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Original Article

ACUTE TOXICITY EVALUATION OF *CURCUMA DOMESTICA* VAHL. RHIZOME VCO CURCUMINOID EXTRACT

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ABSTRACT

Objective: Recently, Curcuma domestica has been reported to have various pharmacological activities, such as antioxidant, anti-inflammatory and antifungal activities. The current study was conducted to evaluate the safety of C. domestica VCO Curcuminoid extract in short term and determine its curcumin content.

Methods: Turmeric extract in VCO was prepared using a Microwave Assisted Extract (MAE) method, acute toxicity tests were carried out using 15 female mice divided into 5 preliminary test groups with 5, 50, 300, 1000, 2000 mg/KgBW doses. Furthermore, 10 female mice divided into 2 groups with 1000, 2000 mg/Kg BW doses as the main test. At the end of the experiments, the blood hematology, blood clinical biochemistry, and histopathology examination were performed.

Results: Turmeric rhizome curcuminoid VCO extract at the dose of 2000 mg/Kg BW indicated signs of toxic symptoms, including diarrhea, changes in fur and skin and walking on the stomach. There are significant difference among the data of blood biochemistry and blood hematology of the CMC-Na group, with extract at the doses of 1000 mg/KgBW.

Conclusion: It can be inferred that the turmeric rhizome VCO curcuminoid extract induces acute toxicity at a dose of 2000 mg/KgBW.

Keywords: Curcuminoid, Curcuma domestica, Toxicity, Acute

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INTRODUCTION

Turmeric, scientifically known as Curcuma longa Linn, is commonly utilized as a component in traditional Indonesian medicine due to its diverse therapeutic characteristics [1]. The herb exhibits pharmacological properties, including its usage in the treatment of hypertension [2]. Hypertension, often known as high blood pressure, is a significant determinant for cardiovascular disease's development [3], which is a prevalent cause of mortality worldwide. Curcuminoids are yellow polyphenols that are slightly soluble in water and acidic solvents and soluble in dimethyl sulfoxide (DMSO), acetone, and ethanols [4]. Curcuminoids have many activities, such as lowering blood sugar (perko *et al.*, 2015) [5], anti-carcinogenic [6], and antioxidant, anti-inflammatory [7],

In this research, the curcuminoid compounds of turmeric rhizome (curcuma domestica Vahl.) was estracted with Virgin Coconut Oil (VCO) using the MAE (Microwave Assisted extraction) method at (270 Watt) for 10 min. Coconut oil pure or what is called Virgin Coconut Oil (VCO) is derived from fresh coconuts (Cocos nicifera) through a process that excludes any chemical additives or high-heat processing [8]. When compared to other vegetable oils like palm, soybean, corn, and sunflower oil, VCO has several advantages, including lauric acid content ($C_{11}H_{23}COOH$) and fatty acid composition high, medium chain fatty acid (MCFA) and molecular weight low [9].

Medicinal plants have been utilized historically for safeguarding health and managing ailments since ancient eras, as it is believed that synthetic medicines often have adverse consequences. Recent surveys show that medical plants do too heve side effects. Due to several concerns that have arisen regarding potential toxix effects caused by the use of this plant, then carried oud toxicological impact evaluation for clinical use or preclinical studies [10]. Base on the description above, this research aims is curcuminoid from turmeric rhizome (Curcuma domestica Val.) (CVE) has acute toxic effects. Testing for toxic effect needs to be carried out to test the safety for a drug that meets established quality requirements, proven effectiveness and safety.

Acute toxicity testing is necessary to evaluate the CVE's safety. The purpose of this test is to gather data on the lethal dosage range for the test subjects, the etiology of mortality, the progression of the dying sequence, and the indications of intoxication [11]. This experiment carried out orally by administering test with various dose variants. In the acute toxicity test, observations were made during the first 24 h continued observation for 14 d and then the test animals were sacrificed.

