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Research Article

Subchronic Toxicity Evaluation of Cincalok Oil Towards Wistar Rats Haematology

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Abstract

Cincalok is a fermented shrimp product which has the potential to be used as pharmaceutical preparations. LD50 test results indicate that the substance is practically non-toxic. However, subchronic toxicity tests have not been performed. Therefore, this study aims to determine its subchronic toxicity assay towards haematology in male and female rats in the Wistar strain. The animals used were divided into six groups: the control group induced with virgin coconut oil (VCO), the treatment groups induced with Cincalok oil (100, 400, and 1000 mg/kgbw), satellite control group induced with VCO and the satellite control group induced with a dose of 1000 mg/kgbw. Haematological parameters evaluated include hemoglobin, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes, basophils, lymphocytes, eosinophils, platelets, and neutrophils. The average level of the haematological profile indicated that hemoglobin, RBC, MCH, MCHC, basophils, eosinophils, stem neutrophils, neutrophil segments, lymphocytes, leukocytes, monocytes, and platelets in female and male rats were still in the normal range. While there was a decrease in levels below the normal range in the parameters of MCV (in female rats) and hematocrit (in male rats), it was reversible.

Key words: Cincalok, Haematology, Subchronic Toxicity

Uji Toksisitas Subkronik Minyak Cincalok Terhadap Hematologi Tikus Galur Wistar

Abstrak

Cincalok merupakan produk fermentasi udang yang berpotensi dimanfaatkan sebagai sediaan farmasi. Hasil uji LD50 menunjukkan praktis tidak toksik. Namun, pengujian toksisitas subkronis belum dilakukan. Penelitian ini bertujuan untuk mengetahui uji toksisitas subkronis terhadap hematologi pada tikus putih jantan dan betina galur Wistar. Hewan coba yang digunakan dibagi menjadi enam kelompok, kelompok kontrol yang diberikan minyak kelapa murni (VCO), kelompok dosis yang diberikan minyak cincalok (100, 400, 1000 mg/kgbb), dan dua kelompok satelit (kelompok kontrol VCO dan 1000 mg/kgbb). Parameter hematologi yang dievaluasi meliputi hemoglobin, *Red Blood Cell Count* (RBC), *Mean Corpuscular Volume* (MCV), *Mean Corpuscular Hemoglobin* (MCH), *Mean Corpuscular Hemoglobin Concentration* (MCHC), leukosit, basofil, limfosit, eosinofil, trombosit, dan neutrofil. Hasil penelitian ini menunjukkan bahwa hemoglobin, RBC, MCH, MCHC, basofil, eosinofil, neutrofil batang, segmen neutrofil, limfosit, leukosit, monosit dan trombosit pada tikus betina dan jantan masih dalam batas normal. Sedangkan, terjadi penurunan di bawah nilai normal pada parameter MCV (pada tikus betina) dan hematokrit (pada tikus jantan), tetapi penurunan ini bersifat reversibel.

Kata Kunci: Cincalok, Hematologi, Toksisitas Subkronik

1. Introduction

Cincalok is a typical West Kalimantan fermented food derived from Acetes sp type shrimp and made by mixing fresh small shrimp, salt, and sugar in a certain ratio and incubating for 3–7 days.[1] Cincalok contains omega 3 and omega 6, which are useful for increasing the growth and development of children and pregnant women and reducing the risk of cardiovascular disease, respectively.[2] Cincalok contains omega 3 and omega 6, which are useful for increasing the growth and development of children, pregnant women, and reducing the risk of cardiovascular disease, as well as astaxanthin, which is useful as an antioxidant with an IC50 value of 12.2 ± 1.5 g/mL and is included in the antioxidant category. It is very strong in reducing oxidative stress in obese people and inhibits aging, besides other ingredients in cincalok in the form of glutamic and is included in the antioxidant category. It is very effective in reducing oxidative stress in obese people and inhibiting aging, besides other ingredients in cincalok in the form of glutamic acid, which is important in the body's metabolism and beneficial for the brain as a neurotransmitter.[3,4,5,6] Therefore, cincalok has the opportunity to be used as a new supplement, in which case the conditions of the drug or supplement must be of high quality, useful, and in accordance with applicable regulations, as well as safe to use. Cincalok oil safety can be tested through a toxicity test.[7]

