



Acute Oral Toxicity of Cincalok Oil in Wistar Rats

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Abstract

Cincalok is a West Kalimantan traditional fermented food that has an opportunity to be developed into supplement products as a source of omega 3 and astaxanthin. This study was designed to observe the acute toxicity of Cincalok oil in female rats for its safety profile using AOT 425 program. Cincalok oil for the acute (2000 and 5000 mg/kg) toxicity studies was administered orally according to the OECD guidelines 425. The rat's body and organs weight were observed then signs of toxicity were assessed. LD₅₀ was being determined at the end of the study. The result showed that a single dose of Cincalok oil at 2000 or 5000 mg/kg had no lethal effect, behavioral change, a significant change in rat's bodyweights, or any lesion on the rat's organs. The LD₅₀ of Cincalok oil for oral administration was higher than 5000 mg/kg and categorized as practically non-toxic.

Keywords: Acute Toxicity, Cincalok Oil, LD₅₀.

Toksisitas Oral Akut dari Minyak Cincalok pada Tikus Wistar

Abstrak

Cincalok adalah makanan fermentasi tradisional Kalimantan Barat yang memiliki peluang untuk dikembangkan menjadi produk suplemen sebagai sumber omega 3 dan astaxanthin. Penelitian ini dirancang untuk mengamati toksisitas akut minyak Cincalok pada tikus betina untuk profil keamanannya menggunakan program AOT 425. Minyak Cincalok untuk studi toksisitas akut (2000 dan 5000 mg/KgBB) diberikan secara oral sesuai dengan pedoman OECD 425. Berat badan dan organ tikus diamati kemudian tanda-tanda toksisitas dinilai. LD₅₀ ditentukan pada akhir penelitian. Hasilnya menunjukkan bahwa dosis tunggal minyak Cincalok pada 2000 atau 5000 mg/KgBB tidak memiliki efek mematikan, perubahan perilaku, perubahan berat badan tikus yang signifikan, atau lesi pada organ tikus. LD₅₀ minyak Cincalok untuk pemberian oral lebih tinggi dari 5000 mg/KgBB dan dikategorikan sebagai praktis tidak beracun.

Kata Kunci: LD₅₀, Minyak Cincalok, Toksisitas Akut.

1. Introduction

Cincalok is a West Kalimantan traditional food made from shrimp which is fermented with microbes.¹ Similar products are also known with different names, like Ronto In South Kalimantan, Cincaluk in Malaysia, Balaobalao in Philippine, Kong chai in Korea, and Kong soom in Thailand.² Cincalok contains shrimp, salt, and sugar and it is usually consumed directly as a side dish with or without cooked.¹ Cincalok has high nutritional content obtained from shrimp such as carbohydrates, protein, and fat.³ According to Khairina (2016), cincalok contains complete amino acids and fatty acids such as glutamic acid, palmitoleic acid, omega 3, and omega 6.² Shrimp as one of the main components in cincalok has a high nutritional content including astaxanthin which is the main carotenoid compound in shrimp that prevent various degenerative diseases such as cancer, cataracts, and the heart due to its antioxidant activity.⁴ Unsaturated fatty acids (omega 3, 6) are important for organs nutrient.⁵ Due to its beneficial ingredients, Cincalok has an opportunity to be developed into supplement products as a source of omega 3 and astaxanthin. This study was designed to find out the safety profile of Cincalok oil by acute oral toxicity study in female rats based on Organization for Economic Cooperation and Development (OECD) guideline 425. Acute oral toxicity is needed as one of the initial safety profile tests to find out the LD₅₀ or the maximum dose that can still be tolerated by the animal subject and several signs as the toxicity symptoms that may occur to the animal subject. Therefore, the safe limit for the consumption of a substance can be obtained to avoid toxic effects.^{6,7}

2. Method

2.1. Equipment

The equipment included surgical instruments (Aesculap M376108, USA), glassware (Iwaky pyrex, Japan), protection test equipment, blenders (Philips, Dutch), hot plates (Torrey Pines Scientific HP 10-2, USA), refrigerators (Toshiba, Japan), analytic scales (Shimadzu AUY-220, Japan), oral sonde, 1

cc syringes, mouse scales, and vacuum dryer (Maksindo VD-4, Indonesia).

