



ACUTE TOXICITY TEST LD₅₀ COMPLEX Fe(II)N-BENZYLMETHYL DITHIOCARBAMATE IN WHITE MICE (*Mus muscle*)

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Abstract

A toxicity test is a test carried out to estimate the degree of damage caused by a compound to biological or non-biological materials. One of the compounds known to have anticancer, antibacterial, and antifungal effects is Fe(II) N-benzyl methyl dithiocarbamate. This compound has cytotoxic activity on liver cells. The purpose of this study was to determine the LD₅₀ value of Fe (II) N-benzyl methyl Dithiocarbamate and to determine the acute toxicity category of Fe (II) N-benzyl methyl Dithiocarbamate. From the results of research that have been carried out the synthesis of compounds capable of forming complex compounds of Fe (II) N-benzylmethyl dithiocarbamate which causes no death in male and female mice with an unknown LD₅₀ value, categorized as non-toxic, meaning that this complex compound does not have a toxic effect on male mice. and female.

Keywords: Acute toxicity test, Fe(II) N-benzyl methyl dithiocarbamate.

INTRODUCTION

Cancer is a disease characterized by abnormal cell growth uncontrolled and the ability of these cells to attack the tissue and other biologics, either by direct growth in that tissue contiguous (invasion) or by migration of cells to distant sites (metastasis). After research, it turns out that iron is often influential in the treatment of cancer¹⁴. Iron is one of the compounds that is being looked at as a drug's future anticancer. Professor Henry Lai of the University of Washington, USA, states that cancer cells need a lot of iron to reproduce DNA if the cancer cells multiply⁶. Benzene Sulfonamide was reported to have activity Anticancer shown through most various mechanisms stands out for the inhibition of carbonic anhydrase (a class of enzymes that serves to catalyze the conversion of carbon dioxide and water into bicarbonate and proton or vice versa so this process is a process reversible or reversible) and induction of apoptosis (mechanism biology which is a type of programmed cell death)⁷. Recent studies show that metal binding dithiocarbamate DNA in different ways, with various cytotoxic activities². Some of the above explanations prove that the compound Fe(II) N-Benzyl methyl Dithiocarbamate can be concluded as an anticancer. In research by Tidjani Rahmouni (2019) the results of anticancer activity against the laryngeal carcinoma cell line (HEP2), indicate that complex Iron is a potential anticancer agent to be developed as an anticancer drug. Based on several pharmacological activities that have been carried out In previous research, it is expected that the compound Fe (II) N-Benzyl methyl Dithiocarbamate can be developed as a drug candidate. But to be Of course, the drug must go through a safety aspect test (toxicity) so that it can be used used in humans, one of which is through acute toxicity tests. The acute toxicity test is part of the designed preclinical test to measure the toxic effect of a compound. Acute toxicity refers to the effect of Toxic reactions that occur after a single oral dose within 24 hours⁸. The value of acute toxicity potential as measured by the Lethal Dose₅₀ (LD₅₀) is a parameter used in the acute oral toxicity test. In addition, the toxicity test can be seen

from changes in the structure and function of vital organs such as the kidney which is the site of excretion of foreign compounds such as drugs or toxic compounds that enter the body¹.

Complex compounds are compounds whose formation involves the formation of coordinate covalent bonds between metal ions or metal atoms and non-metal atoms. Complex compounds or what are often called coordination compounds are compounds composed of a central metal ion with one or more ligands donating one or more lone pairs of electrons¹⁰.

Dithiocarbamates are a class of metal chelating compounds with various applications in medicine. Dithiocarbamate in the body has lower toxicity. The dithiocarbamate class of metal complexing compounds has become a new class of anticancer proteasome inhibitors with great potential to overcome the limitations seen with the use of bortezomib (under the trademark Velcade, which is a type of cancer and lymph node drug, namely mantle cell lymphoma and in injection form). Dithiocarbamate has been used for the treatment of bacterial and fungal infections, possibly the treatment of AIDS, and most recently cancer¹².

