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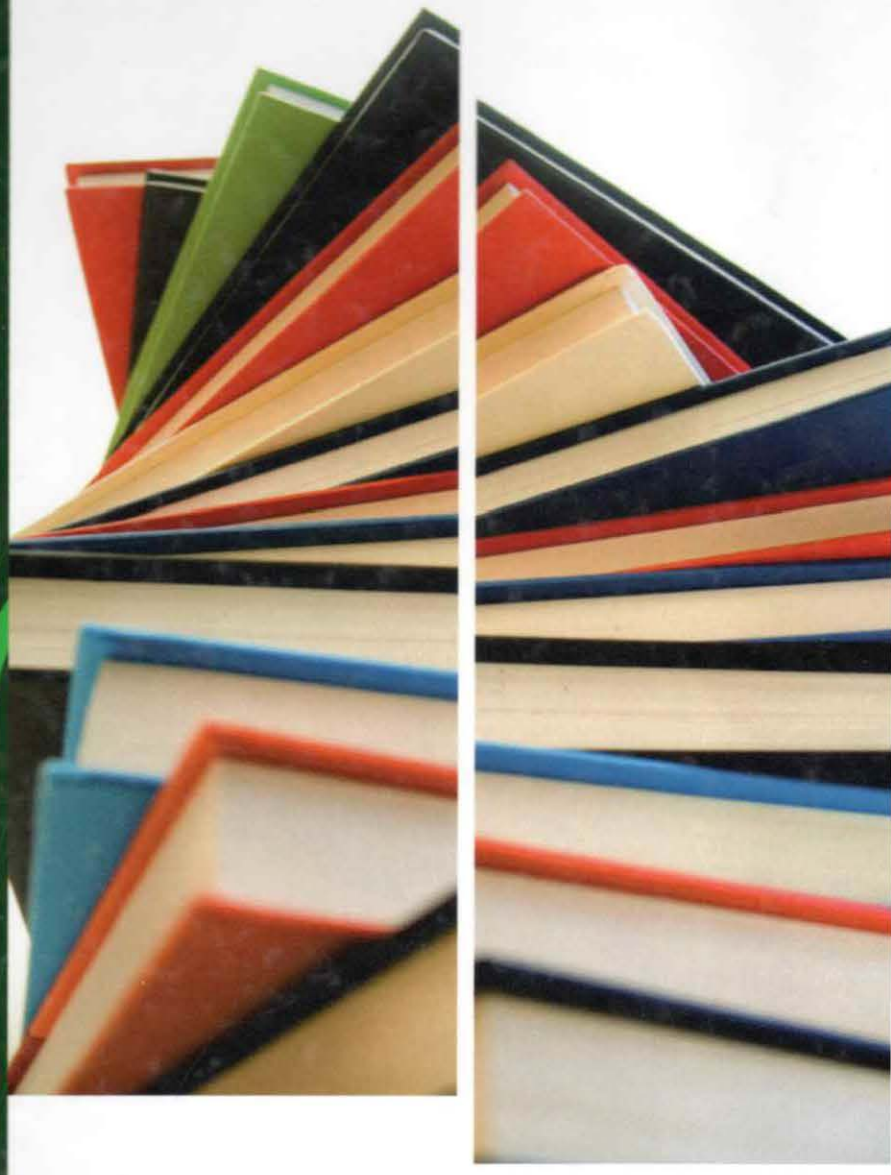

Science for Energy, Food, and Environmental Sustainability

ITS Institut Teknologi Sepuluh Nopember

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PROCEEDING OF

INTERNATIONAL BIOLOGY CONFERENCE

Science for Energy, Food and Environmental Sustainability

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The motor coordination disorder effect of 2-chlorobenzoylthiourea in mice

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Abstract

Recently, stress and mental diseases has increased. The therapies used to treat them are drugs that suppress the central nervous system. To find a new drug that acts on the central nervous system, synthesis of a benzoylthiourea derivative containing acyclic ureide group similar to barbiturates has been conducted. This study used 2-chlorobenzoylthiourea, a benzoiltiourea derivative. The activity assays of 2-chlorobenzoylthiourea as central nervous system suppressant was carried out by observing the motor coordination disorder in mice on the rotarod apparatus. The study used treatment, standard, and control groups. The treatment group were given 2- chlorobenzoylthiourea in five dosage of 15 mg/kgBW, 30 mg/kgBW, 45 mg/kgBW, 60 mg/kgBW and 75 mg/kgBW; the standard group were given 36,4 mg/kgBW of Phenobarbital Na; and control group were given a 0,5% CMC-Na suspension. The mice were left for 60 minutes after the treatments, and then were put on a rotarod apparatus. The parameter measured was how long the mice were able to stay on the rotarod. The results of One-Way ANOVA statistical analysis showed a significant difference between the control group and the treatment and standard groups. There was no significant difference, neither between the treatment and the standard group nor between doses given in the treatment group, but the shortest average lasting time was obtained from the test compound at a dose of 15 mg/kgBW.

