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SHORT COMMUNICATION

LD50 Acute Toxicity Test of the Anticancer Compound of Dibutyltin (IV) Bis-N-Benzyl Methyl Dithiocarbamate in White Mouse (Mus musculus)

MUKHLIS SANUDDIN[1]*, VIRA SRIWULAN DAMAYANTI[2], SISKA EMILIA NASRIL[1], AMELIA SOYATA[1], INDRI MEIRISTA[1], MEDI ANDRIANI[1]

[1]Sekolah Tinggi Ilmu Kesehatana harapan Ibu Company, Jambi Indonesia
[2]Kader Bangsa Palembang University, Indonesia

Abstract

Organotin compounds are organometallic compounds composed of one or more tin-carbon (Sn-C) bonds. Organotin compounds have been shown to have anticancer, antibacterial, and antitumor activities. Based on the several activities of the organotin group, it is hoped that the Dibutyltin (IV) *Bis-N*-Benzylmethyl Dithiocarbamate compound has the potential to be developed as a new drug candidate. To be a candidate for a new drug, the study was aimed to test and determine the safety level of the compound. Study to determine the LD_{50} value and category of toxicity of the Dibutyltin (IV) *Bis-N*-Benzylmethyl Dithiocarbamate compound in white mice (*Mus musculus*). This research is a laboratory experimental study using 40 white mice, consisting of 20 male mice and 20 female mice. The dose of the test substance was 240, 480, and 960mg/Kg body weight. Observations of mice were carried out for 24 hours by observing the number of dead animals and seeing toxic symptoms, body weight, and ROW (Relative Organ Weight) then the data were analyzed statistically. The results showed that the dose of 960 mg caused the most death with LD_{50} values of 776.2mg and 794.3mg of toxic symptoms, weight loss in mice, and an effect on ROW. This compound can cause death in male and female mice, with the LD_{50} value of male mice being 776.2mg/kg body weight and female mice at 794.3mg/ kg body weight, and this compound is categorized as slightly toxic.

Keywords: Dibutyltin (IV), LD_{50}, Toxicity Test Anticancer

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INTRODUCTION

The Organotin compounds are organometallic compounds composed of one or more tin-carbon (Sn-C) compounds[1] these compounds also have a direct link between the tin-carbon atoms of the organic group directly. Organotin (IV) compounds have interesting chemical properties and structures, used as agricultural biocides, antifungals, anticancer agents, and antitumors [2]. One of the compounds belonging to the organotin (IV) group is Dibutyltin(IV) Bis-N-Benzylmethyl Dithiocarbamate. The use of this dithiocarbamate compound depends on the chelating properties of the dithiocarbamate ligand to metal ions [3]. These organotin compounds can provide pharmacological activity in the pharmaceutical field. A previous study by [4] has succeeded in synthesizing 3 organotin compounds, namely triphenyl tin (IV), dibutyltin (IV), diphenyl tin (IV), and triphenyl tin (IV) compounds providing anticancer activity against L-1210 leukemia cancer cells. In another study [5] an organotin compound was synthesized which produced the compound Diphenylstanum (IV) N-Methylbenzil dithiocarbamate as an antifungal against the fungus Candida albicans. The compound Dibutyltin (IV) *Bis-N*-Benzylmethyl Dithiocarbamate has been patented as an anticancer P388 [6].

Other dithiocarbamate compounds also function as leukemia anticancer [7]. dithiocarbamate compounds as antibacterial inhibitors [8]. agricultural industry and biology of alkyl triphenylene (IV) compound phenyl dithiocarbamate can be used as an insecticide [9], N-methylN-phenyl dithiocarbamate as an anti-microbial[3][10]. Dithiocarbamate also fungsions as anticancer leukemia, and berast cancer[11][12][13]

Based on several pharmacological activities of the organotin (IV) group carried out in previous studies, the Dibutyltin (IV) *Bis-N*-Benzylmethyl Dithiocarbamate compound is expected to be developed as a drug candidate. To become a drug candidate one must go through a test for its safety aspect or its toxicity effect so that it can be used in humans one of the methods used to test this safety is using a toxicity test. It can be done in the acute toxicity test. The advantage of this acute toxicity test method is the short time

*Correspondence to: Mukhlis Sanuddin, MSc, 1Sekolah Tinggi Ilmu Kesehatana harapan Ibu Company, Jambi Indonesia.
Tel: 085268464461, Email: mukhlissanuddinmsc@gmail.com

used in detecting toxic effects compared to the chronic toxicity test method. An acute toxicity test is a test carried out to detect the toxic effect of a substance or compound on biological or non-biological systems. This toxicity test itself uses test animals to see the presence of toxic substances or compounds that will be given in a single dose. The toxic effect of a substance or test can be seen within 24 hours after the administration of the substance or compound to test animals. the acute toxicity parameter of a substance or compound can be seen from the median lethal dose value or LD_{50}[14]). The LD_{50} value is a single dose of a substance or compound that can statistically require 50% of the test animals. LD_{50} is determined by administering a substance or compound in varying doses (levels) to a group of test animals.

