifecycle as sedative hypnotic that can cause damage on neural system. The aim of this study is to know the effect of combination omega-3 and vitamin E on cognitive and memory function in induced diazepam male rats. This study was a randomized post test control group trial. First, 20 male wistar rats (weight 180-200 gram) kept under standardized diet and condition for 7 days. On day 8th until 10th, all of they were trained in Morris maze water for 10 minutes duration each day. On every training, that search hidden platforms and memorized it for 20s. Escape Latency Time (ELT) was an index memory and cognitive. On day 11 until 20, they given diazepam 1 mg via IM into a control positive group (I1), (I2), and (I3). Then the rats treated with 1 soft gel for (I2), 2 soft gel for (I3) food supplement omega 3 and vitamin E via oral for 10 days. The result showed the escape latency time (ELT) between control group and treatment groups was significantly different ($p<0.05$). The results indicate that combination omega-3 and vitamin E is useful to enhance cognitive and memory function in induced diazepam male rats.

Keywords: Neurodegenerative, Diazepam, Omega-3, Vitamin E, Memory and Cognitive.

INTRODUCTION

Memory define as brain capability to translate, store, and recall information (Fotuhi. 2004). When someone not able to recall an information, that means there is disruption of memory process that pass through those three steps. Memory and learning process had strong relation one to another so then can be stated as same process. Learning process refer to any translation process of information, whereas memory refer to storing and recalling of information (Baddeley. 2005).

Life style alteration of society and global competition are things that we cannot avoid. Stress, emotion, even anxiety is often happening in society and can cause sleep disturbance or insomnia. Today, diazepam is first drug choice to encounter this problem and can keep their quality of life. In 2011, sales of diazepam in Indonesia reach 2.3 billion tablets, where 82% of it is benzodiazepine (Gjerde. 2011).

The side effects that often happen to diazepam user are sedation, muscle weakness, and ataxia. Diazepam utilization in high dose can cause central nervous system disturbance (neurodegenerative) and damage in other organs like liver and kidney (Regnehed. 2014). Memory disturbance is damage that most often happen as side effect of diazepam use. Memory disturbance is early sign of amnesia, dementia, and even any neurodegenerative disease like alzheimer.

Neurodegenerative is kind of disease that if not treated as soon as possible can cause paralyze and death.

To perform its function in case of memory, needed a neurotransmitter called Acetylcholine (Ach). Ach is part of cholinergic nerve system that important in memory process and learning process. Ach will be hydrolyzed by Acetylcholinesterase (Ache) enzyme to become choline. There is also glutathione (GSH) as brain tissue protector from free radicals or antioxidant. Diazepam will inhibit Ach activity through chain inhibition of GABAnergic receptor and generate free radicals in brain that can induce brain damage or neurodegenerative. All of this can reduce Ach level in nerve so that cause reduces in learning process and memory. In the other hand, diazepam reduces GSH level because its function to neutralize free radicals.

The newest treatment for memory disturbance still as attribute of pharmatheutical, that is piracetam. Piracetam (2-oxo-1 pyrolidine-acetemid) is no tropic agents group that form odorless white crystal powder. Piracetam increases effectiveness of brain hippocampus function through raising function of cholinergic neurotransmitter. According to study that perform by Kosta in 2013, using piracetam induced oxidative stress and excretion 98% through urine and may cause nephrotoxic.

Omega-3 PUFA consist of α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Study by Sarsilmaz in 2003 indicate that ω-3 PUFA is able to modulate several process in secondary degeneration central nervous system through antioxidant and anti-inflammation effect with process that inhibit production of pro-inflammation cytokines. (Sarsilmaz et al. 2003) Meanwhile, vitamin E or alpha-tocopherol is sort of antioxidant that dissolved in lipid and involve in forming inhibition of free radical. Beside of antioxidant effect, vitamin E also has protective effect toward lipid peroxidation that has important role to maintain functional integrity from all of biologic membrane (Merih et al. 2006). The monoteraphy effectivity of ω-3 or vitamin E already proven in several studies that assess ω-3 PUFA + vitamin E effects in diazepam-induced amnesia cases.

5. METHODS AND MATERIAL

5.1. Experimental Animals

Local wistar male rats, weighing about 200gr (3 months), were purchased from Eka Animal house, Veterinary Faculty of Udayana University. Since estrogens (female sex hormone) have found effect on memory, we excluded female mice and used only male rats for the study. Animals were housed separately in groups of 5 per cage (30 x 25 x 15 cm) under laboratory conditions with alternating light and dark cycle of 12 hours cycle. Animal had free access to food and water that refill every 24 h in the evening.