Keywords: *2-chlorobenzoylthiourea, motor coordination disorder, central nervous system suppressant, rotarod.*

INTRODUCTION

In Indonesia, the issue of mental health disorders such as anxiety disorders and depression in adults reaches 11.6%. With the adult population of approximately 150 million, there were an estimated 1.74 million people in Indonesia who have mental disorders due to emotional stress (Haryadi, 2011). Generally, people with stress and mental disorders are treated with drugs that can suppress the central nervous system. However, in Indonesia the development of central nervous system-suppressing drugs in an effort to get the desired effects is still not much done, yet in a country that is undergoing rapid development, such as Indonesia, stress and mental illness is increasing. Therefore, it is necessary to develop new drugs that suppress the central nervous system to tackle the issue (Siswandono, 1998).

One of the efforts to get active compounds with optimal activity as a suppressor of central nervous system is to modify the structure of a compound which has the central nervous system suppressant effect. Starting from a study by Siswandono in 1998, the synthesis of benzoylurea has performed through acylation reaction between one of primary amine of urea with benzoyl chloride derivative.

The benzoylurea contains acyclic ureide group which has central nervous system suppressant effect, so it is expected that the compound also has effects on central nervous system (Siswandono, 1998). Kesuma (2004) conducted a synthesis of benzoylthiourea by replacing the oxygen atom at position C2 with sulfur atom in thiourea (H2N-(C = S)-NH2), which later showed a central nervous system suppressant effect (hypnotic effect) from the results of activity assays. Therefore, 2-chlorobenzoylthiourea, a benzoylthiourea derivative then was created through acylation reaction between thiourea with the benzoyl group of 2-chlorobenzoyl chloride. The lipophilic and electronic properties of benzoylthiourea can be increased by inserting Cl atom at the ortho position, and hopefully it will increase the central nervous system suppressant activity (Kesuma, 2006).

The central nervous system suppressant activity in this study was examined by observing motor coordination impairment of mice on rotarod apparatus. The motor coordination disorder test is a pharmacological tests that are easy to use and understandable to figure out if a compound can work on the central nervous system. Experimental animals used in this study were BALB/c mice as they were sensitive to the central nervous system suppressants (Basori, 2003). The stimulation was given in the form of round rods where mice stand and and the response was shown by the ability of mice to stay in the rotarod until they fell. The motor activity is generated by muscle contractions that produce force to move the limbs. The muscles function normally if the nervous system, spinal cord, and muscles are completely connected and working properly. Therefore, the observation of motor coordination impairment in this study was done because the compounds that can suppress the central nervous system will affect the neuromuscular stimulation which finally lowers motor coordination.

The results of this study are expected to provide information on the motor coordination impairment effect of 2-chlorobenzoylthiourea as a potential central nervous system-suppressing drug and provide useful additional information in designing new, rational, and potent drugs.

MATERIALS AND METHODS

Materials

2-chlorobenzoylthiourea, Phenobarbital Na, CMC-Na, aquadest.

Instruments

Rotarod (round rod), oral syringe, animal scale, measuring cylinder, beaker glass.

Animals

The animals used were adult male BALB/c mice (Mus musculus), with the age of 2-3 months and weighing 20-30 grams; with no abnormalities visible on the body. The mice were fed standard food during caged. One week prior to treatment, the mice were adapted to the room where the study was conducted. The mice were fasted for 12 hours before treatment and every mouse was only used once.

Experimental methods

The research was divided in two parts:

1. The determination of Time Peak Effects (TPE)
   The mice were randomly divided into six groups, with each group consisting of 2 mice. All mice in each group were given 45 mg/kg of 2-chlorobenzoylthiourea orally and then were left for 15 minutes (group I), 30 minutes (group II), 45 minutes (group III), 60 minutes (group IV), 75 min (group V) and 90 minutes (group VI). After that, the mice were placed on a rotarod and it was rotated at a speed of 6 rpm. The fastest faling time was considered as the time peak effects (TPE) of 2-chlorobenzoylthiourea.