2.2. Materials

VCO (Virgin Coconut Oil) were obtained from PT Dwi Centra Cahaya Wiguna, Pontianak, Indonesia. The Cincalok was purchased from a home industry in Singkawang City, West Kalimantan with characteristic paste form, pink color, has a mixed taste of salty and sour, and has a specific aroma of shrimp..

2.3. Procedure

The experimental animal protocols was authorized by the Animal Ethics Committee of Medicine Faculty, Tanjungpura University, West Kalimantan (number of ethical clearance: 6627/UN22.9/TA.00.03/2019). Animal subjects that used in this research were female Wistar rats (*Ratus norvegicus*), aged 3 months, weighing 150-200 g (no weight variation more than 20%), not pregnant, not disabled/have any physical abnormalities, and did not experience significant weight loss during acclimatization.

Wet cincalok was filtered, and the residue was dried at 50°C for 3 hours using a vacuum dryer. It was ground using a blender to become a fine powder. Cincalok fine powder was soaked using VCO solvent, and it was ground again for 30 minutes. The mixture was filtered after 24 hours passed.

Cincalok oil is induced in rats at a dose 2000 mg/KgBW and 5000 mg/KgBW, with the use of rats in this study a maximum of 5 mice for each test. The rats were observed during the first 24 hours for the beginning of any immediate toxic signs or mortality. If there was no mortality, the experiments were continued being observed during 14 days without any dosing, to observe mortality, behavioral changes, and any acute delayed effect. The data analyzed using AOT 425 program and SPSS application with a 95% confidence level.^{8,9}

3. Result

The test animals were observed for 30 min, 60 min, 120, and then for 24 h and

continued for 14 days. The body weights of all the animals both in the dose of 2000 mg/Kg BW and 5000 mg/Kg BW are shown in Fig. 1.

Behavioral observations for the dose of 2000 mg/Kg BW showed that all parameters were normal except the motoric and platform activities, Haffner parameter, grooming, defecation, and urination. The motoric activity decreased after being given the cinalok oil for 1 hour but increased again after 24 hours. The platform activity also decreased after being given the cinalok oil until 24 hours. The Hafner parameter decreased only after 14 days of administrations. The parameters of grooming, defecation, and urination had fluctuation results including pineal and corneal reflex, flexion induction, hanging, reestablishment, straub, piloerection, ptosis, lacrimation, catalepsy, respiratory, salivation, vocalization, tremor, convulsions, and writhing were normal during the study. The detailed observations are showed in Table 1.

Behavioral observations for the dose of 5000 mg/Kg BW showed that all parameters were normal except platform activity, Hafner parameter, grooming, defecation, and urination. The platform activity decreased after being given the cinalok oil until 14 days after administration. The parameters of hafner, grooming, defecation, and urination had

fluctuation results. The detailed observations are showed in Table 2.

No lesion was found on the examination of isolated vital organs such as heart, kidney, liver, lung, and spleen. The organ to body weight index was summarized in Fig. 2.

Based on the results, most of the organ index results showed the decreasing of organ index in dose 2000 mg/kg BW Cinalok oil compared to the 5000 mg/kg BW, except in the liver organ index. But statistically, there was no significant variation present among the groups.

The results of the limit test with dose of 2000 mg/Kg BW and 5000 mg/Kg BW on 5 rats exhibited no mortality. LD₅₀ value is obtained through data processing with AOT 425 StatPgm software and showed that LD₅₀ of Cinalok oil was higher than 5000 mg/kg BW (Fig 3 and Fig 4). According to globally harmonized classification system, cinalok oil can be put in lower toxicity class on practically non-toxic categories.

4. Dissscussion

Cinalok has characteristics shaped like pasta, pink in color, mixed salty and sour, and a specific aroma of shrimp.⁸ The pink color of cinalok is presumably from the astaxanthin component which undergoes the hydrolysis and proteolysis during the