In previous research by [15] Toxicity and Anti-inflammatory tests have been carried out with the compound 1,5-Bis(3'-Ethoxy-4'-Hydroxyphenyl)1,4-Pentadine-3-on (EHP) which has a The LD_{50} of the EHP compound is 6.8675 g/kg body weight, which is classified as mildly toxic.[16] Conducted an in vivo assessment of the acute toxicity of a new quinoxalinone (6-nitro-2(1H)-quinoxalinone) using animals. Wistar rats were tested at doses of 20, 40, 60, 120, 200, and 300 mg/Kg body weight. The test results show that the LD_{50} value 161.16 mg/kg body weight with a dose of 40 mg/kg body weight does not cause toxicity and affect the weight of organs (liver, kidney, spleen, pancreas, heart, brain).

MATERIALS

Equipment (animal scales, analytical balance, stirrer (IKA C-MAG HS7), aluminum foil, filter paper, funnel, volume pipette, suction ball, 1 cc syringe, oral sonde, surgical instrument, measuring cup, stirring rod, Erlenmeyer, mortar, mechanic, glass vial, tweezers, cotton swab, scalpel, surgical scissors). Materials (Primary amine is N-benzyl methyl-amine (Sigma Aldrich), carbon disulfide (CS_2) (Merck KGaA), metal dibutyltin (IV) (Sigma Aldrich), Na CMC, ammonia, and test animals used male and female white mice).

Methods Sample Setup

The Primary Amines (N-Benzylmethylamine), CS_2 ,and dibutyltin(IV)dichloride Synthetic Compounds[17]

2.58 Ml of N-Benzylmethyl Amine ($C_8H_{11}N$) was pipetted into an Erlenmeyer and added to 15 ml of methanol. A solution of carbon disulfide (CS_2) was pipetted as much as 1.2 mL was put into an Erlenmeyer and added to 15 mL of methanol. Metal Dibutyltin Weighed 3.03 g Then 15 mL Methanol. After dissolving, each solution slowly added, then added a little ammonia and then the mixture stirred for ± 2 hours. After a precipitate formed, it filtered and put into a vial, and then stored in a desiccator. The Samples Formed Will Perform Acute Toxicity Test.

In this study, 40 male mice, 20 male mice, and 20 female mice were used and divided into four groups consisting of five male mice and five female mice with separate cages between male and female mice.

Acclimatization

Mice were acclimatized for 7 days and given adequate food and water.

Treatment

The treatment stage for mice (Mus musculus) with the acclimatization of mice for 7 days. After that,weighed. Then the mice were fasted (not eating but still drinking) for 18 hours. After fasting, the mice were given preparations according to their weight of the mice. The test given at various doses of 240, 480, and 960 mg/kg body weight and negative control of Na-CMC orally.

Observation

Clinical symptoms were evaluated after presenting the test preparation for the first 4 hours. The counting of dead mice was carried out from the time of treatment until 24 hours after treatment, and the observed toxic symptoms included fur, enlarged pupils, weakness, convulsions, tremors (shaking), and death. Then the value of LD_{50} and ROW (Relative Organ Weight) with the formula below

$$\text{Log } LD_{50} = \text{Log } D + d\,(f+1)$$

Information

D = smallest dose administered d = log r (multiples of dose) f = factor (Weil table) LD_{50} = anti log of LD_{50}

$$Ratio\ Organ\ Weight = \frac{Organ\ Weight}{Body\ Weight} \times 100\%$$

RWO = Relative Organ Weight
MOW = Mice Organ Weight
BW = Body Weight

Data Analysis

The data obtained were the number of experimental animals that died, which then calculated the LD_{50} value using the Thomson Weil method after which the toxicity category was seen. The parameter taken the body weight of the test animals where the results processed using a paired T-Test. Paired T-Test test aims to see if there is an average difference between BW before treatment and after treatment. The value of % ROW (Relative Organ Weight) after that the results processed using Two Way aims to be able to see the effect of dose + gender on organ ROW values.

The compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate was formed with a weight of 4.81 grams. Furthermore, an acute toxicity test was carried out using mice as test animals.

In this study, male and female mice were used as test animals. Animals in the form of mice because they are economical, easy to obtain, and easy to care for (Radji, 2008). The mice used were first acclimatized for 7 days, acclimatization was carried out so that the mice adapted to the new environment.

Based on table 1, visual observations of symptoms in symptomatic mice showed that the test animals that were given 240, 480, and 960 mg/Kg body weight preparations experienced tremors, weakness, enlarged pupils, tremors, and hair standing when compared to the negative control group. This means showing the presence of toxic symptoms.

Table 1. Result of Acute Toxicity Test Symptoms

Symptom	Negative Control		240mg/ kg body weight Mice		480mg/ kg body weight Mice		960mg/ kg body weight Mice	
	Male	Female	Male	Female	Male	Female	Male	Female
Convulsions	-	-	-	-	-	-	-	-
Weakness	-	-	+	+	+	+	+	+
Eye Pupil	-	-	+	+	+	+	+	+
Tremors	-	-	+	+	+	+	+	+
Hair standing	-	-	+	+	+	+	+	+

Notes Information: (+) : experiencing symptoms, (-) : not experiencing symptoms

Furthermore, after 24 hours of testing, the LD_{50} value was calculated. Based on table 2, it can be seen that a dose of 240mg/ kg body weight did not cause the death of test animals in male mice, while in female mice it caused the death of 1 mouse, and at a dose of 480mg/Kg body weight, it caused the death of test animals in 4 male mice and then in female mice as much as mice. At a higher dose of 960mg/ kg body weight when given to test animals, male mice caused the death of 5 mice and in female mice, it caused the death of 4 mice. The results showed that more male mice died than female mice. When compared to giving control, it did not cause death in male mice female mice. The LD_{50} value was calculated.

Based on table 3. LD_{50} calculation using the Thomson Weil method where the LD_{50} value for male mice 776.2 mg/ kg body weight and in female mice, it 794.3mg/kg body weight, so it can be said that the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate according to BPOM (2014) included in the criteria of slightly toxic.

In addition to LD_{50}, another parameter that was observed was the effect of the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate on the body weight of mice. Can be seen that there was a decrease in body weight of mice after being given the preparation of dibutyltin (IV) *Bis-N*-benzyl methyl. Based on the statistical test using SPSS with paired T-test, it obtained a significance value of <0.001 ($p<0.05$), meaning that there was a difference in body weight before being given treatment and after being given treatment. In toxicity studies, test animals that received high doses usually lost weight due to decreased appetite. Then the experimental animals were operated on to observe the organs after being given the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate. According to [18] in the toxicity test, there can be several categories for example the toxicity of a substance, and one of the categories is the affected organ. In this category the affected organs such as the CNS, heart, liver, kidneys, and so on. In this study, the organs observed were the kidneys, liver, and heart.

Observations on the kidneys, liver, and heart of mice were carried out because the liver is the largest and most complex organ in the body. This organ is involved in the metabolism of food [19]. The liver is a detoxifying organ most drugs and toxins. While the kidneys were observed because the kidneys are important organs that filter blood, maintain fluid balance, and remove metabolic waste [20]. According to [21], the main function of the kidney is the main route of excretion of most toxins. Therefore, the kidney has a large blood flow, concentrates toxins in the filtrate, carries toxins through the renal tubular cells, and activates certain toxins. In addition, the function of the heart is to pump blood from the heart to all parts of the body. The heart is one of the target organs and

Table 2. Death Count Result

Treatment	Number of Mice	Number of Test Animal Death	
		Male	Female
Negative Control	5	0	0
(240mg)	5	0	1
(480mg)	5	4	2
(960mg)	5	5	4

Table 3. Results LD_{50} Value and Toxicity Category

Gender	Score LD_{50}	Category
Male	776,2mg/kg body weight	Slightly Toxic
Female	794,3mg/kg body weight	Slightly Toxic

Table 4. Results of the Average Body Weight of Mice

Treatment	Gender	Body Weight Beginning	Body Weight End
		Average (gr)	Average (gr)
Control (-)	Male	23,60	21,80
	Female	25,80	24,40
I (240mg)	Male	28,20	25,40
	Female	29,20	26,60
II (480mg)	Male	26,20	23,60
	Female	27,00	25,00
III (960mg)	Male	27,60	25,00
	Female	26,00	24,20

Table 5. Results Average ROW Value (Weight Ratio of Organs)