MATERIALS AND METHODS

Plants material and reagents

The materials used include CVE, CMC Na 0.5%, NaCl physiology (Otsuka), buffer formalin 10%, EDTA 10%, ketamine, Hematoxylin Eosin (Merck) 1, and female Wistar rats. Turmeric herbs are taken from the Laucih Village area, Deli Serdang Regency, North Sumatra Province. The tools used in this research include glassware labpratory (Pyrex), Rotary evaporator (Heidolph) Germany, autoclave (Hirayama) Japan, blender (Panasonic), microscope (Carl Zeiss) Germany, Hematology analyzer (Sysmex) Japan, micropipette (Thermo scientific) Finland, oral sonde, microwave (Sharp) Indonesia, animal blance (Sartorius) Indonesia, analytical balance (Sartorius) Indonesia. Centrifuge (Zenith Lab) China.

Sample preparation

The research utilizes turmeric rhizomes as the primary material. These rhizomes were collected and meticulously washed with running water. Subsequently, they was drained and put on paper to allow absorption of any remaining water. Once this is complete, the material was weighed and subsequently cut into pieces. Then, the material was dried by placing it in a drying cabinet. The weight of

the dry material was weighed. Next, store it in an airtight plastic bag in a place protected from sunlight [12].

Making curcuminoid VCO extract

Making turmeric extract in VCO was done using a microwave-assisted exctractin (MAE), namel weighing 6 gs of simplicia powder, then adding VCO to reach a volume of 60 ml, then put in the microwave for 10 min at medium temperature (270 watt), Stir until homogeneous, Wait until it cools then filter with filter paper [13].

Simple characterization

The characterisation of Simplicia involved many methods, such as microscopic examination, macroscopic examination, the determination of total ash content, measurement of water content, determination of soluble essence content in water, determination of acid insoluble ash content, and determination of soluble essence.

Identification of curcumin compounds

Identification of curcumin compounds was carried out qualitatively applying thin layer chromatography with the mobile phase benzene: chloroform: ethanol (45:45:10) and then viewed using a 254 nm and 366 nm UV lamp [14].

Biochemical analysis

Blood samples from the orbital sinus were collected and transferred into Eppendorf tubes. Subsequently, the samples underwent centrifugation to separate the serum, which was then analyzed to assess the activity levels of Creatinine, SGPT (Serum Glutamic Pyruvate Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), and BUN (Blood Urea Nitrogen). SGOT as well as Enzymatic reactions were utilized to ascertain the amounts of SGPT by combining a 100 µl sample solution with 1000 µl of the respective reagent kit. Creatinine analysis involved mixing a 50 µl sample solution with a 1000 µl reagent kit, and BUN measurement included combining a 10 µl sample solution with a 1000 µl reagent kit. The mixture then assessed utilizing a Microlab 300 instrument at a wavelength of 340 nm.

Hematology analysis

Blood samples were transferred into test tubes containing an anticoagulant known as ethylenediaminetetraacetic acid (EDTA) for hematological assessment. Test parameters encompass hemoglobin concentration, platelets, erythrocyte count, segment neutrophils, MCHC (mean Corpuscular Hemoglobin Concentration), lymphocytes, MCV (mean Corpuscular Volume), MCH (mean Corpuscular Hemoglobin), hematocrit, and leukocytes [15].

Histopathological analysis

The liver, lungs, kidney, and heart specimens underwent a paraffin wax coating process for a duration of two hours at temperatures ranging from 60 to 700 degrees Celsius. Subsequently, the paraffin blocks were sliced using a microtome to achieve of around 5-7 µm thickness. These slices were then printed and subjected to freezing. The organ components are set on a surface that was heated to a temperature of 56–58 °C for around ten seconds in order to elongate and attach to the slide. Modifications were implemented to prevent the folding of tissues. In addition, hematoxylin and eosin stains were performed. The tissue was immersed in a xylene solution for a duration of twelve minutes. Following a five-minute exposure to ethanol solutions of several concentrations (70%, 80%, 90%, and 100%), the tissue was dehydrated by rinsing it with running water. Following duration of 5 min in the hematoxylin solution, the samples underwent rinsing with flowing water. The sample was stained with eosin and thereafter submerged in ethanol solutions at progressively higher concentrations (70%, 80%, 90%, and 100%) for a duration of 10 min each. During the final stage, the samples were immersed in xylene for a duration of 12 min prior to examination under the microscope at a magnification of 40×.

Statistical analysis

The research data were examined utilizing the SPSS software. The determination of homogeneity and normalcy relies on the statistical analysis employed. The results of the acute toxicity examination were scrutinized through the One-way ANOVA test to ascertain the average variances among treatments. Assuming that a substantial disparity exists, proceed with employing Tukey's post hoc test to identify the variables that exhibit differences. The threshold of $p<0.05$ is utilized to indicate statistical significance.