Toxicity is defined as a condition that shows toxic or toxic effects that can cause damage to sensitive parts inside or outside the bodies of living things.[8] Cincalok oil was tested for acute toxicity by researchers and found to have an LD50 > 5000 mg/kgbw, placing it in the practically non-toxic category. Long-term use and repeated use encourage the need to determine subchronic toxicity.[9]

Determination of subchronic toxicity is necessary because cincalok also has content that can be toxic if damaged, such as the content of unsaturated fatty acids and astaxanthin in cincalok oil, which are easily oxidized, and glutamate content, when mixed with salt, will form MSG, which, if consumed in excessive amounts, triggers free radicals that can cause cell damage and can also cause cell death (excitotoxicity) in the brain.[3,9,10] Free radicals also cause lipid peroxidation in cell membranes that facilitate erythrocyte cells undergoing hemolysis.[11,12] This encourages researchers to test the subchronic toxicity of cincalok oil, which is observed through the parameters of blood hematology profiles (hemoglobin, erythrocytes, leukocytes, neutrophils, basophils, eosinophils, lymphocytes, monocytes, hematocrit, platelets, MCV, MCHC), which aims to see changes in blood profile due to metabolic disorders, lymphocyte disease, and the effect of test preparations after 28 days of administration in Wistar strain rats.

2. Method

2.1. Equipment

The equipment included surgical instruments (Aesculap M376108, USA), glassware (Iwaki Pyrex®, Japan), blender (Philips®, Dutch), porcelain dishes (Iwaki Pyrex®, Japan), hot plates (Thermo Scientific®, USA), micropipette (Rainin E1019705k®, Swiss), centrifuge (Hettich®, Germany), 1 cc and 3 cc syringes (Terumo®, Japan), spectrophotometer (Mindray BA-88A®, Germany), eppendorf tube, refrigerators (Toshiba, Japan), analytic scales (Shimadzu AUY-220, Japan), oral sonde, mouse scales, and vacuum dryer (Maksindo VD-4, Indonesia).

2.2. Materials

Sodium citrate (Merck®, Germany), 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, for mujair fish.

2.3. Methods

2.3.1. Preparation of Cincalok Oil

Wet cincalok was filtered, and the residue was dried at 50ºC for 3 hours using a vacuum dryer. It was ground in a blender

for 15 minutes to a fine powder. Cincalok fine powder was soaked in VCO solvent. The mixture was filtered after 24 hours.

2.3.2. Experimental Animals

The subjects used in this study were male and female Wistar strain rats with bodyweight 150-200 grams (no weight variation more than 20%), age of approximately 6-8 weeks (adults). The animals were obtained from the pharmacology laboratory, pharmacy study program, faculty of medicine, Tanjungpura University, Pontianak. Their specific characteristics are no anatomic abnormalities, no visible appearance of dull, falling, or bald hair, and active moves, and not pregnant in female mice. Before testing, the subjects were acclimatized under laboratory conditions for 7 days, and were given a standard diet and drinking water ad libitum. The Animal Ethics Committee of the Faculty of Medicine Universitas Tanjungpura peer-reviewed the protocol, which was granted the ethical clearance number 6630/UN22.9/TA.00.03/2019.

2.3.3. Subchronic Toxicity Testing

The test animals used in this study were 60 white Wistar rats. Test animals are grouped into six groups of male rats and six groups of female rats, each consisting of 5 rats. Behavioral test observations to determine the impact of test preparation include the number of subjects on the platform, motor activity, straub, piloerection, ptosis, pineal reflex, corneal reflex, lacrimation, catalepsy, hanging, retabilism, flexion, hafner, mortality, grooming, defecation, urination, respiration, salivation, vocalization, tremor, seizures, and writing.