Iron (II) is a metal that has the symbol Fe (Latin: Fero) in the first transition and is not corrosive resistant. Iron (either in the form of iron (II) or iron (III)) needs to be complexed with iron complexing to form a specific color. Some of the iron complexes used are molybdenum (Mo), selenite (H_2SeO_3), diphenylcarbazone ($C_{13}H_{12}N_4O$) and phenanthroline ($C_{12}H_8N_2$). Of the several types of reagents, phenanthroline is the most widely used because the Iron (II) - phenanthroline complex can form a complex with a stable color for a long time¹².

Carbon disulfide (CS_2) also called dithiocarbonate anhydride is a colorless liquid but when exposed to the sun it turns yellowish, odorless, flammable and volatile, soluble in benzene, alcohol and ether, very slightly dissolved in water, about 0.014%. This compound has an odor like the smell of chloroform which is toxic to the nervous and cardiovascular systems. Carbon disulfide is volatile and flammable, with a high solubility in blood and fat. It has a melting point of $-111.61^\circ C$ and a boiling point of $46.24^\circ C$. But usually these compounds are not present in a pure state, so they smell bad due to other sulfur compounds⁶.

Benzylmethylamine ($C_8H_{11}N$) is a chemical compound having a molecular weight of 121.18 g/mol, boiling point $184-189^\circ C$, flash point $75^\circ C$, vapor density 4.85 g/ml, relative density 0.939 g/ml at $25^\circ C$, not suitable for use together with carbon dioxide, acids, anhydrous acids and a heavy atomic number of 9³.

Methanol (CH_3OH) known as methyl alcohol, wood alcohol or spirit is the simplest form of alcohol. It has a melting point of $-97^\circ C$ and a boiling point of $64.7^\circ C$. Methanol is commonly used as an organic solvent, is a type of alcohol that has the simplest structure, but is the most toxic to humans because it is toxic⁴. Lethal Dose₅₀ is a statistically derived quantity or a certain dose expressed in mg/KgBW. Description: mg (milligram), kg (kilogram), BW (Body weight) Which produces a 50% death response in the test animal population within a certain period of time. To express a single dose of a compound that is expected to kill or cause significant toxic effects in 50% of experimental animals after treatment. Lethal Dose₅₀ (LD_{50}) orally is 115 mg/kgBW. The LD_{50} is the dose that causes 50% of the experimental animals to die⁸. Mice (*Mus musculus*) are a member of the animal kingdom animalia. These animals are characterized by docile, afraid of light, active at night, easy to breed, short life cycle and classified as polyestrus. Mice (*Mus musculus*) are the most commonly used animals in laboratory research as experimental animals, which are around 40-80%. Mice have many advantages as experimental animals (especially used in biological research), namely a relatively short life cycle, large number of offspring, high variation in traits and easy handling⁵.

Test materials available in solid form must be prepared in the form of a solution or suspension so that they can be administered by gastric tube. The test material was given orally with a blunt-tipped tube, slightly lumpy at the end and made slightly curved. The blunt end of the sonde aims to avoid getting into the trachea and avoid trauma to experimental animals.

Mice were restrained by pulling the skin of the neck with the left hand in such a way that the skin was pinched by the thumb and forefinger. This is reinforced by the clamp at the base of the thumb with the other fingers on the skin of the back and tail attached to the little finger of the left hand. Insert the sonde carefully until it reaches the stomach. After making sure that the probe has entered the stomach and not into the lungs, then the test material is pumped out. A good volume is a maximum of 1 mL because the stomach capacity of mice weighing 20 grams is only 1.0 mL. Generally given half of the maximum volume in experimental animals⁹.



METHOD

This study using experimental methods conducted trials of LD₅₀ acute toxicity of complex compound Fe(II) N benzyl methyl dithiocarbamate in white mice (*Mus musculus*).

Material and Instrumentation

Materials used in this study include: Test animals used Male and female white mice, Primary amine n Benzylmethylamine (C₈H₁₁N) (Sigma Aldrich), carbon disulfide (CS₂), metal iron (II) chloride (FeCl₂) (Sigma Aldrich), methanol PA(analysis), Na CMC 0.5% (Sodium Carboxymethyl Cellulose), NaCl (Sodium Chloride). Animal scales, analytical scales, aluminum foil, 1 cc syringe, oral probe, surgical instruments, porcelain cup, measuring cup, stir bar, erlenmeyer, tweezers, scalpel, surgical scissors.