Provision of test preparation

Ethical Considerations, the perfomence of this study was cunducted based on the guideline and approval of the Ministry of Health and The Ethics Committee. In addition, all operations were approved by the Animal Research Ethics Committees (AREC) of the University of Sumatera Utara`s Faculty of Mathematics and Natural Science, Biological Department, with the approval number 0162/KEPH-FMIPA/2024. 26, Maret 2024.

During the testing phase, the experimental animals underwent a 24 h period of fasting before to receiving the test preparation. Subsequently, their weights were measured. Subsequently, a preliminary examination was conducted on five test subjects, administering 5, 50, 300, 1000, as well as 2000 mg/kg body weight (BW) doses. Moreover, the toxic symptoms were detected within the initial 4 h period and persisted for the subsequent 24 h following the therapy. The primary acute toxicity assessment was conducted on a sample of five test subjects, administering dosages of 1000 and 2000 mg/kg BW. Subsequently, toxic effects manifested within the initial 4 h period and persisted throughout the subsequent 24 h period following the therapy. Monitoring of hazardous symptoms persisted on a daily basis until the 14th d.

RESULTS AND DISCUSSION

Simple characterization

Curcumin extracted from turmeric with the structural formula 1,7-bis (4-hydroxy-3 methoxyphenol)-1,6-hepdiene-3,5-dione is a natural yellow pigment used in cosmetics and medicine [9].

Table 1: Results of simplicia characterization

No	Parameter	Result	FHI
1.	Macroscopic	Elongated, branched, yellow in color, smells like turmeric, bitter taste	Elongated, branched, yellow in color, smells like turmeric, bitter taste
2.	Microscopic	Turmeric transport bundles, peridem tissue and covering hair	Turmeric transport bundles, peridem tissue and covering hair
3.	Determination of water content	6.98±0.99	No more than 10%
4.	determination of soluble essence content in water	17.58±0.23	Not less than11.5%
5.	determination of soluble essence content in ethanol	13.2±0.66	Not less than11.4%
6.	determination of acid-insoluble ash content	7.7±0.09	No more then 8.2%
7.	determination of total ash content	0.53±0.11	No more than 0.9%

From table 1, the results of the characterization of turmeric simplicia show that the results are in accordance with the 2017 Indonesian Herbal Pharmacopoeia. Characteristics that are in accordance with the FHI indicate that the sample to be used meets official standards to be used as medicine.

Identification of curcumin compounds

Identification of curcumin compounds was carried out qualitatively employing thin layer chromatography utilizing benzene as the mobile phase: chloroform: ethanol (45:45:10) and then viewed using a 254 nm and 366 nm UV lamp [11]. As in fig. 3, 4, and 5 below:

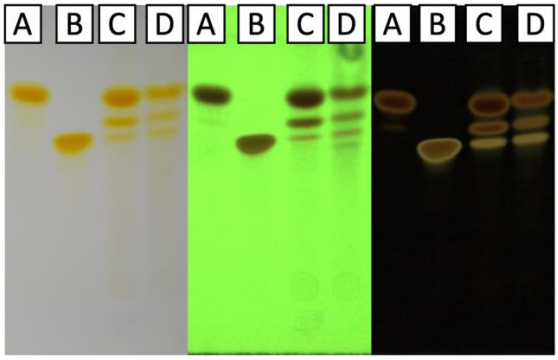

Fig. 1: A: Curcumin, B: Bisdemetoxicurcumin C: Curcuminoids, D: Mae

Mobile Phase Benzene: Chloroform: Ethanol (45:45:10). Extraction of turmeric simplicia powder extracted using VCO solvent using medium conditions for 10 min can extract curcuminoid compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) as proven by the results of klt analysis.

Acute toxicity test results

Observation results of toxic symptoms in preliminary tests

Symptoms of toxicity were detected within each group and subsequently juxtaposed with those of the control group. Notable toxic symptoms include tremors, diarrhea, excessive salivation, muscular weakness, alterations in hair and skin condition, changes in the eye mucosa, and abnormal animal locomotion such as retrograde walking and belly crawling. Prior to conducting the primary test, to establish the proper dosage for the primary test at the outset, a preliminary test is first performed [16].