The oral subchronic toxicity test was conducted based on OECD 407 guideline for 28 days and continued for 14 days for the satellite groups.[13] Behavior and motor activity tests were carried out by observing the rat's behavior before and after the experiment. Moreover, weight monitoring of rats was observed in all groups. Rats were acclimatized for 7 days, and the healthy ones without any physical defect were included as animal subjects. Rats were grouped into 6 groups consisting of VCO (virgin coconut oil), cincalok oil dosage 100 mg/kgbw, cincalok oil dosage 400 mg/kgbw, cincalok oil dosage 1000 mg/kgbw, vco (virgin coconut oil), cincalok oil dosage 1000 mg/kgbw.

2.3.4. Haematological Profile Examination

A haematological profile was performed with rats under anesthesia then blood was drawn through the rat's heart using a one cc syringe. Then, 0.5 mL of blood was put into Eppendorf, which contained an EDTA anticoagulant of 0.5 mL. Blood and EDTA

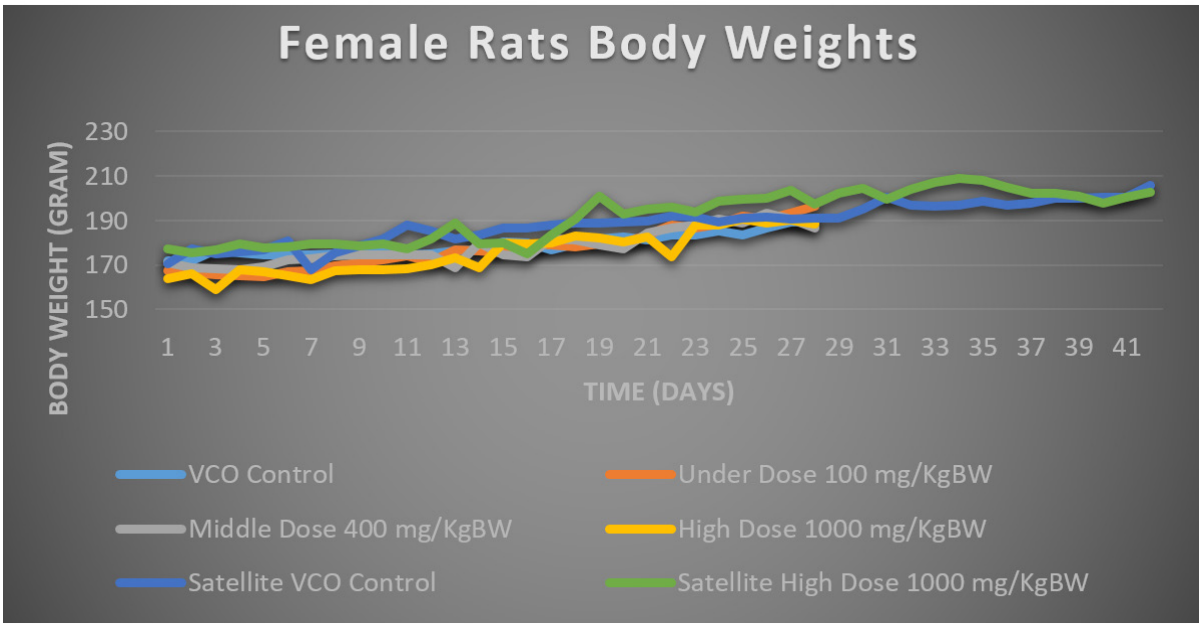

Figure 1. Graph of Body Weight of Female Rats.