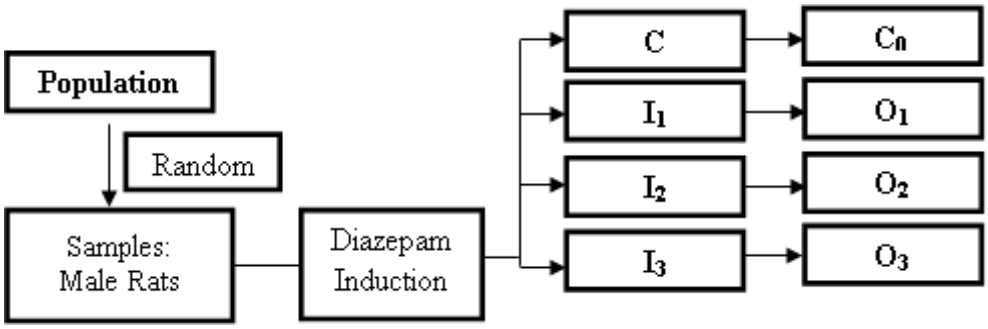
5.2. Drug and chemicals

Stesolid (diazepam) injection 10 mgr/2cc (Actavis, Indonesia), Omega-3 and Vitamin E food suplement (Marine Nutriceutical Inc, Pennsylvania 18343, USA)

5.3. Experimental Design

The formula sample used was Frederer formula: (p-1)(n-1)> 15 with p (intervension) is 5, and the obtained n (minimum sample size) in each goup is 5.

This research was carried out purely experimental with randomized posttest control group.



Notes:

C = Control, given only vehicle

I1 = Control positive, given 1 mg diazepam intramuscularly

I2 = Intervension 2, Diazepam 1 mg and ω-3 PUFA + vitamin E (omega-3 2000 mg and vitamin E 1,5 IU)

I3 = Intervension 3, Diazepam 1 mg and ω-3 PUFA + vitamin E (omega-3 4000 mg and vitamin E 3 IU)

C0 = Posttest group control

O1,O2, O3, and O4 = Posttest of four group (C, I1, I2,and I3) after giving intervention of ω-3 PUFA + vitamin E.

5.4. Research Procedure

5.4.1. Experiment of Morris water maze learning function

Morris water maze (MWM) for mice consisted of a circular pool (60 cm in diameter, 25 cm in height) filled to a depth of 20 cm with water maintained at 25◦C. A submerged platform (with top surface 6 cm × 6 cm) was placed inside the target quadrants of this pool 1 cm upper surface of water. The position of platform was kept unaltered throughout the training session. While in assessment memory, the water was made opaque with nontoxic white colored dye and the platform 1 cm upper surface.

The water maze task is divided into two phases:

1. Acquisition trials: Each animal was subjected to five consecutive trials on each day (from 8th to 10th day) with an interval of 10 minutes, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In a preliminary study, the trial was conducted to familiarize the mouse with the task and was not counted.
2. Retrieval trial: On the 11th day, the platform was submerged and each mouse was allowed to explore the pool for 90 seconds. Mean time spent by the mouse in each of four quadrants was noted. The mean time spent by the mouse in target quadrant for searching the hidden platform was noted as an index of retrieval.

5.4.2. Administration of Diazepam, Omega-3 and vitamin E

Group 2: rats was given Diazepam 1 mg intramuscularly with spuite 1cc for 10 days starting from 11th day to 20th day. Group 3: Diazepam 1 mg and ω-3 PUFA + vitamin E (omega-3 2000 mg and vitamin E 1,5 IU. Group 4: Diazepam 1 mg and ω-3 PUFA + vitamin E (omega-3 4000 mg and vitamin E 3 IU.

5.4.3. Statistical analysis

All the results are expressed as Mean + S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered a significant.

6. RESULT

The effect of oral administration Omega-3 and vitamin E food supplement on diazepam induced amnesia using Morris maze water in rats.

The animal that treated with diazepam (1mg/dose, i.m) has shown significantly increased ELT on day 11th to 15th due to positive control group, group I_2 and I_3. The treatment animal group with Omega-3 and vitamin E 1 soft gel (group 3) and 2 tablet (group 4) per oral on day 13th and 14th has shown significantly decreased ELT compared to control group (Table 1). The summarized of significantly result of ELT by effect Omega-3 and vitamin were on Graph 1.