2. The motor coordination impairment test of 2-chlorobenzoylthiourea
   The mice were divided randomly into 7 groups and each group consisted of 10 mice. The standard group was given Phenobarbital Na, the control group was given 0.5 % suspension of CMC-Na, and the treatment group were given 2-chlorobenzoylthiourea suspension at a dose of 15 mg/kgBW (treatment I), 30 mg/kgBW (treatment II), 45 mg/kgBW (treatment III), 60 mg/kgBW (treatment IV) and 75 mg/kgBW (treatment V). All treatments were administered orally. Furthermore, the mice were left in place for the time gained from TPE determination, then the mice were placed on the rotarod and the duration of mice being able to stay on the rotarod were observed.

Data analysis

The data from TPE determination were obtained by observing the shortest time in which the mice were able to stay on a rotating rod (rotarod). The data of motor coordination impairment of mice which showed motor activity were obtained by observing the duration of mice lasted on the rotating rod in each treatment group, and the data were analyzed using One-Way Anova.

RESULTS AND DISCUSSION

The observation result from TPE determination can be seen on Table 1.

Table 1. The Results from TPE Determination of 2-Chlorobenzoylthiourea

Mice number	Average time of mouse staying on the rotarod (sec)						
	Control (CMC Na 0,5%)	Standard (Phenobarbital-Na 36,4 mg/kg BB)	T_1 (15 mg/kg BB)	T_2 (30 mg/kg BB)	T_3 (45 mg/kg BB)	T_4 (60 mg/kg BB)	T_5 (75 mg/kg BB)
1	155	9	12	9	26	30	18
2	177	8	10	5	26	42	28
3	242	11	13	9	14	33	46
4	339	14	9	8	20	14	14
5	39	19	13	14	19	24	25
6	32	14	17	10	13	38	42
7	50	9	24	13	8	24	28
8	20	10	6	18	14	33	25
9	32	18	19	29	9	31	28
10	23	18	17	28	14	30	19
Mean ± SD	**110.9 ± 111.98**	**13 ± 4.19**	**14 ± 5.31**	**14.3 ± 8.30**	**16.3 ± 6.31**	**29.9 ± 7.85**	**27.3 ± 10.05**

The data of the mice's motor coordination impairment that was shown by its motor activity can be seen on Table 2 and the diagram that shows the average time of mice staying on the rotarod can be seen on Figure 1.

Table 2. The Results from Observation of Movement Coordination Impairment of the Mice that was Given 2-Chlorobenzoylthiourea

Mice number	Average time of mouse staying on the rotarod (sec)						
	Control (CMC Na 0,5%)	Standard (Phenobarbital-Na 36,4 mg/kg BB)	T_1 (15 mg/kg BB)	T_2 (30 mg/kg BB)	T_3 (45 mg/kg BB)	T_4 (60 mg/kg BB)	T_5 (75 mg/kg BB)
1	155	9	12	9	26	30	18
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6	32	14	17	10	13	38	42
7	50	9	24	13	8	24	28
8	20	10	6	18	14	33	25
9	32	18	19	29	9	31	28
10	23	18	17	28	14	30	19
Mean ± SD	**110.9 ± 111.98**	**13 ± 4.19**	**14 ± 5.31**	**14.3 ± 8.30**	**16.3 ± 6.31**	**29.9 ± 7.85**	**27.3 ± 10.05**

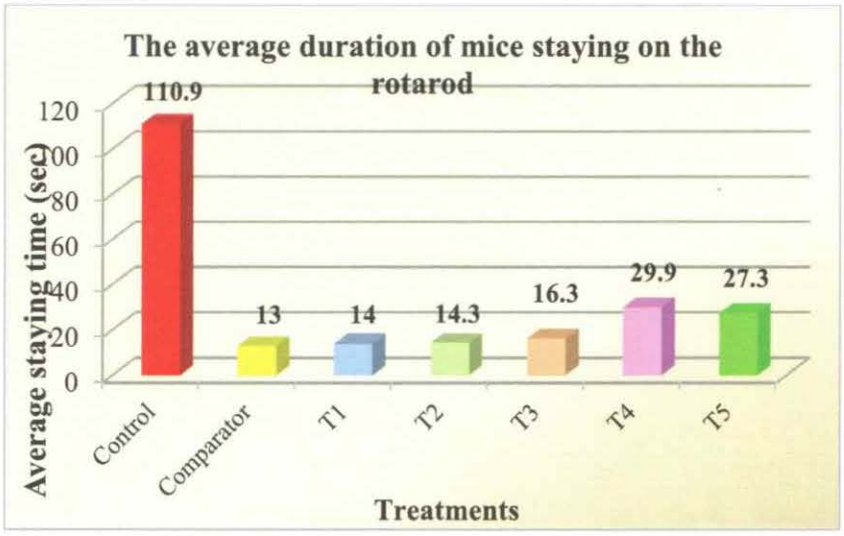

Figure 1. The diagram of the mean duration of mice staying on the rotarod of the control, standard and treatment group