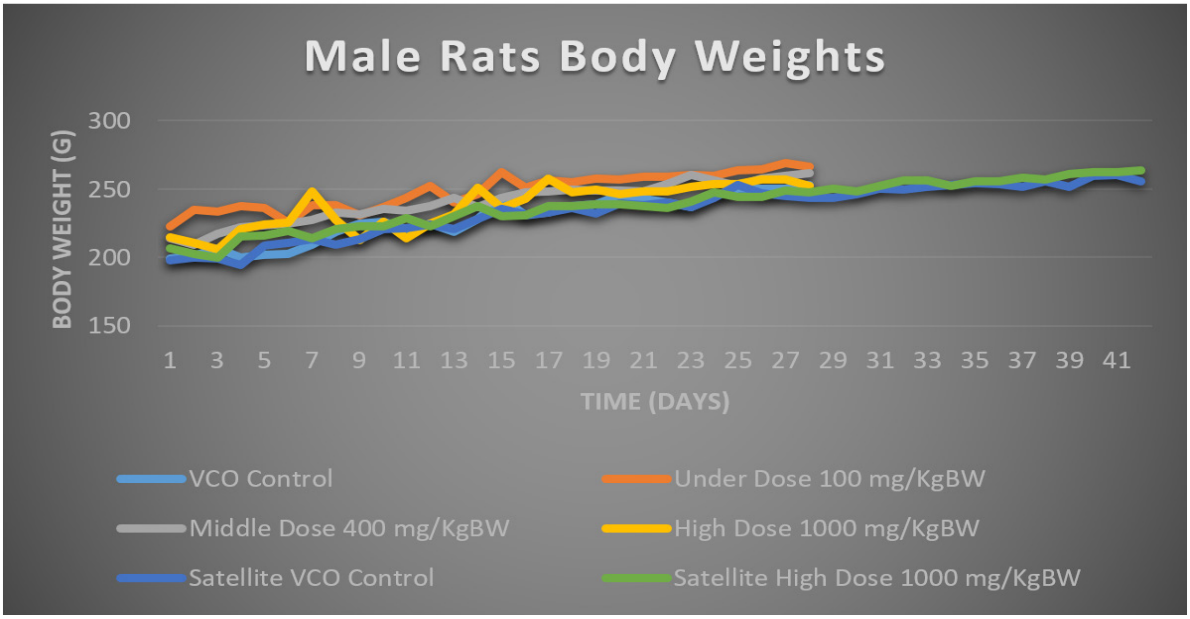

Figure 2. Graph of Body Weight of Male Rats.

were shaken mixed, and there was no blood clotting. Blood containing EDTA was then tested for hemoglobin, erythrocytes, MCV, MCH, MCHC, leukocytes, basophils, lymphocytes, eosinophils, platelets, neutrophils.

2.3.5. Data Analysis

Data obtained from observations of behavior and motor activity were analyzed descriptively. While the bodyweight and the hematology results were analyzed using the SPSS program with the ANOVA method with a confidence level of 95%.

3. Result

3.1. Behaviour, Motoric and Weight Profiles

All groups' behavioral parameters, including groming, defecation, and urination, showed significant variation from the control group of male and female rats. The rats' respiratory systems were both normal, and the test dose group's rats' parameters for the pineal reflex, corneal reflex, hafner, flexion, hanging, retablismen, defecation, and urination did not differ significantly from those of the control group. Ptosis, lacrimation, straub, piloerection, catalepsy, salivation, vocalization, tremor, convulsions, and writhing are other behavioral traits that were not observed in all groups. Not all test animal groups exhibited writhing. According to the results of the behavioral tests, the treatment with cincalok oil has a typical impact on the test animals' motor activity. All groups' behavioral and motoric observations showed that all parameters were normal before and after the experiment. Thus, there was no effect of cincalok and VCO on the behavioral and motoric of subjects. Behavioral observation test result data is not shown.

The next parameter observed was the bodyweight of the test animals. Data on the bodyweight of test animals was weighed for 29 days for the test group and 43 days for the satellite group before and after treatment, then analyzed with an independent sample t-test. Based on Figures 1 and 2, there was a significant increase in body weight in all test and satellite control groups after oral administration of cincalok oil. The statistical analysis results obtained a significance value of $p > 0.05$ in the female and male test animal groups after oral administration of cincalok oil, which showed no significant differences between groups.

3.2. Haematological Profile

The average level of haematological profile in this study indicated that hemoglobin, RBC, MCH, MCHC, basophils, eosinophils, stem neutrophils, neutrophil segments, lymphocytes, leukocytes, monocytes and platelets in female and male rats were still in

the normal range ($p<0.05$). Meanwhile, there was a decrease in levels below the normal range in the parameters of MCV (in female rats) and Hematocrit (in male rats). These differences were statistically significantly different compared to the normal group.

