



Toxicity Test of LD50 Zinc (II) N-Benzyl Methyl Dithiocarbamate Complex Compounds on White Mice (*Mus muscules*)

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Abstract:

Dithiocarbamate complex compounds can be used as an antibacterial and antifungal. Zinc compounds function as antioxidants, antibacterials, and zinc can stop the proliferation of cancer cells. Based on some of the activities of these groups, it is expected that the Zinc (II) N-Benzylmethyl Dithiocarbamate complex compound has the potential to be developed as a new drug candidate. A toxicity test must be carried out to determine the level of safety of the compound. The objective of this study was to determine the LD50 value and determine the category of toxicity of the Zinc (II) N-Benzyl methyl Dithiocarbamate compound in white mice (*Mus muscules*). Methods This research is a laboratory experimental study using 40 white mice, consisting of 20 male mice and 20 female mice. As for the treatment, namely the Na CMC group as a negative control, the dose of the test substance was 240, 480, 960mg/Kg BW. Observations of mice were carried out for 24 hours by observing the number of dead animals and seeing toxic symptoms, body weight, and OWR (Organ Weight Relative) then the data were analyzed statistically. Results The results showed that the dose of 960mg caused the most death with LD₅₀ values of 954mg and 794 mg of toxic symptoms, weight loss in mice, and an effect on OWR

Keywords: Zinc (II), LD₅₀, Toxicity Test

1. Introduction

Introduction Zinc is a chemical element with the chemical symbol Zn, atomic number 30, and a relative atomic mass of 65.39. It is the first element of group 12 on the periodic table. Some of the chemical properties of zinc are similar to magnesium. This is because the ions of these two elements are almost the same size. According to the Indonesian Pharmacopoeia edition III (1989) in the pharmaceutical world Zinc chloride in the form of powder, amorphous is very fine, white, odorless, and practically soluble in water and in ethanol. Zinc chloride works as a bacteriostatic and eradicates microorganisms that are on the surface of the skin, or as a local antiseptic. Zinc compounds are also able to inhibit the process of converting H₂O₂ into OH, besides that Zn functions to produce antioxidant molecules, namely metallic thionic to protect the body from radiation exposure, anti-bacterial, anti-cancer, and various other oxidative stress conditions. In addition, zinc also can stimulate androgen hormones (testosterone) in Leyden cells, so that in the process of spermatogenesis zinc plays a role (1). The synthesis of Dithiocarbamate complex compounds is



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known to be able to be used as an antibacterial, based on the results of research produced that this compound has antibacterial activity and also does not cause cytotoxic in liver cells (2). Previous research (2) reported that the 6-coordinated organotin (IV) Dithiocarbamate complex compound (Octahedron, i.e. the N-methyl Dithiocarbamate compound Dithiocarbamate di butyl tin(IV)) has anticancer activity.

Study (3) reported that several complexes synthesized organotin (IV) from N-methyl-N- phenyl dithiocarbamate tested in vitro proved to be able to resist bacterial growth (*S. aureus*, *B. cereus*, *K. pneumonia*, *P.aeruginosa*), and phenyl and diphenyl tin complexes showed good antibacterial activity with inhibitory power ranging from 10 – 21 mm. A study (2) reported that the synthesis of diphenyl tin (IV) N-methyl benzyl Dithiocarbamate compounds was proven to have antifungal activity against *Candida albinos* and *Aspergillus Niger* fungi with an average inhibitory power of 17 mm (dose 0.050 grams) and 16.58 mm (dose 0.050 grams) respectively

Based on several pharmacological activities carried out in previous studies, it is expected that the compound Zinc (II) N-Benzyl methyl Dithiocarbamate has the potential to be developed as an efficacy. To become a drug candidate, of course, one must go through a test of its safety aspects or the effects of toxicity to be used in humans, one of the methods used to test this safety uses a toxicity test. The toxicity test that can be done is an acute toxicity test. The advantage of this acute toxicity test method is the short time used in detecting toxic effects compared to chronic toxicity test methods.

A toxicity test is a test to detect the toxic effects of a substance on biological systems and to obtain typical dose-response data from test preparations useful to see the presence of biochemical, physiological, and pathological reactions in humans to test preparation. Toxicity test results cannot be used absolutely to prove the safety of preparation in humans, but they can provide clues to relative toxicity and help identify toxic effects in the event of human exposure. The factors that determine the reliable in vivo toxicity test results are the selection of the test animal species, strain, and several animals; mode of administration of test preparations; test dose selection; side effects of test preparations; Testing techniques and procedures including how animals are handled during experiments (4).

Research Methods In this study using experimental methods carried out acute toxicity tests of Zinc (II) N-benzyl methyl Dithiocarbamate complex compounds in white mice. The materials used in this study include: Test animals used male and female white mice, primary amine n-Benylmethylamine ($C_8H_{11}N$) (Sigma Aldrich), carbon disulfide (CS_2), metal diphenyl tin (IV) dichloride (C_6H_5)₂ SnCl₂ (Sigma Aldrich), methanol, Na CMC 0.5%, NaCl.



