
Subchronic Toxicity Profile of Ifalmin[®] Product Containing Toman Fish Extract (*Channa micropeltes*) in *Rattus Novergicus*

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Article

Subchronic Toxicity Profile of Ifalmin[®] Product Containing Toman Fish Extract (*Channa micropeltes*) in *Rattus Novergicus*

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Abstract

Ifalmin[®], an oral extract of *Channa micropeltes* (Toman fish) that have registered on Indonesian FDA (BPOM RI) for herbal medicine. Toman fish is known for anti-inflammatory, antidiabetic, wound-healing, and antioxidant activities, but its safety profile requires verification; therefore, this study evaluated the in vivo subchronic toxicity of *Channa micropeltes* extract. Male and female *Rattus norvegicus* were allocated to control (0 mg/kg BW), three treatment groups receiving 270, 540, or 1000 mg/kg BW, and a satellite group given 1000 mg/kg BW, with all doses administered orally once daily for 14 consecutive days; animals were observed and assessed on day 15 after dosing cessation. Subchronic toxicity endpoints included clinical signs, body-weight changes, macropathology, relative organ weights, histopathology of heart, liver, kidneys, spleen, and lungs, and standard biochemical and hematological parameters. Across all dose levels, no treatment-related abnormalities or organ damage were detected, and physiological and laboratory measures remained comparable to controls. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg BW. These findings indicate that Ifalmin derived *Channa micropeltes* extract is relatively non-toxic under the tested subchronic oral exposure conditions.

Keywords: Ifalmin[®]; *Channa micropeltes* extract; subchronic toxicity; NOAEL; *Rattus norvegicus*; histopathology; biochemical parameters; hematological parameters

1. Introduction

Ifalmin is a standardized preparation containing *Channa micropeltes* (Toman fish) extract, a freshwater species indigenous to Southeast Asia with a long record of ethnomedicinal use. Pharmacological evidence indicates that *Channa micropeltes* exhibits anti-inflammatory, antidiabetic, wound healing, and antioxidant activities [1,2], supporting its potential as a health promoting natural product. In addition to these bioactivities, the extract is characterized by a high albumin content. Albumin is a major plasma protein involved in regulation of oncotic pressure, transport of endogenous and exogenous compounds, immune modulation, and tissue regeneration, so its presence may contribute to the proposed therapeutic value of fish derived supplements [3]. However, despite the expanding utilization and commercialization of fish based nutraceuticals such as Ifalmin, their toxicological characterization under repeated dose exposure remains limited.

Subchronic toxicity testing is a key component of preclinical safety assessment for products intended for continuous intake. Repeated oral administration for approximately 14 consecutive days is designed to detect early signs of cumulative or organ specific toxicity, including changes in body weight, gross and relative organ weights, histopathological lesions, and alterations in biochemical and hematological homeostasis, especially within hepatic, renal, cardiovascular, splenic, pulmonary, and gastrointestinal systems [4]. Importantly, natural origin does not guarantee safety, because bioactive constituents may elicit adverse effects at high doses or with prolonged exposure [5]. *Rattus norvegicus* (Wistar rats) is an established model in toxicology due to its reproducible physiological responses and translational relevance for predicting human safety outcomes [6].

Most prior investigations on *Channa micropeltes* have concentrated on elucidating its beneficial bioactive properties [1,2], whereas evidence delineating its subchronic safety profile is limited. This evidentiary gap is clinically and regulatory relevant given the increasing prevalence of daily supplement consumption and the risk of unintended toxicity from inappropriate dosing or duration of use. Therefore, the present study aimed to evaluate the subchronic oral toxicity of Ifalmin in male and female Wistar rats following 14 days of graded-dose administration, employing comprehensive clinical, morphometric, histopathological, biochemical, and hematological endpoints [4]. The outcomes of this study are expected to provide objective safety data to support the responsible development and use of Ifalmin within healthcare contexts.

Additionally, Ifalmin is a traditional medicine product from Indonesia, bearing the Jamu logo, and it is registered with the Indonesian National Agency of Drug and Food Control (BPOM RI). The selection of a 14-day period for the subchronic toxicity study is in accordance with the regulations of BPOM RI, which stipulate this duration for subchronic testing on traditional medicine products [4]

2. Materials and Methods

The Ifalmin product, which contains *Channa micropeltes* extract, is produced by PT. Ismut Fitomedika Indonesia (PT. IFI) in Takalar Regency, South Sulawesi. It has been registered as herbal medicine with the Indonesian National Agency of Drug and Food Control (BPOM) on April 26, 2023, under Registration Number TR183313911. The product contains *Channa micropeltes* extract as its active ingredient.

2.1. Preparation of Animals:

Thirty male and thirty female *Rattus norvegicus*, with body weights of 120-130 grams for males and 110-120 grams for females, were obtained and acclimatized for one week prior to the commencement of the study. The animal care and study protocols followed the guidelines established by the Indonesian National Agency of Drug and Food Control (BPOM). The study protocols were reviewed and received ethical clearance (Registration RG.02.05.42.423.01.8.2024.1450 and Protocol No.: A02/PUPK/A04/VIII/2024/Rev.3).