Table 1. ELT in mean (±SD) compared to control group (*<0,05), **<0,01, *<0,001)**

Treatment	Dose (kg^{-1})	ELT (±SD)				
		Day 11	Day 12	Day 13	Day 14	Day 15
Control	-	7,11 (1,44)	6,44 (2,94)	6,93 (2,39)	5,18 (2,12)	11,60 (6,54)
Diazepam	1 (i.m.)	11,69 (2,43)*	11,32 (1,80)*	10,67 (2,44)*	11,17 (1,73)***	7,20 (7,15)***
Omega-3, Vitamine E, Diazepam	2000mg (p.o.), 1,5 IU (p.o.), 1mg (i.m.)	4,79 (2,59)	4,57 (1,92)	3,98 (1,49)	3,84 (0,89)	4,40 (1,94)
Omega-3, Vitamine E + Diazepam	4000mg (p.o.), 3 IU (p.o.), 1mg (i.m.)	4,38 (1,26)	3,58 (2,84)	2,28 (0,40)**	2,24 (0,38)*	6,80 (4,65)

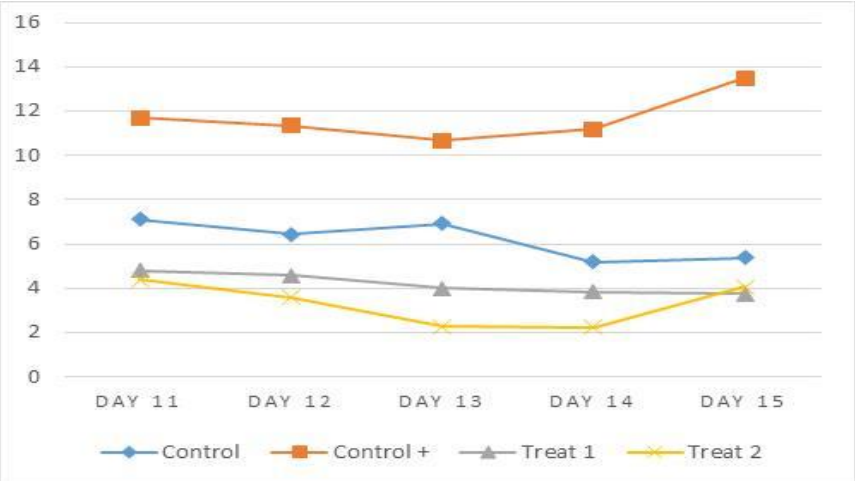


Figure 1 : Effect of oral administration omega-3 and vitamine e on diazepam induced amnesia of escape latency using morris water maze

7. DISCUSSION

In the present of study, we focused on the efficacy of the Omega3 and Vitamin E reversing the memory deficit and improving aquisition and memory retention in diazepam induced amnesia in rat by Morris maze water

ELT were the parameter assesed in diazepam induced amnesia in rat using Morris maze water. On early process, the animal were trained to find the hidden platform for a period 3 days. Then animal induced diazepam and treated by food suplement on day 4th until day 14th

Diazepam works on the GABA (Gamma Amino Acid Butiric) system which is a neurotransmitter inhibitor, meaning that would block impulse conduction in nerve fibers. Gamma Amino Acid Butiric will open the gates of negatively charged chlorine ions so that nerve fibers will be highly negative charged. Impuls will be hard to be delivered through nerve fibers, by strengthening the barrier function of GABA neurons. Benzodiazepines receptors in the central nervous system are a high density, especially in the frontal and occipital cortex of the brain, in the hippocampus and the cerebellum. At this receptor, benzodiazepine will work as agonist. There is a high correlation between the various pharmacological activities by binding to benzodiazepine binding site. With the interaction of benzodiazepines, GABA to its receptor affinity will increase, and with this GABA works will increase. Activation of GABA receptors, chloride ion channels will open so chloride ions will be more likely to flow into the cell. This reaction sequence results in all the nerves and its neurotransmitter will be inhibited causing inhibition of acetylcholine activity.

The activity of GABA receptor also increasing oxidative stress by releasing free radical that damage the tissue called secunder proceess. The brain tissue that damaged by free radical were assosiated with neurodegeneratif disorder. The potential of ω-3 PUFA is able to modulate several process in secondary degeneration CNS through antioxidant and anti inflamation effect with process that inhibit production of pro-inflamation citokines while vitamin E also has protective effect toward lipid peroxidation that has important role to maintain functional integrity from all of biologic membrane. Thus the combination of vitamin E optimize the functions of omega-3 in its use as therapy.

8. CONCLUSION

The result showed the escape latency time (ELT) between control group and treatment groups was significantly different ($p<0.05$). The results indicate that combination omega-3 and vitamin E is useful to enhance cognitive and memory function in induced diazepam male rats.

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