2. Method

In this study, using samples of N-Benzyl methyl amine compounds as much as 2.58 ml, carbon disulfide (CS₂) as much as 1.52 ml, and zinc chloride 2.72 grams, then added with methanol as much as 15 ml to each of these compounds. FTIR testing of the results of synthetic compounds in this study is by the expected formula, this can be seen from several spectrum absorption results. Based on NMR testing of complex compounds tested on the ¹³C NMR spectrum, carbon values are obtained at CH₃, CH₂, and C–N, and for aromatic carbon values this is also following the expected formula and following the range of carbon shift regions determined by the ChemDraw application. Based on the test compound test results against the ¹H NMR spectrum, the shift region of CH₃, CH₂, and aromatic protons is also by the estimated ChemDraw application. Then formed the compound Zinc (II) N-Benzyl methyl Dithiocarbamate with a weight obtained of 7.2 grams. Furthermore, an acute toxicity test was carried out using mouse test animals.

This toxicity test was carried out because the complex compound Zinc (II) N-Benzyl methyl Dithiocarbamate has been shown to have activity as, an active antibacterial at 100 ppm and to be a candidate for new drugs, it is necessary to conduct a toxicity test to determine the level of safety of the compound. The toxicity test in this study used the acute toxicity test method. This acute toxicity test is to detect the drug effect of a toxic substance that appears within a short time after being administered orally in a single within 24 hours. When compared to other toxicity test methods, this acute toxicity test uses a shorter timeframe than other toxicity tests (5). In this study, male and female mice were used as test animals because one of the factors that affect the work of the drug, namely sex, hormonal differences, and absorption (absorption) is different, then later it will be compared, the test animals used are mice because they are more economical, easy to obtain, and easy to care for (6). The mice used are first acclimatized for 7 days, acclimatization is done so that the mice adapt to the new environment. During acclimatization, mice are fed and watered, and placed separately between male mice and parrot mice.

Based on Table 4.4, visual observation of symptoms in symptomatic mice showed that test animals given preparations of 240mg/Kg BW 480mg/Kg BW, and 960mg/Kg BW experienced seizures, weakness, dilated pupils, tremors, standing hair when compared to the negative control group. This means that it shows the presence of toxic symptoms. Furthermore, after 24 hours of testing, the LD50 value is calculated. Based on Table 4.5, it can be seen that at a dose of 240mg / Kg BW does not cause the death of test animals in male mice, while in female mice causes the death of 1 mouse, and at a dose of 480mg/ Kg BW causes the death of test animals in male mice as many as 2 heads than in female mice as many as 2 mice while at a higher dose of 960mg / Kg BW when given to male mice test animals cause the death of 4 mice and in mice, The female caused the death of as many as 4 mice. When compared with the provision of control did not cause death in both male mice and female mice, and then the calculation of LD50 values was carried out. Based on the calculation of LD50 using the Thomson Weil method where the male mice LD50 value is 954mg / Kg BW and in female mice is 794.3mg / Kg BW, it can be said that the compound Zinc (II) N- Benzyl

methyl Dithiocarbamate according to BPOM (2014) is included in the criteria of slightly toxic with a range of 500-5000mg / Kg BW.

In addition to LD50, another parameter observed was the body weight of mice, based on Table 4.7 it was seen that there was a decrease in mouse weight after being given a preparation of the complex compound Zinc (II) N-benzyl methyl. Based on statistical tests using SPSS with paired T-tests, it obtained a significance value of <0.001 ($p < 0.05$), meaning that there was a difference in body weight before treatment and after treatment. In toxicity studies, test animals receiving high doses typically lost weight due to decreased appetite (8). Then surgery was carried out on experimental animals to observe the organs after being given doses. According to (9) the toxicity test can be several categories for example toxic a substance, and one of the categories is the affected organ. In this category the affected organs e.g. CNS, heart, liver, kidneys, and so on. In this study, the organs observed were the kidney, liver, and heart.

3. Results and Discussion

The result of the synthesis of compounds in the form of white odorless powder weighing 7.2 grams.

Results of functional group analysis using a Fourier Transform Infrared (FTIR) spectrophotometer, the results of the FTIR spectroscopic analysis, yielded a functional group of the compound Zink(II) N-benzyl methyl dithiocarbamate, so that the compound bonds formed by bidentate can be predicted, the expected compounds can be characterized by FTIR, the expected functional group number results data can be seen in Table 1

Table 1 FTIR Analysis Results of Synthesis of Complex Compounds Zinc (II) N- Benylmethyl Dithiocarbamate

Absorption Region (cm^{-1})	Complex	Wave number(cm^{-1})
	Functional group	
1020 – 1250	C – N	1028,06
2840 – 3000	C – H	2918,30
700 – 1563	C – S	696,30
486-670	Zn-S	565,14

Measurement analysis results of ¹H spectrum NMR spectroscopy, the results of proton NMR analysis of the Zink (II) N-Benzylmethyl Dithiocarbamate compound, found methyl peaks, ethyl peaks, and benzyl peaks, confirm that this compound was successfully synthesized, the peak results can be seen in the table2.