Table 2: Results of observations of toxic symptoms in the preliminary test

Toxic symptoms	Normal control (CMC Na 0.5%)	dose 5 mg/Kg BB	Dose 50 mg/Kg BB	Dose 300 mg/Kg BB	Dose 1000 mg/Kg BB	Dose 2000 mg/Kg BB
Tremors	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-
Salivation	-	-	-	-	-	-
Weak	-	-	-	-	-	-
Changes in fur and skin	-	-	-	-	-	-
Changes in the eye mucosa	-	-	-	-	-	-
Walk backwards	-	-	-	-	-	-
Walk using your stomach.	-	-	-	-	-	-

Table 2 demonstrates that the animals are engaging in regular activities without experiencing any harmful symptoms. The CVE's components were administered at doses of 5, 50, 300, 1000, as well as 2000 mg/Kg BW in both the control group as well as the treatment group. This shows that there is no relationship between dose and toxic effects, where no toxic symptoms were seen in any treatment group. The toxic properties of a compound are determined by the dose. Increasing the dose can cause more organ systems to produce very different effects [17].

Table 3: Average body weight of mice in the preliminary test

Group	Days to		
	0	7	14
Normal control (CMC Na 0.5%)	156.4±2.07	156.6± 1.14	157±1.52
5 mg/Kg BB	155.8±2.28*	156.6±2.28	157.3±1.45
50 mg/Kg BB	158.6±1.82	158±2.92	158.3±2.24
300 mg/Kg BB	157.0±1.58	158.8±1.48	160.3±1.48
1000 mg/Kg BB	154±1.58	160±2.12*	161.8±1.79
2000 mg/Kg BB	155.6±2.07	153.2±2.39*	149.8±1.58*

Results are given in mean±SD, n=5. (*) There is significant different with the control group ($p<0.05$).

In toxic symptoms, body weight parameters are a sensitive indicator. Test animals were observed and body weight was measured periodically every day (Gupta and Bhardwaj, 2012) [18]. Before and after 14 d of giving each animal the CVE's, their body weight was recorded. Statistical results of weight observations. Table 3 displays the initial test.

According to the data shown in table 3, the statistical analysis demonstrates a notable disparity in weight reduction between the control group as well as the group administered with CVE's derived from turmeric rhizome ($p>0.05$), namely on the first day p= 0.024, on the 7th day p= 0.017 and on day 14 p=0.0. Significant differences were shown in test animals by giving a dosage of 2000/mg Kg BW CVE's. According to [19], it is said to be toxic if there is a 10% change in body weight.

Based on table 4 above, it is known that no animals died during the administration of the test preparation within the 14 d observation period. This shows that the CVE's did not cause death during 14 d of observation after treatment in test animals.

Table 4: Results of observations of mouse death and preliminary tests

Treatment	Number of rats	Number of dead mice
Normal control (CMC Na 0.5%)	5	0
5 mg/Kg BB	5	0
50 mg/Kg BB	5	0
300 mg/Kg BB	5	0
1000 mg/Kg BB	5	0
2000 mg/Kg BB	5	0

Table 5: Results of observations of toxic symptoms in the main test

Toxic symtopms	Normal control (CMC Na 0.5%)	Dose 1000 mg/Kg BB	Dose 2000 mg/Kg BB
Tremors	-	-	-
Diarrhea	-	-	√
Salivation	-	-	-
Weak	-	-	-
Changes in fur and skin	-	-	√
Changes in the eye mucosa	-	-	-
Walk backwards	-	-	-
Walk using your stomach	-	-	√

Information; (-) does not show toxic symptoms, (√) shows toxic symptoms

Symptoms of toxicity were noted in every group and subsequently contrasted with those of the control group. Notable toxic symptoms include tremors, diarrhea, excessive salivation, muscular weakness, alterations in hair and skin condition, changes in the eye mucosa, and abnormal animal locomotion such as retrograde walking and belly crawling. According to the data in table 3.5, it is clear that the test animals treated with control substances (CMC Na 0.5%) and CVE's at a dosage of 1000 mg/Kg BW exhibited normal behavior and did not experience any toxic symptoms. This indicates that there is no correlation between the dosage and toxic effects, as no overt toxicity indicators were found throughout the research. This differs from the experimental animals that received CVE's at a dosage of 2000 mg/Kg BW. Table 5 indicates that the administration of a 2000 mg/Kg BW test dose resulted in the manifestation of toxic symptoms, including diarrhea, alterations in fur and skin, and ambulation utilizing the stomach. In accordance with previous research which demonstrates that substances can cause undesirable effects related to the dose given, namely side effects, adverse effects and toxic effects [20].