Treatment	Kidney (%)		Liver (%)		Heart (%)	
	Male	Female	Male	Female	Male	Female
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

Table 6. Statistic Test Results Value ROW

No	ROW	P Value	Information	Conclusion
1	Kidney	p>0,05	H1 accepted	There is no difference in renal ROW by sex*treatment
2	Liver	p>0,05	H1 accepted	There is no difference in liver ROW by sex*treatment
3	Heart	P<0,05	H0 rejected	There is no difference in heart ROW by sex*treatment

is easily damaged by various chemicals. Chemicals act directly on the heart muscle or indirectly through the nervous system or blood vessels, If the heart function decreases, it can affect the oxygen and nutrient requirements needed by the whole body [21].

The results of ROW (Relative Organ Weight) can be seen in table 5, the average kidney ROW for male mice has a decrease in kidney organ weight and female mice also has a decrease in kidney organ weight, the greater the dose given, the lower the kidney ROW. This means that the dose given with the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate can affect the kidney organs of mice compared to the kidney organs that were given the control preparation. Furthermore, the test was carried out using SPSS with two-way which is seen in table 6. The obtained significance value of 0.263 (p>0.05) means that value of kidney ROW. Between the dose and the renal ROW value a significance value of <0.001 (p<0.05) meaning that there between the dose and the renal ROW value. Meanwhile, for gender with a kidney ROW value, a significance value of 0.019 (p<0.05) was obtained, meaning that gender could affect the kidney ROW value.

The average liver ROW value can be seen in table 5. where male and female mice have a decrease in liver weight, the greater the dose given, the lower the liver ROW, meaning that the dose given with the compound Dibutyltin (IV) *Bis-N*-benzyl methyl Dithiocarbamate can affect the liver of male and female mice compared to the liver that was given the control preparation. Furthermore, if the SPSS test was carried out with two-way ANOVA, it can be seen in Table 6. The significance value was 0.597 (p>0.05), meaning that there was no between gender and dose on the ROW value of the liver. You look at the significance value between the dose and the liver ROW value, it has a significant value of <0.001 (p<0.05), which means that the dose can affect the liver ROW value. Then the gender with the liver ROW value obtained a significance value of 0.003 (p <0.05), meaning that gender can affect the liver ROW value.

The average value of heart ROW can be seen in table 5, mice have a decrease in heart organ weight. greater the lower the heart ROW, meaning the dose given with the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate can affect the heart of mice compared to the heart of mice that were given the control preparation. Then, a two-way test was performed, which is shown in table 6. Significance value of 0.050 (p<0.050) has an effect between sex and dose on the ROW value of the heart. The dose and the heart ROW value obtained a significance value of <0.001 (p<0.05), meaning that the dose can affect the heart ROW value. Meanwhile, for the gender with the cardiac ROW value, a significance value of 0.911 (p>0.05) was obtained, meaning that gender could not affect the cardiac ROW value.

From the results of the research that has been carried out, it can be concluded that the compound dibutyltin (IV) *Bis-N*-benzyl methyl dithiocarbamate can cause death in male and female mice, with an LD_{50} value of 776.2mg/ kg body weight in male mice and female mice 794.3 mg/kg body weight. This compound is categorized as slightly toxic, meaning that this complex compound has a slightly toxic effect on male and female mice.

REFERENCES

1. Asrial. Senyawa Turunan Organotimah: Sintesis dan Struktur Kristal BIS (Trimetil Timah) Krokonat [(CH3)3Sn]2C5O5 2H2O. J Ind Soc Integ Chem. 2014;6(1):1–8.
2. Hadi AS dan S. AKTIVITAS IN VITRO DAN STUDI PERBANDINGAN BEBERAPA SENYAWA ORGANOTIMAH(IV) 3-HIDROKSI BENZOAT TERHADAP SEL KANKER LEUKEMIA, L-1210. SNSMAIP III. 2012;1(978):382–7.
3. Adeyemi JO, Onwudiwe DC. Organotin(IV) dithiocarbamate complexes: Chemistry and biological activity. Molecules. 2018;23(10):1–27.
4. M. Graisa A, A. Husain A, H. Al-Mashhadani M, S. Ahmed D, Adil H, Yousif E. The Organotin Applications in Biological,