MCV levels decreased below the normal range in the 1000 mg/kgbw dose group of female rats. However, MCV levels in the satellite control group with a dose of 1000 mg/kgbw were in the normal range. Thus cincalok oil does not irreversibly affect MCV levels in female rats.

HCT levels in the treated male rats decreased in the dose groups of 100, 400, and 1000 mg/kgbw compared to the control VCO group. The normal HCT levels in the 1000 mg/kgbw satellite control group. cincalok oil does not irreversibly affect HCT levels in male rats.

The results obtained for leukocyte levels were a decrease below normal levels

Table 1. Profile haematology of Cincalok, control and satellite group for 28 days of female rats.

Parameter	Normal Dose of Rats		VCO Control	Female Rats Group				
				Cincalok oil dose of 100 mg/ Kgbw	Cincalok oil dose of 400 mg/ Kgbw	Cincalok oil dose of 1000 mg/ Kgbw	Satellite control VCO	Satellite control cincalok oil dose of 1000 mg/ Kgbw
RBC	6.76-9.20 x 106/µL	Mean	6.6	7.53	7.32	7.86	7.19	7.07
		SD	0.651	1.002	0.665	1.939	0.472	0.463
Hb	11.5-16.1 g/dL	Mean	11.886	13.006	12.652	12.166	11.832	11.978
		SD	1.033	1.570	0.731	1.287	0.728	0.543
Hct	37.2-50.6%	Mean	35.658	39.018	37.926	36.498	35.496	36.04
		SD	3.099	4.710	2.163	3.863	2.185	1.745
MCV	49.8-65.6µ3/sel	Mean	54.424	52.014	52.076	47.654*	49.42	50.898
		SD	5.061	4.360	4.795	7.083	2.155	1.958
MCH	14.2-19 pg/sel	Mean	18.136	16.782	17.37	15.88	16.47	16.876
		SD	1.687	2.190	1.587	2.358	0.717	0.738
MCHC	26.2-36.1 g/dL	Mean	33.234	33.676	33.522	33.06	33.066	33.14
		SD	0.540	0.724	0.448	0.695	0.202	0.164
Leukocytes	6.60-12.6 x 103/µ	Mean	7.28	9.1	6.86	5.96	6.36	8.12
		SD	2.688	4.110	3.491	1.066	1.837	3.140
Basophils	0-0.6 %	Mean	0	0	0	0	0	0
		SD	0	0	0	0	0	0
Eosinophils	0-1.96 %	Mean	0.8	0.4	0.8	0.8	0.8	0.6
		SD	0.447	0.547	0.447	0.447	0.447	0.547
Bands Neutrophils	2-4 %	Mean	2.8	2	2	2.4	2.8	2.2
		SD	0.836	1.224	1.224	0.894	0.836	0.447
Segment Neutrophils	4.4-49%	Mean	30	30	36.4	39.2	32.6	38.4
		SD	7.778	7.314	8.820	7.496	9.633	7.765
Limphocytes	50.2-84.5%	Mean	67.6	66,8	62.2	57	65	58.2
		SD	7.334	7,463	10.733	7.810	11.379	8.136
Monocyte	0-1.81 %	Mean	0.8	0,8	0.6	0.6	0.8	0.6
		SD	0.447	0,447	0.547	0.547	0.447	0.547
Platelets	150-460 x 103/µl	Mean	180	166	220	162	204	178
		SD	22.360	24,083	41,833	13.038	31.304	31.144

*$p < 0.05$.

Table 2. Profile haematology of Cincalok, control and satellite group for 28 days of Male rats.