Results of weight observations in the main test

The body weight of each animal was monitored before being treated with 0.5% CMC Na and CVE's. Statistical results of rat body weight in the main acute toxicity test can be observed in table 6.

Based on table 3.6, the results of observations of body weight in the main test mice above show that the statistical results show that there is a significant difference in weight loss in mice between normal controls (CMC Na 0.5%) and treatment with administration of CVE's at a 1000 mg/kg BW ($p<0.05$) dose, and substantially distinct from administering a dose of VCO curcuminoid extract at a 2000 mg/kg BW dose, because there was a change in body weight or weight loss of more than 10%. This shows that the CVE's affects the development of body weight in mice.

Table 6: Results of weight observations in the main test

Group	Days to		
	0	**7**	**14**
Normal control (CMC Na 0.5%)	159.6±1.82	158.4± 2.33	158.5±1.58
1000 mg/Kg BB	168.8±2.49*	167.2±2.86	161.8±2.07
2000 mg/Kg BB	169.2±2.49*	162±1.41*	151.8±2.24*

Results are given in mean±SD, n=5. (*) There is significant different with the control group ($p<0.05$)

Table 7: Results of observations of mouse death in the main test

Treatment	Number of rats	Number of dead mice
Normal control (CMC Na 0.5%)	5	0
1000 mg/Kg BB	5	0
2000 mg/Kg BB	5	1

Based on table 7 above, it is known that one test animal died, namely the test animal given a test dosage of 2000 mg/Kg BW. The former also indicates symptoms of diarrhea also weight loss before death. This shows that the test preparation of CVE's at a 2000 mg/kg BW dose caused mild toxicity, this is in accordance with the toxicity classification criteria for test preparations in [21]. Based on the results of the main test at a 2000 mg/Kg BW dose, one test animal died and caused toxic symptoms, which is based on the hazard criteria of the "GHS (Globally Harmonized Classification System for Chemical Substances and Mixtures)" listed in the "Thirteenth Addendum to The OECD Guidelines for The Testing of Chemicals" (2001) is included in category 5 or not classified. Because the LD50 is higher than 2000 mg/kg, assuming 3 or more animals survive.

Results of organ macro pathology observations

The dissection of experimental animals was conducted on the 15th d. The organ macropathology observations involve assessing the hue, texture, and firmness of heart, spleen, liver, kidneys, and lungs. Color change is a metric used to assess the toxic effects of a test chemical on target organs and their corresponding consequences. Its purpose is to gather information about the substance's toxicity [22]. The macropathological observations of the heart, kidney, liver, spleen, as a well as lungs are presented in table 8 through table 12.

Table 8: Results of liver macro pathology observations

Treatment	Observation		
	Color	**Surface**	**Consistency**
CMC Na 0.5%	Brownish red	Slippery	Springy
Dose 1000 **m**g/Kg BB	Brownish red	Slippery	Springy
Dose 2000 **m**g/Kg BB	Brownish red	Slippery	Springy

Based on the results of table 8, liver macro pathology observations show that the color of the liver is dark red, the surface of the liver looks slippery, and the consistency of the liver is rubbery in all groups of test animals. A normal liver looks brownish red. The red-brown color is caused by blood flow entering the liver [23].

Based on table 9, macro pathological observations of the kidney organs in the control and groups given CVE's did not show any changes, namely blackish red in color. The blackish-red color of the kidneys is a normal condition [24].

Based on table 10, the results of observations on the rat heart organ, there is no change in color in contrast to the control, namely brownish red, b smooth surface shape, as well as rubbery concentration. This shows that administration of CVE's has no effect on the heart organ.