To see if there is a significant difference between the groups, One-Way Anova was used for statistical analysis. The results from the statistical analysis can be seen on Table 3

Table 3. The Results from Statistical Analysis

Groups (I)	Groups (J)	Significance value
Control	Treatment 1	0,000 *)
	Treatment 2	0,000 *)
	Treatment 3	0,000 *)
	Treatment 4	0,000 *)
	Treatment 5	0,000 *)
	Standard	0,000 *)
Standard	Treatment 1	0,959
	Treatment 2	0,946
	Treatment 3	0,864
	Treatment 4	0,381
	Treatment 5	0,458
Treatment 1	Treatment 2	0,988
	Treatment 3	0,905
	Treatment 4	0,410
	Treatment 5	0,490
Treatment 2	Treatment 3	0,917
	Treatment 4	0,419
	Treatment 5	0,500
Treatment 3	Treatment 4	0,481
	Treatment 5	0,568
Treatment 4	Treatment 5	0,893

Notes:
* the difference was significant ($\alpha < 0,05$)

Theoretically, benzoylthiourea has lipophilicity (log P) of 1.12. The lipophilicity (log P) of the compound can be increased to 1.68 by incorporating the Cl atom on the aromatic ring, while the optimal log P value for the central nervous-suppressant drugs system is 2 (Siswandono & Soekardjo, 2008). Therefore, the study was conducted to prove that benzoylthiourea derivative (2-chlorobenzoylthiourea) has central nervous system suppressant effect through an experiment by observing motor coordination impairment of mice on rotarod apparatus. The motor coordination impairment test is a test used to observe motor disorders in mice, demonstrated by the inability of mice to maintain its position on the rotarod. Stimulation was given by rotating the rod where the mice stood on a certain speed. Normal mice will be able to maintain its position in a long time.

In order to perform some motor movements, good coordination between the nervous system, neurotransmitter release, and muscle is required. In muscle contraction mechanism, action potentials that runs along the nerves is needed, resulting in neurotransmitter release. The neurotransmitters then bind to the receptors on muscle cell membrane and the movement of the muscle cell miofilamen seen as muscle contraction occurs (Martini Frederic H., 2006). The the central nervous system suppressant may affect neuromuscular stimulation resulting in motor movement lowering.

The mice used in this study were white male BALB/c mice because they are sensitive to the effects of CNS (Central Nervous System) drugs and used as animal standard for various CNS experiments. The mice used must be naive, which have never received any treatment, because mice which have recognized the container or experimental area show different behavior compared to naive mice.

Based on Table 1, it is known that Time Peak Effect (TPE) or peak activity time of 2-chlorobenzoylthiourea was 60 minutes because at that time the mice could stay on the rotarod within the minimum average duration at 7.5 seconds.

Based on Table 2 and Figure 1, the minimum average duration of mice staying on the rotarod obtained by the standard group which was given Phenobarbital Na and treatment group I which was given 15 mg/kg BW of 2-chlorobenzoylthiourea. The results of One-Way ANOVA analysis in Table 2 to determine if there was a significant difference between the effects of the standard, control, and treatment group. From the analysis, it was found that there are significant differences between the control group and the treatment group to the standard group; there was no significant difference between the standard group and the treatment group; and there was no significant difference between the treatment groups. The results suggest 2-chlorobenzoylthiourea can provide central nervous system suppressant effect by disrupting the motor coordination of mice on the rotarod apparatus. The structure of 2-chlorobenzoylthiourea contains ureida acyclic group similar to barbiturates. Therefore, 2-chlorobenzoylthiourea can suppress synaptic transmission thus reducing cell stimulation at the post-synaptic membrane.

2-chlorobenzoylthiourea is able to penetrate into the brain and is believed to bind to $GABA_A$ receptor because of structural similarities to barbiturate (ureida group content) so it can prolong the opening of Cl-channels and causes the post-synaptic membrane to hyperpolarize. In this state, the action potential may not occur so that there won't be any muscle contraction.

CONCLUSION

Based on these results, it can be concluded that 2-chlorobenzoylthiourea can disrupt motor coordination of the mice so that the compound can act as a central nervous system suppressant, and a dose of 15 mg/kg gave the shortest duration to stay on the rotarod.

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CERTIFICATE of APPRECIATION

is hereby presented to

Aguslina Kirtishanti

for valuable participation as **PRESENTER** with poster entitled

The motor coordination disorder effect of 2 - Chlorobenzoylthiourea in mice

at **International Biology Conference (IBOC) 2012**
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