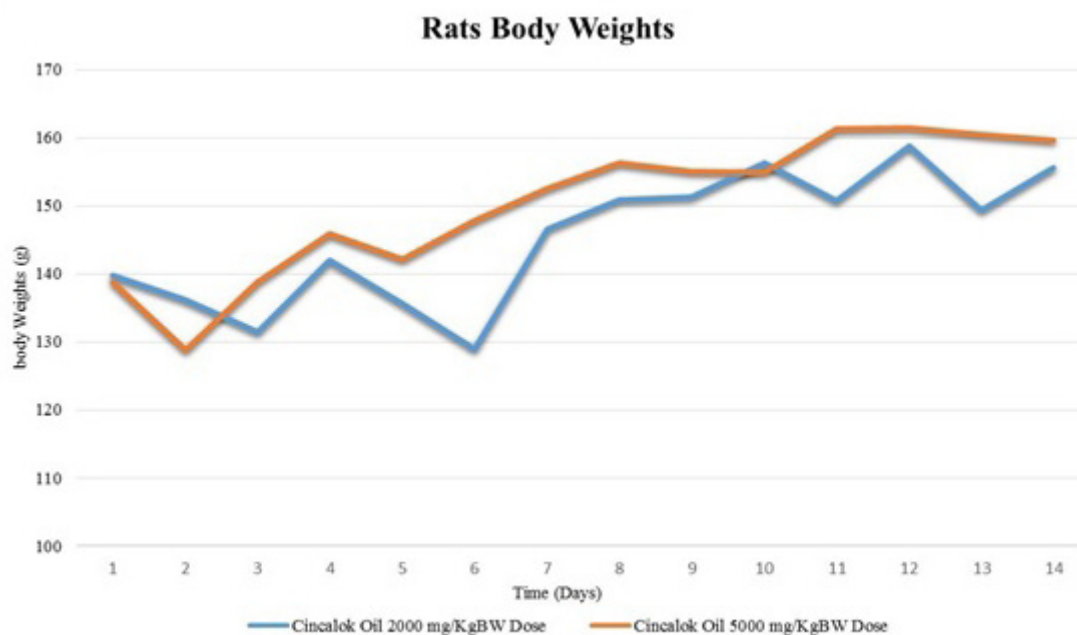


Figure 1. Effect of cinalok oil on body weight of Wistar female rats in acute toxicity studies

Table 1. Effect of 2000 mg/Kg BW cinalok oil on the behaviour of white Wistar female rats in acute toxicity studies.

The Parameters of Behavioural Observation		The Average of Cinalok Oil Behavioural Observation					
		Dose of 2000 mg/KgBW					Day-15
		0	30 minutes	60 minutes	120 minutes	24 hours	
Platform		7,8	3,6	2,2	1,8	3,6	5
Motoric Activity	Up (%)	0	0	0	0	0	0
	N (%)	100	100	40	0	40	100
	Down (%)	0	0	60	100	60	0
	No Activity (%)	0	0	0	0	0	0
Straub (%)		0	0	0	0	0	0
Piloerection (%)		0	0	0	0	0	0
Ptosis (%)		0	0	0	0	0	0
Pineal Reflex (%)		100	100	100	100	100	100
Corneal Reflex (%)		100	100	100	100	100	100
Lacrimation (%)		0	0	0	0	0	0
Catalepsy (%)		0	0	0	0	0	0
Gestures	N (%)	100	100	100	100	100	100
	NN (%)	0	0	0	0	0	0
Hanging (%)		100	100	100	100	100	100
Retablishment (%)		100	100	100	100	100	100
Flexion(%)		100	100	100	100	100	100
Hafner (%)		100	100	100	100	100	60
Mortality (%)		0	0	0	0	0	0
Grooming (%)		40	20	20	20	90	0
Defecation (%)		100	20	20	20	90	80
Urination (%)		40	40	60	60	20	60
Respiration	Fast (%)	0	0	0	0	0	0
	N (%)	100	100	100	100	100	100
	Breathless (%)	0	0	0	0	0	0
Salivation (%)		0	0	0	0	0	0
Vocalization (%)		0	0	0	0	0	0
Tremor (%)		0	0	0	0	0	0
Convulsions (%)		0	0	0	0	0	0
Writhing (%)		0	0	0	0	0	0

Note: N= Normal, NN= Not Normal

shrimp fermentation process. Astaxanthin is the carotenoid compound in shrimp that can prevent various degenerative diseases such as cancer, cataracts, and heart due to its antioxidant activity.⁴ Khairina (2016) tested Ronto (the term of Cinalok in South Kalimantan) also found the complete amino acids and fatty acids contain such as glutamic acid and polyunsaturated fatty acids (omega 3 and omega 6).² Due to the various useful

compounds in it, Cinalok has the opportunity to be developed into a supplement.