Table 2. Results of NMR Analysis of ¹H spectrum Synthesis of complex compounds Zinc (II) N-Benylmethyl Dithiocarbamate

n (ppm) (Silverstein <i>et al.</i> , 2005)	Results of proton analysis Complex compound Zinc (II) N-Benylmethyl dithiocarbamate
δ 0 – 3 ppm (CH ₃)	0 (CH ₃) (s)
δ 3 – 5 ppm (<i>β</i> – substituted aliphatic)	3,36-5,12 (CH ₂) (m)
δ 6 – 9 ppm (Aromatik)	7,32– 7,41 (C ₆ H ₅) (m)

NMR spectral measurement analysis results in ¹³C, the results of carbon NMR analysis of the Zink (II) N-Benylmethyl Dithiocarbamate compound, found methyl peaks, ethyl peaks, and benzyl peaks, this confirms that this compound was successfully synthesized, the peak results can be seen in table 3.

Table 3. Results of NMR Analysis of ¹³C spectrum Synthesis of complex compounds Zinc (II) N-benzyl methyl Dithiocarbamate

Carbon Shift Areas (ppm) (Silverstein <i>et al.</i> , 2005)	Analysis of the complex compound Zinc (Results II) N-Benylmethyl dithiocarbamate
δ 0 – 50 ppm (C alkyl)	0 (CH ₃) (s)
δ 40 – 75 ppm (CH ₂)	41,76 (CH ₂) (s)
δ 45 – 70 ppm (C – N)	60,41 (C – N) (s)
δ 100 – 150 ppm (C Aromatic)	127,99– 134,70 (C ₆ H ₅) (m)

Toxicity Test

The results of the toxicity test can be seen in the following table.4 - 8

Toxicity Symptoms

Table 4. Acute Toxicity Test Symptom Results

Symptom	Negative Control		240mg/ Kg BW Mice		480mg/ Kg BW Mice		960mg/ Kg BW Mice	
	Male	Female	Male	Female	Male	Female	Male	Female
Stiff	-	-	-	-	-	-	-	-
Weak	-	-	+	+	+	+	+	+
Dilated pupils	-	-	+	+	+	+	+	+
Tremor	-	-	+	+	+	+	+	+

Hair stand up - - + + + + + +
information: + = experiencing symptoms, - = not experiencing symptoms

Table 5. Results of the Number of Deaths

Treatment	Number of Mice	Number of Deaths of Test Animals	
		Male	Female
Control (-)	5	0	0
I (240mg)	5	0	1
II (480mg)	5	2	2
III (960mg)	5	4	4

Table 6. Results LD50 Value and Toxicity Category

Gender	Value LD ₅₀	Category
Male	954mg/Kg BW	Slightly toxic
Female	794mg/KgBW	Slightly toxic

Weight Loss in Mice

Table 7. Average Results of Mouse Weight Loss

Treatment	Gender	BW Average Start Average (G)	BW End Average (G)
Negative control (-)	Male	28,20	26,40
	Female	26,80	25,80
I (240mg)	Male	28,00	25,40
	Female	26,60	24,00
II (480mg)	Male	28,00	24,80
	Female	27,40	24,90
III (960mg)	Male	27,20	23,80
	Female	27,60	23,20

1. Organ Weight Ratio (OWR)

Table 8. Average Grade Results OWR (Organ Weight Ratio)

Treatment	Kidney (%)		Heart (%)		Lung (%)	
	Male	Female	male	Female	Male	Female
Negative control (-)	1,75	1,64	9,57	8,82	1,27	1,15

I (240mg)	1,05	1,58	9,18	8,70	1,13	1,16
II (480mg)	1,27	1,47	8,44	7,79	1,00	1,08
III (960mg)	1,47	1,26	7,80	7,28	1,01	0,98

Conclusion

1. From the results of research that has been done, it can be concluded that: Zinc Complex Compound (II) N-Benzyl methyl Dithiocarbamate can cause death in male mice and female mice, with LD50 values of 954mg / Kg BW in male mice and female mice 794.3 mg/ Kg BW. This compound is categorized as slightly toxic with a range of 500-5000mg/Kg BW, meaning that this complex compound has a slightly toxic effect on male mice and female mice From the results of research that has been done it can be concluded that: Zinc Complex Compound (II) N-Benzyl methyl Dithiocarbamate can cause death in male mice and female mice, with LD50 values of 954mg / Kg BW in male mice and in female mice 794.3 mg / Kg BW. This compound is categorized as slightly toxic with a range of 500-5000mg/Kg BW, meaning that this complex compound has a slightly toxic effect on male mice and female mice.

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A Brief Author Biography

1st Mukhlis Sanuddin – (author 1) Mastered chemistry by research as a lecturer in study pharmacy, experienced in research on, he played a role in characterizing compounds with FTIR and FTNMR.

2nd Medi Andriani – (master of pharmacy) experienced in pharmaceutical Pharmacology, both as testing the toxicology of complex compounds against white mice

3rd Febri Vitriasari (BSc pharmacy) in this study played a role in the synthesis of Zink (II) N-Benzylmethyl Dithiocarbamate Complex Compounds