Parameter	Normal Dose of Rats		VCO Control	Male Rats Group				
				Cincalok oil dose of 100 mg/ Kgbw	Cincalok oil dose of 400 mg/ Kgbw	Cincalok oil dose of 1000 mg/ Kgbw	Satellite control VCO	Satellite control cincalok oil dose of 1000 mg/ Kgbw
RBC	6.76-9.20 x 106/µL	Mean	8.26	7.3	7.46	7.278	6.83	8.1
		SD	0.568	0.968	0.521	1.045	0.349	0.845
Hb	11.5-16.1 g/dL	Mean	13.518	12.106	11.97	12.132	12.004	13.748
		SD	0.792	1.254	1.334	0.942	0.658	0.906
Hct	37.2-50.6%	Mean	40.554	36.318*	36.13*	36.396*	36.012	41.244
		SD	2.377	3.763	3.546	2.827	1.975	2.719
MCV	49.8-65.6µ3/sel	Mean	49.2	49.938	48.432	49.856	52.718	51.114
		SD	3.210	3.173	3.596	2.826	0.296	2.993
MCH	14.2-19 pg/sel	Mean	16.396	16.644	16.036	16.618	17.57	17.034
		SD	1.071	1.058	1.391	0.945	0.095	0.997
MCHC	26.2-36.1 g/dL	Mean	33.826	33.344	33.344	33.324	33.302	33.848
		SD	0.335	0.401	0.344	0.188	0.734	0.302
Leukocytes	6.60-12.6 x 103/µ	Mean	9.6	7.5	8.01	6.06	9.14	6.62
		SD	3.073	2.681	2.340	1.101	2.580	2.071
Basophils	0-0.6 %	Mean	0	0	0	0	0	0
		SD	0	0	0	0	0	0
Eosinophils	0-1.96 %	Mean	0.6	0.8	0.6	0.8	0.8	0.6
		SD	0.547	0.447	0.547	0.447	0.447	0.547
Bands Neutrophils	2-4 %	Mean	2.2	2.6	2.8	2.6	2.6	3
		SD	0.836	0.894	0.836	1.140	1.140	1
Segment Neutrophils	4.4-49%	Mean	31.2	33	39.4	38.2	34	37
		SD	12.755	5.385	7.231	9.833	10.198	10.344
Limphocytes	50.2-84.5%	Mean	65.4	61.2	56.4	58	62	58.8
		SD	13.390	6.685	7.829	9.327	10.931	10.545
Monocyte	0-1.81 %	Mean	0.6	0.8	0.8	0.4	0.6	0.6
		SD	0.547	0.447	0.447	0.547	0.547	0.547
Platelets	150-460 x 103/µl	Mean	190	184	178	204	172	242
		SD	27.386	20.736	14.832	21.908	19.235	63.796

*$p < 0.05$.

in female and male rats in the 1000 mg/kgbw treatment group, although statistically not significantly different. However, in the 1000 mg/kgbw satellite control group, leukocyte levels returned to the normal range.

4. Discussion

Based on the results of 28 days of subchronic toxicity evaluation for the test group and 43 days for the satellite group, no mortality was found. In testing the motor activity of female and male rats, there were no significant changes in the test group and satellite group. Observation of body weight in female and male test groups showed no significant differences between groups. The increase in body weight in all groups of test animals is thought to be due to the content of cincalok glutamic acid that reacts with salt to form MSG, where MSG can increase the appetite of test animals so that their body weight also increases.[14,15]

Haematological profiles were obtained, which showed decreased levels below normal in the parameters MCV (in female rats) and hematocrit (in male rats). A kind of anemia known as microcytic anemia, in which the red blood cells are smaller than normal, is indicated if the MCV value is below the normal level. These patients frequently experience fatigue, weakness, loss of stamina, shortness of breath, lightheadedness, and pale skin.[16,17,18] The hematocrit measures the quantity of red blood cells in the body. We may determine the ratio of the quantity of red blood cells (erythrocytes) to the blood volume in percent by examining at the hematocrit. Diagnosis for anemia, leukemia, dehydration, and nutritional deficits is done using hematocrit tests. Leukemia, hemolytic anemia, and bone marrow disorders can all be indicated by low hematocrit levels.[19,20] However, the decrease in MCV and hamatocrit parameters is temporary, as evidenced by normal MCV and hematocrit levels in the 1000 mg/kgBW satellite control group.

5. Conclusion

Consumption of cincalok oil did not statistically affect the hematological profile of the test animals. there was a decrease in MCV and hematocrit levels, but it was only temporary or reversible effect and would return to normal if the consumption of cincalok oil was stopped.

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