Although Cinalok has been consumed as a side dish and has various benefits from its compounds, it does not mean that it is completely safe, especially if it will be developed as a medicine or supplement. Herbal ingredients are known to be safe as a medicine, nowadays they have increased in the number of reported cases of acute and

Table 2. Effect of 5000 mg/Kg BW cinalok oil on the behaviour of white Wistar female rats in acute toxicity studies.

The Parameters of Behavioural Observation		The Average of Cinalok Oil Behavioural Observation					
		Dose of 5000 mg/KgBW					
		0	30 minutes	60 minutes	120 minutes	24 hours	Day-15
Platform		7	5	4,2	3,4	3,6	3,4
Motoric Activity	Up (%)	0	0	0	0	0	0
	N (%)	100	100	100	100	100	100
	Down (%)	0	0	0	0	0	0
	No Activity (%)	0	0	0	0	0	0
Straub (%)		0	0	0	0	0	0
Piloerection (%)		0	0	0	0	0	0
Ptois (%)		0	0	0	0	0	0
Pineal Reflex (%)		100	100	100	100	100	100
Corneal Reflex (%)		100	100	100	100	100	100
Lacrimation (%)		0	0	0	0	0	0
Catalepsy (%)		0	0	0	0	0	0
Gestures	N (%)	100	100	100	100	100	100
	NN (%)	0	0	0	0	0	0
Hanging (%)		100	100	100	100	100	100
Retablishment (%)		100	100	100	100	100	100
Flexion(%)		100	100	100	100	100	100
Hafner (%)		60	90	90	90	100	40
Mortality (%)		0	0	0	0	0	0
Grooming (%)		100	90	100	60	40	60
Defecation (%)		100	100	60	90	100	100
Urination (%)		60	0	60	20	20	60
Respiration	Fast (%)	0	0	0	0	0	0
	N (%)	100	100	100	100	100	100
	Breathless (%)	0	0	0	0	0	0
Salivation (%)		0	0	0	0	0	0
Vocalization (%)		0	0	0	0	0	0
Tremor (%)		0	0	0	0	0	0
Convulsions (%)		0	0	0	0	0	0
Writhing (%)		0	0	0	0	0	0

chronic poisoning due to their use.⁹ Shrimp as a basic ingredient in Cinalok can also have the potential to cause toxic effects due to contamination of waste in the sea such as Pb, Cu, and Cd that are harmful to the body.¹⁰ Therefore, toxicological evaluation is needed to know the safety of herbal medicines, which according to FDA and WHO recommendations, the validation of the effectiveness and safety of using herbal therapy can be done through scientific-based studies. Research on the safety of cinalok

has never been done before, so this study was conducted to assess the acute toxicity of Cinalok oil following the Organization for Economic Cooperation and Development (OECD) 425 guideline, to determine a safe dosage range in its development as a drug.^{11,12} In this study, the extraction of Cinalok used an oil solvent because the compounds contained in cinalok, including astaxanthin, are oil-soluble pigments. Virgin Coconut Oil (VCO) in the research of Sindhu (2011) and Phromthong (2012) can extract

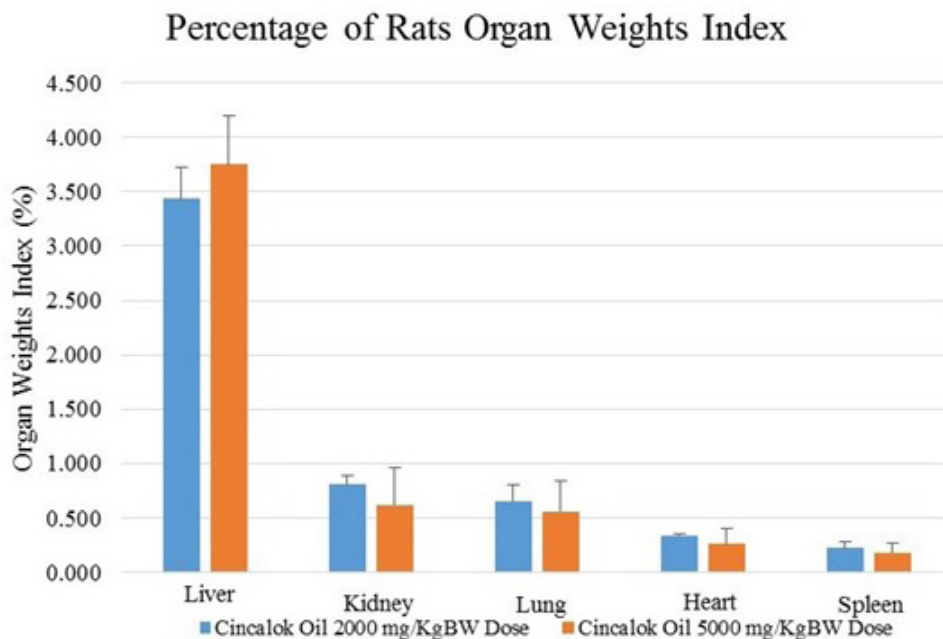


Figure 2. The percentage of organ index after being given cincalok oil.