PREPARATION AND CHARACTERIZATION

The sample consisted of primary amines namely N-Benzylmethyl amine (C₈H₁₁N), carbon sulfide (CS₂), and metal Iron (II) chloride (FeCl₂), prepared in situ everything was dissolved in pure methanol in 15 minutes.

Before testing began, 40 mice were divided into 4 Each treatment group consisted of 10 mice (5 mice male mice and female mice) with a weight of 20 grams - 30 grams and experienced a more adaptation period first for 7 days. All mice are adapted to the place of their new residence with the provision of a food and drink routine. This acclimatization aims so that the test animals are not in a condition stressed and in the same state at the start of the study, then the mice that were dissected were removed for their organs (liver, kidney, and heart).

The data collected in the research is data from the results observation of experimental animals, both the control group and the treatment group. LD₅₀ data was taken from the number of mice that died in each group. Furthermore, statistical analysis was tested with a normality test on organ weight, test homogeneity on organ weight, and a two-way ANOVA test.

Nuclear Magnetic Resonance (NMR) is a "research tool" fundamental and has been widely used in various fields of science and technology. NMR spectroscopy is a tool available for determining the structure of organic compounds. NMR is often used in There are two confirmations of the structure of a compound, namely, ¹³C Nuclear Magnetic Resonance (¹³C-NMR) and ¹H-Nuclear Magnetic Resonance (¹H-NMR).

Fourier transform infrared (FTIR) is one such technical analysis that is important to researchers. This type of analysis can be used for characterizing samples in the form of liquids, solutions, pastes, powders, films, fibers, and gases. This analysis is also possible to analyze the material on the substrate surface. Compared with this type of characterization analysis, FTIR is quite popular. This characterization analysis is quite fast, has good accuracy, and is relatively sensitive. In the FTIR analysis procedure, the sample is exposed to infrared radiation. The IR radiation then has an impact on the vibrations of molecular atoms in the sample, resulting in absorption and/or transmission energy. This makes FTIR useful for determining molecular vibrations contained in the sample.

RESULT AND DISCUSSION

Fe(II) N- Benzyl methyl Dithiocarbamate Complex Compounds, successfully synthesized with 98% purity by FTIR spectroscopy, produced the desired spectral peaks as in Table 1, and characterization using FTNMR (¹H protons) produced methyl peaks in the range of 0 ppm, -CH₂- peaks at delta 4.26 ppm and aromatic protons at 6.26 ppm as in table 2. The results of FTNMR Carbon (¹³C) characterization show a peak of -CH₃ at 0-44.3 ppm, a peak of -CH₂- at 58-77 ppm, C aromatic at 129.4 – 129.8 ppm, and CS₂ at 198 – 206 ppm as in table 3. The results of this NMR show that synthetic compounds have been successfully formed with molecular peaks in Fe(II) N- Benzyl methyl Dithiocarbamate Complex Compounds. The results of observing the LD₅₀ value of Fe(II) N-Benzylmethyl Dithiocarbamate complex compounds on the number of mice deaths after administration of Fe(II) N-Benzyl methyl Dithiocarbamate Complex Compounds showed that with doses of 240 mg/KgBW, 480mg/KgBW, 960 mg/KgBW, 1500 mg/KgBW, 3000 mg/KgBW, 4500 mg/KgBW and 5000 mg/KgBW could not cause death in male and female mice so that LD₅₀ values above 5000 mg body weight, So it is concluded that this compound is classified as slightly toxic.

Table 1. FTIR Analysis Results of Synthesis of Fe(II) N- Complex Compounds Benzylmethyl Dithiocarbamate

Absorption Direction Wave Number (cm ⁻¹)	Analysis Results of Complex Compounds Fe (II) N-Benzylmethyl Dithiocarbamate	
	functional groups	Wavenumber (cm ⁻¹)
2000 – 1900	C=C	1600,92
1250 – 1020	C-N	1244,09
3100 – 2990	C-H	3026,31
700 – 1563	C-S	694,37
515 – 605	Sn - C	547,78
350 – 450	Sn - S	337,54