Industrial and Agricultural Sectors: A Systematic Review. J Serambi Eng. 2021;7(1):2631–8.
5. Anggraini SM, Hadriyati A, Sanuddin M. Sintesis dan Uji Aktivitas Senyawa Dibutil Timah (IV) Bis-Metil Ditiokarbamat Pada Bakteri Salmonella Typhi dan Escherichia Coli Synthesis and Activity Test of Dibutyl Tin (IV) Bis-Methyl Dithiocarbamate on Salmonella Typhi and Escherichia Coli Bacteria. J Healthc Technol Med. 2020;6(1):308–17.
6. Sanuddin M, Purnamasari L, Soyata A. Sintesis dan Uji Aktivitas Senyawa Dibutil Timah (IV) Bis-Metil Ditiokarbamat Pada Bakteri Salmonella Typhi dan Escherichia Coli Synthesis and Activity Test of Dibutyl Tin (IV) Bis-Methyl Dithiocarbamate on Salmonella Typhi and Escherichia Coli Bacteria. 2022;19(1):169–80.
7. Hamid A, Azmi MA, Rajab NF, Awang N, Jufri NF. Cytotoxic effects of organotin(IV) dithiocarbamate compounds with different functional groups on leukemic cell line, K-562. Sains Malaysiana. 2020;49(6):1421–30.
8. Wang MM, Chu WC, Yang Y, Yang QQ, Qin SS, Zhang E. Dithiocarbamates: Efficient metallo-β-lactamase inhibitors with good antibacterial activity when combined with meropenem. Bioorganic Med Chem Lett [Internet]. 2018;28(21):3436–40. Available from: https://doi.org/10.1016/j.bmcl.2018.09.028
9. Sebatian K, Iv D, Culicidae D, Effectiveness T, Alkylphenyldithiocarbamate TI V. Alkilfenilditiokarbamat sebagai Insektisid ke Atas Aedes aegypti LINN . 2014;12(1):35–9.
10. Onwudiwe DC, Nthwane YB, Ekennia AC, Hosten E. Synthesis, characterization and antimicrobial properties of some mixed ligand complexes of Zn(II) dithiocarbamate with different N-donor ligands. Inorganica Chim Acta. 2016;447.
11. Xuan H, Zhang J, Wang Y, FU C, Zhang W. Anti-tumor activity evaluation of novel chrysin-organotin compound in MCF-7 cells. Bioorganic Med Chem Lett. 2016;26(2):570–4.
12. Awang N, Ehlam SNF, Chan KM. Cytotoxicity and genotoxicity assessments of batik industrial wastewater on V79 cells. J Chem Pharm Sci. 2016;9(4):3221–6.
13. Xuan HZ, Zhang JH, Wang YH, Fu CL, Zhang W. Anti-tumor activity evaluation of novel chrysin-organotin compound in MCF-7 cells. Bioorganic Med Chem Lett [Internet]. 2016;26(2):570–4. Available from: http://dx.doi.org/10.1016/j.bmcl.2015.11.072
14. BPOM R. Peraturan Kepala Badan Pengawas Obat Dan Makanan Republik Indonesia Nomor 7 Tahun 2014. Tentang Pedoman Uji Toksisitas Nonklinik Secara Vivo. 2014;66–8.
15. Waruwu SB, Harahap U, Yuandani Y, Purnomo H, Satria D. Anti-inflammatory activity and toxicity evaluation of 1 , 3-bis (p-hydroxyphenyl) urea [version 2 ; peer review : 1 approved with reservations , 1 not approved]. 2022;1–22.
16. Nakache R, Touil T, Hessni A El, Ouichou A, Bahbiti Y, Berkiks I, et al. In vivo acute toxicity assessment of a novel Wistar rats. Cogent Chem. 2017;43:1–11.
17. Sanuddin M, Permatasari J, Rahmadina J, Fitria N. SENYAWA DIBUTILTIMAH (IV) BIS-N-BENZILMETIL DITIOKARBAMAT SEBAGAI ANTIKANKER P-388. Vol. 388. indonesia: Direktorat Jenderal Kekayaan Intelektual Kementerian Hukum dan Hak Asasi Manusia Republik Indonesia; ID S000003538, 2020. p. 2020.
18. FKUI DIF dan T. Farmakologi dan Terapi. 6th ed. Gunawan SG, editor. jakarta: Badan penerbit FKUI; 2016.
19. Saidu Y, LS Bilbis, M Lawal SI and RAU. Hematotoxicity Study of the Leaf Extract of Albizia chevalieri. Biochem Medica. 2007;17(2):139–270.
20. Churchill J. Caring for pets and Native Fauna. Angus and Robetson Publisher. 1990;
21. Lu F. Toksikologi Dasar. 3rd ed. jakarta: Univertias INdonesia Press; 1995. 224–236 p.