more astaxanthin than other vegetable oil solvents, therefore VCO solvent was used to optimize the uptake of existing content.^{13,14} In this study, Wistar strain rats were used as the tested animals because these rats are easy to obtain and maintain, also have good growth.¹⁵ The reason for using only female rats is because male rats are more sensitive to the compound and the OECD has established

guidelines that recommend that in acute oral toxicity testing.¹⁶

During 14 days of acute toxicity evaluation, no mortality was found while there were some changes in behavioral patterns like decreased motoric activity, decreased platform activity, and fluctuation of Hafner parameters, grooming, defecation, and urination. The decreased in motoric

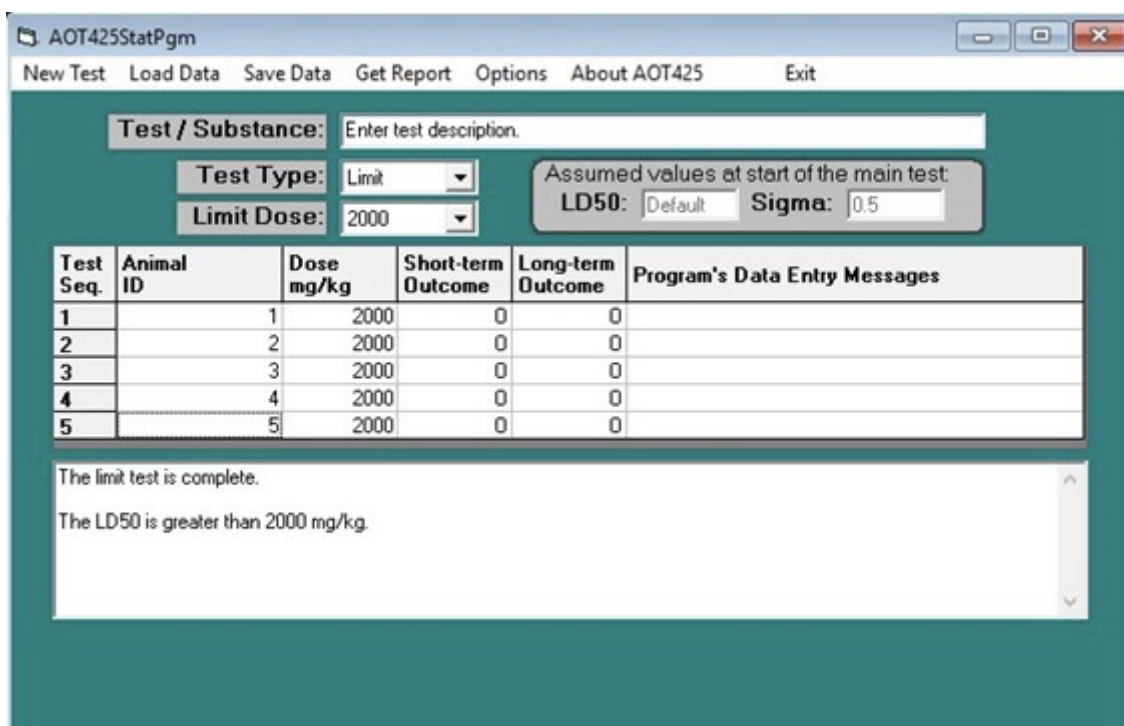
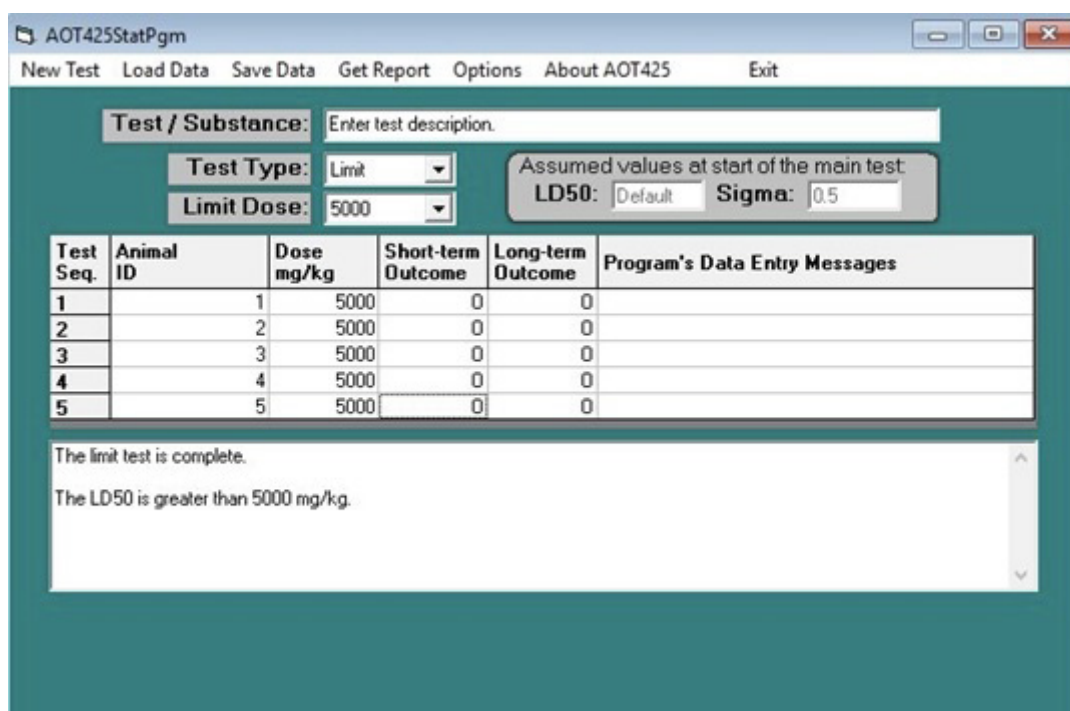