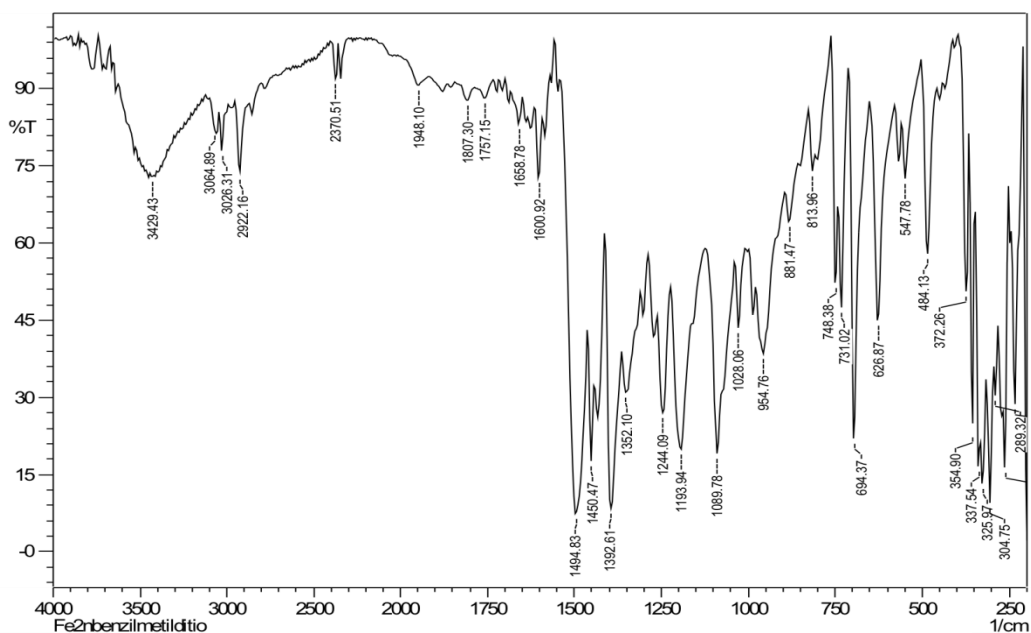


Figure 1. Peak spectrum FTIR Compound Fe (II) N-Benzylmethyl dithiocarbamate

Table 2. NMR Spectrum Analysis Results of ^1H Synthesis of Complex Compounds of Fe (II) N-Benzylmethyl Dithiocarbamate.

Proton Shift Area (ppm)	Results of Proton Analysis of Complex Compounds of Fe (II) N-Benzylmethyl Dithiocarbamate
$\delta 0 - 4$ ppm (CH_3)	0(CH_3) (s)
$\delta 4 - 6$ ppm (β - substituted aliphatic)	4,26 - 6,94 (CH_2) (m)
$\Delta 6 - 8$ ppm (aromatic)	6,26 - 7,28 C_6H_5 (m)

Table 3. NMR Spectrum Analysis Results of ^{13}C Synthesis of Complex Compounds of Fe (II) N-Benzylmethyl Dithiocarbamate.

Carbon Shift Area (ppm)	Results of Proton Analysis of Complex Compounds of Fe (II) N-Benzylmethyl Dithiocarbamate
$\delta 0 - 50$ ppm	0 - 44,3 (CH_3)(s)
$\delta 60$ ppm - 80 ppm	58,1- 77,16 (CH_2)(s)
$\delta 130 - 140$ ppm	129,40 - 129,80(C_6H_5)(m)
$\delta 135 - 140$ ppm	136,4 - 141, 96 (C-N)(s)
$\delta 198 - 105$ ppm	198,4 - 206,90 (C-C)(m)