Table 9: Results of macro pathological observations of kidney organs

Treatment	Observation		
	Color	Surface	Consistency
CMC Na 0.5%	Blackish Red	Slippery	Springy
Dose 1000 **m**g/Kg BB	Blackish Red	Slippery	Springy
Dose 2000 **m**g/Kg BB	Blackish Red	Slippery	Springy

Table 10: Results of macro pathological observations of the heart organ

Treatment	Observation		
	Color	Surface	Consistency
CMC Na 0.5%	Brownish red	Slippery	Springy
Dose 1000 mg/Kg BB	Brownish red	Slippery	Springy
Dose 2000 mg/Kg BB	Brownish red	Slippery	Springy

Table 11: Results of macro pathological observations of the spleen organ

Treatment	Observation		
	Color	Surface	Consistency
CMC Na 0.5%	Brownish red	Taper	Springy
Dose 1000 **m**g/Kg BB	Brownish red	Taper	Springy
Dose 2000 **m**g/Kg BB	Brownish red	Taper	Springy

Based on table 11, the macro pathology results of the spleen organ are dark red in color. The surface is sharp and the consistency is chewy. The normal spleen organ is dark red to blackish blue with sharp or crescent-shaped edges. The damaged spleen organ will experience swelling, brown and almost black in color, with blunt edges [25].

Table 12: Results of macro pathological observations of lung organs

Treatment	Observation		
	Color	Surface	Consistency
CMC Na 0.5%	Pink	Slippery	Springy
Dose 1000 mg/Kg BB	Pink	Slippery	Springy
Dose 2000 mg/Kg BB	Pink	Slippery	Springy

Based on table 12, it shows the results of observations of lung organ macro pathology in control experimental animals and administration of CVE's, pink turmeric rhizomes with a smooth surface and a spongy consistency like a sponge.

Table 13: Results of observations of relative organ weights

Group	Liver (%)	Heart (%)	Spleen (%)	Lung (%)	Right kidney (%)	Left kidney (%)
CMC Na 0.5 %	4.93 ±0.42	0.52±0.04	0.60±0.04	1.53±0.05	0.64±0.02	0.61±0.02
1000 **m**g/Kg BB	5.18±0.41	0.44±0.03	0.67±0.03	1.55±0.03	0.62±0.02	0.59±0.03
2000 **m**g/Kg BB	5.54±0.25	0.61±0.04	0.64±0.03	1.48±0.04	0.53±0.02	0.53±0.01

Results are given in mean±SD, n=5.

The statistical analysis of rat organ weights, as presented in table 13, was conducted by applying the one-way ANOVA test. The findings suggest that there was no notable variance in liver weights between the standard control group (CMC Na 0.5%) and the remaining groups, as evidenced by a p-value of 0.965 (p>0.05). When CVE's is administered at a dosage of 1000 mg/Kg BW, it did not yield a substantial distinction in response, as indicated by a p-value of 0.747 (p>0.05) in the statistical analysis. However, When the dosage was raised to 2000 mg/Kg BW, there was a notable difference seen, with a p-value of 9.47 (p>0.05). When CMC Na 0.5% was administered in the cardiac organ, there was no nitable difference observed with a p-value of 0.939 (p>0.05) for the administration and dosage of 1000 mg/kg BW with a p-value of 0.990 (p>0.05). However, a notable variance was observed with the administered dosage. The CVE's was provided at a dosage of 2000 mg/kg BW. Statistical analysis revealed a p-value of 0.895, surpassing the significance level of 0.05. In contrast, when CMC Na 0.5% was administered in the spleen, there was no notable distinction noted, with a p-value of 0.999 (p>0.05)