Figure 3. Acute toxicity result of cincalok on limit test of dose of 2000 mg/kg BW



Test / Substance: Enter test description.

Test Type: Limit

Limit Dose: 5000

Assumed values at start of the main test:
LD50: Default **Sigma:** 0.5

Test Seq.	Animal ID	Dose mg/kg	Short-term Outcome	Long-term Outcome	Program's Data Entry Messages
1	1	5000	0	0	
2	2	5000	0	0	
3	3	5000	0	0	
4	4	5000	0	0	
5	5	5000	0	0	

The limit test is complete.
 The LD50 is greater than 5000 mg/kg.

Figure 4. Acute toxicity result of cincalok on limit test of dose of 5000 mg/kg BW

and platform activities is a manifestation of sedative activity, muscle relaxants, paralysis, or anesthesia. The behavior parameter of Haffner, grooming, defecation, and urination is associated with fearful and stressful situations. As a conclusion, Cincalok oil can be considered affecting on nervous system because it decreased motoric and platform activities.¹⁷

The observations of body weight showed that there were no significant body weight variations in both doses. It indicated the normal processing of carbohydrate, protein, and lipids metabolism and normal functions of vital organs in the body, which are the major targeted area of any toxic substance metabolically.¹¹

There were no lesions found on macroscopic examination of the heart, kidney, liver, lung, and spleen. Statistically, no significant variations were found in organ to body weight index of rats. LD₅₀ value was obtained through data processing with AOT 425 StatPgm software and showed that LD₅₀ of Cincalok oil was higher than 5000 mg/kg BW. According to a globally harmonized classification system, Cincalok oil can be put in a lower toxicity class on practically non-toxic categories.¹⁸ If we see from the

toxicity of the individual components, these results are appropriate because of the astaxanthin obtained from *P. carotinifaciens* has no significant observed side effects when observed in mice wat dose levels much higher than the levels currently used by the supplement industry.¹⁹ Another result for astaxanthin from *Haematococcus pluvialis* in pregnant rats is the LD₅₀ reaching a value greater than 20 g / kg.²⁰

5. Conclusion

The cincalok oil has an LD₅₀ greater than 5000mg/kg BW which is categorized as practically non-toxic. However, the preliminary results suggested that it should be further evaluated for long term use and repeated dose effects to ensure the safety of cincalok oil.

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