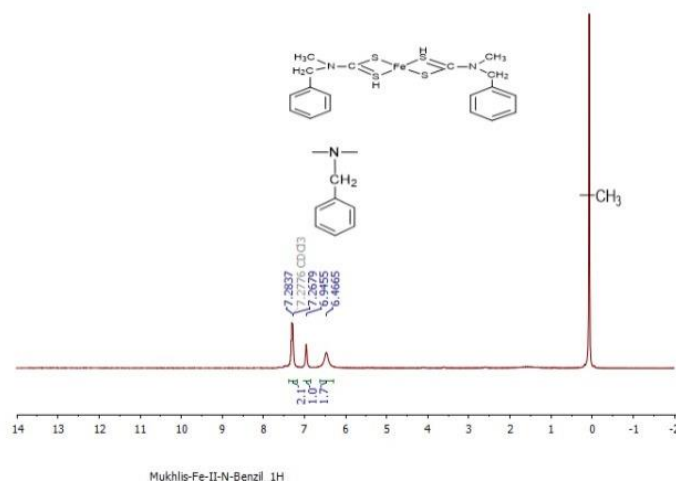


Figure 3. Peak spectrum of results of ^1H NMR Compound Diphenyltin(IV)N-benzyl methyl dithiocarbamate

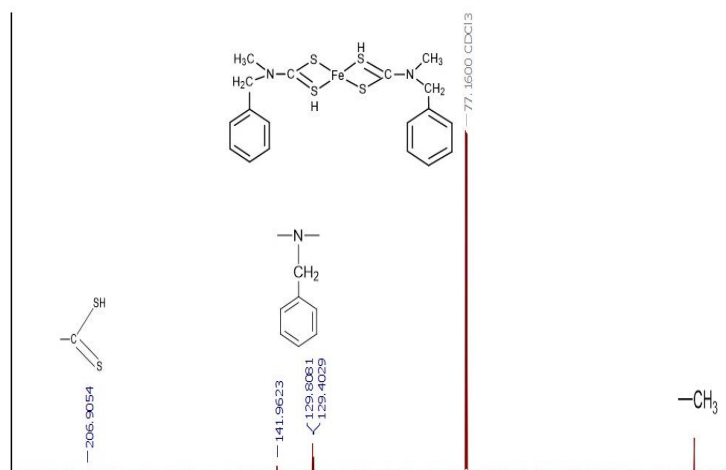


Figure 4. Peak spectrum of results of ^{13}C NMR Compound Diphenyltin(IV)N-benzyl methyl dithiocarbamate

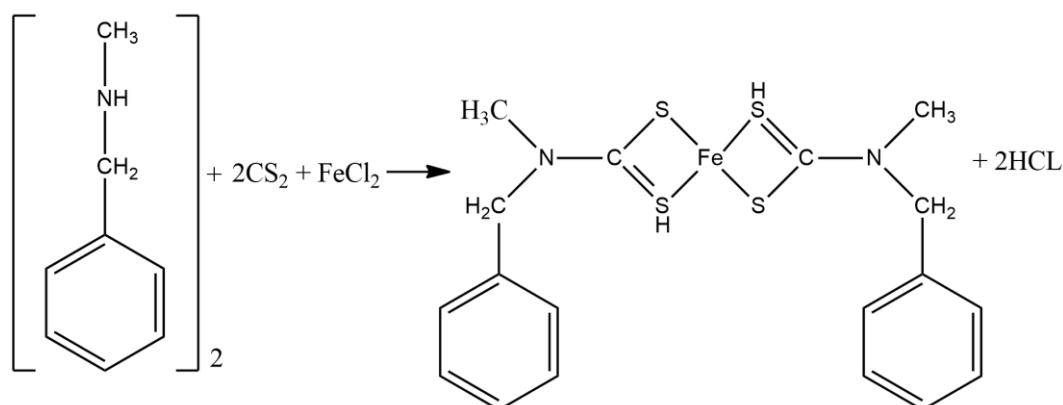


Figure 5. Compound structure of Fe (II) N-benzyl methyl dithiocarbamate complex

CONCLUSION

From the results of research that has been done can be concluded that:

1. Complex compound Fe(II) N-Benzylmethyl Dithiocarbamate does not cause death in male mice and female mice, so LD₅₀ is unknown.
2. This compound is categorized as non-toxic, meaning this complex compound does not have a toxic effect on male and female mice.

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A Brief Author Biography

1. 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.
2. Indri Meirista (master of pharmacy) experienced in pharmaceutical technology, the synthesis of organotin compounds, both as anti-bacterial, and anti-fungi
3. Ranny Adia Kinanti (BSc pharmacy) in this study played a role in Acute Toxicity Test LD₅₀ Complex Fe(II)N-Benzylmethyl Dithiocarbamate In White Mice (*Mus Muscle*).