for the administration as well as a dosage of 1000 mg/kg BW with a p-value of 0.962 (p>0.05). However, a significant difference was evident with the given dosage. The CVE's was provided at a dosage of 2000 mg/kg BW. Statistical analysis indicated a p-value of 0.940, signifying no substantial distinction (p>0.05). The one-way ANOVA test was employed to analyze rat organ weights based on the data presented in table 13. The findings indicated no notable variance in liver weights between the normal control group (CMC Na 0.5%) and the other groups, with a p-value of 0.965 (p>0.05). The CVE's was administered at a dosage of 1000 mg/kg BW. Statistical analysis indicated no significant change (p=0.747, p>0.05). However, when the dosage was increased to 2000 mg/kg BW, a significant difference was observed (p=9.47, p>0.05). When CMC Na 0.5% was applied to the cardiac organ, there was no significant disparity noted, with a p-value of 0.939 (p>0.05). Likewise, a dosage of 1000 mg/kg BW exhibited no notable difference, with a p-value of 0.990 (p>0.05). Nonetheless, a notable discrepancy was observed with the provided dosage. The CVE's was given at a dosage of 2000 mg/kg BW. The statistical analysis yielded a p-value of 0.895, suggesting no substantial distinction (p>0.05). Upon administering CMC Na 0.5% in the spleen, no notable difference was found, with a p-value of 0.999 (p>0.05) for the administration method, as well as a dosage of 1000 mg/kg BW showed a p-value of 0.962 (p>0.05). However, a significant difference was evident with the administered dose. The CVE's was given at a dose of 2000 mg/kg BW. The statistical analysis revealed a p-value of 0.940, surpassing the significance level of 0.05.

Table 14: Hematology observation results

Group	CMC Na 0.5 %	1000 mg/Kg BB	2000 mg/Kg BB
Hemoglobin	15.18±0.6*	6.89±0.15	7,91±0,23
Hematocrit (31-59)	45.24±1.29*	20.90±0,08	23.25±0.50
WBC (10-30)	2.19 ±0.63	3.84±0.25	5.89±0.17
RBC (4,3-6,3)	5.96± 0.23*	4.42±0.55	3.91± 0.13
Platelets (150-440)	801.4±13.5	337.92±2.20	487±5.29
MCV (88-123)	53.2±1.30*	54.89±0.54	57.77± 0.29
MCH (31-37)	20.53±0.3*	18.15±0.25	18.40±0.18
MCHC (28-36)	34.6±0.77*	33.01± 0.14	32.21± 0.19
Monocytes (2-8)	3.98±0.39	0.02±0.02*	4.51±0.18
Eosinophils (1-6)	2.06±0.57*	0.79± 0.15	0.17±0.02
Basophils 0-1)	0.28±0.16*	0.22±0.11	0.78±0.15

(*) There is a significant different with the control group (p<0.05).

Based on table 14, the hematological measurements of the mice above were analyzed statistically using one way Anova, showing that the control test was significantly different from the test animals with results (p=0.005); subsequently, a Post Hoc Test in the form of the Tukey Honestly Significant Difference (HSD) test was conducted on mice, revealing a notable distinction between the control group and the test group (p>0.05) with each significance value for Platelets p=1,000 (p>0.05); RBC p = 0.13 (p>0.05); HB p=1,000 (p>0.05); HCT p = 1,000 (p>0.05); MCV p=1,000 (p>0.05); MCHC p = 0.06 (p>0.05); MCH p = 0.419 (p>0.05); Mon p = 0.166 (p>0.05); EOS p= 0.789 (p>0.05); BAS p=0.981 (p>0.05); WBC p=1,000 (p>0.05). It can be concluded that administration of VCO curcuminoid extract affects the hematological values of animal tests between each group. Good body condition will be characterized by good blood levels and blood components that are within the normal range [26].

Table 15: Results blood biochemical observations

Group		CMC Na 0.5 %	1000 mg/Kg BB	2000 mg/Kg BB
Liver	Total Protein	6.06±0.03	7.59± 0.39	7.6± 0.27
	Direct Bilirubin	0.024±0.01	0.17±0.06	0.04±0.03*
	SGOT	112.72±0.48*	137.28± 0.47*	186.35± 1.26*
	SGPT	34.4±0.71*	91.60±1.14	86.64± 0.41
	ALP	145.48±0.24	168.40± 1.52	102.25±1.26*
Kidney	urea	23.18±0.43	98.23± 0.38*	83.61± 0.35*
	creatinine	0.368±0.03	0.34±0.03	0.34±0.05

(*) There is a significant difference with the control group (p<0.05).

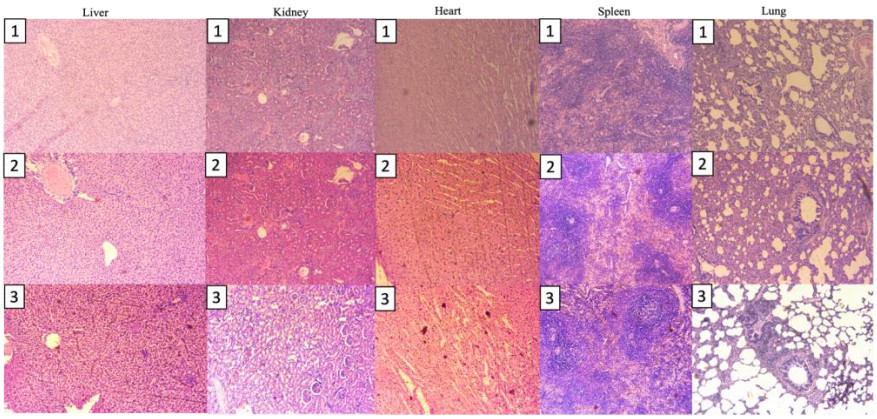

Fig. 2: Microscopic organs observation in acute toxicity 1. CMC Na 0.5%; 2. Dose 1000 mg/Kg BW; 3. Dose 2000 mmg/Kg BW

The clinical, biochemical test results from table 15 were analyzed using statistical methods, specifically One Way Anova. Following this analysis, a post hoc test (HSD) Test was conducted. The results from these tests revealed a noteworthy distinction ($P<0.05$) in the levels of Bilirubin Direct, SGOT, SGPT, ALP, and urea between the normal control group and the group administered with CVE's. However, the examination outcomes for total protein ($p = 0.339$, $P>0.05$) as well as creatinine ($p = 0.444$, $P>0.05$) did not demonstrate any substantial variance. The test results indicate that the ingestion of CVE's has an impact on the parameters of blood biochemistry.

Histological observations

Histological analysis of organs is conducted to establish the correlation between the symptoms manifested and the structural composition of the organ that has been subjected to the experimental substance. The extent of harm induced by the test substance over a period of 14 d was demonstrated through the examination of liver, kidney, heart, spleen, and lung tissue at a microscopic level. Histological preparations are made by slicing the organ using a special cutting machine (microtome) then placing it on a glass slide, after which a staining procedure is carried out using Hematoxylin-Eosin (HE), then covering it with a cover glass and glued using entellan. The preparations were examined using a microscope and documented by photography [27].

Based on fig. 2, it can be seen that the histopathological picture [in the normal group and the dosage of 1000 mg/Kg BW is still in normal condition, namely the hepatocytes are arranged radially in the liver lobules and there is no visible hydropic degeneration or necrosis, whereas in the 2000 mg/Kg BW test group it can be seen that Microscopically, the liver begins to bleed with bleeding in the central vein, but the hepatocytes have not yet experienced necrosis. The gaps between these plates contain capillary sinusoids called hepatic sinusoids. Sinusoids are irregularly dilated vessels and consist of only one discontinuous layer of endothelium [28]. Microscopic picture of the kidney organs in normal controls and test animals given CVE's at a dosage of 1000 mg/Kg BW shows that they are still in a normal condition. When administering the test preparation at a dosage of 2000 mg/Kg BW, there was visible dilation of the Bowman's capsule. It is evident that within the control group and those given CVE's, there was no visible damage to the heart muscle; microscopic examination of the heart organ showed a normal shape of myocytes and myofibrils. The results of the microscopic examination of the spleen organ show that it is in a normal condition, with the red and white markings also showing that it is still in a normal condition. So it can be concluded that normal control and administration of CVE's did not affect the spleen, the microscopic results on the lung tissue of the control were normal and those given CVE's were in normal condition, with alveolar, bronchial, bronchiole and blood vessel tissue in normal condition.

CONCLUSION

Providing treatment of CVE's caused acute toxic symptoms at certain doses; this was proven in hematological examination, clinical blood biochemistry and organ histology, which was significantly different from normal controls or administration of CMC Na 0.5% and in this experiment a dosage of 2000 mg/Kg BW indicated toxic symptoms.

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AUTHORS CONTRIBUTIONS

Every author contributed an equel contribution.

Ardana T: Data Curation, Formal Analysis Writing–Original Draft Preparation; Yuandani: Funding Acquisition, Supervision, Validation, Writing–Review and Editing; Rosidah: Formal Analysis, Investigation; Satria D: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology; Effendy: Methodology, Project Administration; Mahatir; Software, Validation, Writing – Review and Editing.

CONFLICT OF INTERESTS

Declared none

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