2.2. Subchronic Toxicity Testing:

Following acclimatization, the rats were randomly selected and divided into six groups, each consisting of five animals. The groups included: the control group with a dose of 0 mg/kg body weight (BW) (Group I), the 270 mg/kg BW dose group (Group II), the 540 mg/kg BW dose group (Group III), the 1000 mg/kg BW dose group (Group IV), the satellite control group with a dose of 0 mg/kg BW (Group V), and the satellite control group with a dose of 1000 mg/kg BW (Group VI). The extract was administered orally for 14 days. On day 15, all rats were subjected to necropsy for analysis, which included body weight, organ macropathology, relative organ weight, histology, blood hematology, and blood biochemistry.

2.3. Body Weight Monitoring:

The body weight male and female of *Rattus norvegicus* was monitored by weighing the animals once a week throughout the treatment period.

2.4. Organ Macropathology Analysis:

On day 14, the main organs of male and female *Rattus norvegicus* were collected and cleaned using a saline solution. The organs were then visually examined for any changes in shape, size, color, texture, or the presence of hemorrhages. Each organ was photographed for macropathological analysis.

2.5. Measurement of Relative Organ Weight:

The main organs, including the heart, lungs, liver, kidneys, and testes (for males), were evaluated for relative organ weight percentage using the following formula:

$$\text{Relative Organ Weight (\%)} = (\text{Organ Weight (g)}/\text{Body Weight (g)}) \times 100$$

2.6. Histopathological Analyses:

The main organs of male and female *Rattus norvegicus* were collected post-euthanasia, cleaned with a saline solution, and fixed in 10% buffered formalin for 24-48 hours to preserve tissue structure. The fixed tissues were processed through graded ethanol series for dehydration, cleared with xylene, and embedded in paraffin. Thin tissue sections (3–5 μm) were prepared using a microtome, mounted on glass slides, and stained with Hematoxylin and Eosin (H&E). The stained slides were examined under a light microscope to identify histopathological changes such as necrosis, inflammation, fibrosis, or degeneration. Microscopic images were captured for documentation and analysis [7].

2.7. Serum Biochemical Analyses:

Blood samples were collected from male and female *Rattus norvegicus* via venipuncture post-euthanasia. The blood samples were collected into vacutainer tubes and subsequently centrifuged at 3000 rpm for 20 minutes to separate the serum from the cellular components. An aliquot of 100 μL of blood serum was combined with 100 μL of buffer and thoroughly mixed, then incubated at 37°C for five minutes. Following this, 250 μL of the kit substrate was added to the mixture, homogenized, and incubated at 37°C for one minute, and various biochemical parameters were analyzed using a Humalyzer 3500 spectrophotometer (Human, Germany) were read at 340 nm [7]. Parameters tested included liver function (AST/SGOT, ALT/SGPT), kidney function (creatinine, and BUN), lipid profile (total cholesterol, and triglycerides), and glucose levels. Data were analyzed statistically to compare control and treatment groups, and results were interpreted in the context of potential physiological or toxicological effects.

2.5. Hematological Analysis

Blood samples were collected from male and female *Rattus norvegicus* via venipuncture into tubes containing EDTA as an anticoagulant. Hematological parameters, including red blood cell count (RBC), hemoglobin (Hb) concentration, hematocrit (Hct), white blood cell count (WBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Red Cell Distribution Width – Standard Deviation (RDW-SD), Neutrophils (%), Lymphocytes (%), and Monocytes (%) were analyzed using an automated hematology analyzer. Data were analyzed statistically to compare treatment and control groups [9].

3. Results

3.1. Body Weight Monitoring

In this subchronic toxicity study, body weight changes in both male and female rats were monitored weekly to assess the potential impact of *Channa micropeltes* extract are shown in Table.1. No substantial differences in relative organ weights were observed across the treatment and control groups, indicating the absence of organ-specific toxicity in this study, although the body weight of the rats tended to increase naturally over the study period, there were no statistically significant differences between the control group and the groups administered the extract at various doses [5] [10].

Table 1. Body Weight Monitoring 14 Days Subchronic Toxicity.

Group	Male	Female
G1	128 ± 1.91	120.7 ± 2.28
G2	136 ± 8.24	132.9 ± 12.82
G3	137.5 ± 9.83	118.98 ± 2.04
G4	132.7 ± 5.95	120.98 ± 2.12
G5	133.5 ± 7.46	125.9 ± 6.51
G6	129.38 ± 5.39	124.98 ± 5.86

3.2. Toxicological and Clinical Observations

During the 14-day observation period, all treatment groups (G1-G6) administered with toman fish extract exhibited consistent results, with no signs of toxicity observed in the clinical parameters assessed. These parameters, including skin condition, fur, mucous membranes, secretions, excretions, behavior, and seizures, remained normal across all groups throughout the observation period. No significant physiological or behavioral changes were detected in the groups receiving toman fish extract compared to the control group [5].

Table 2. Toxicological and clinical observations over 14 days.

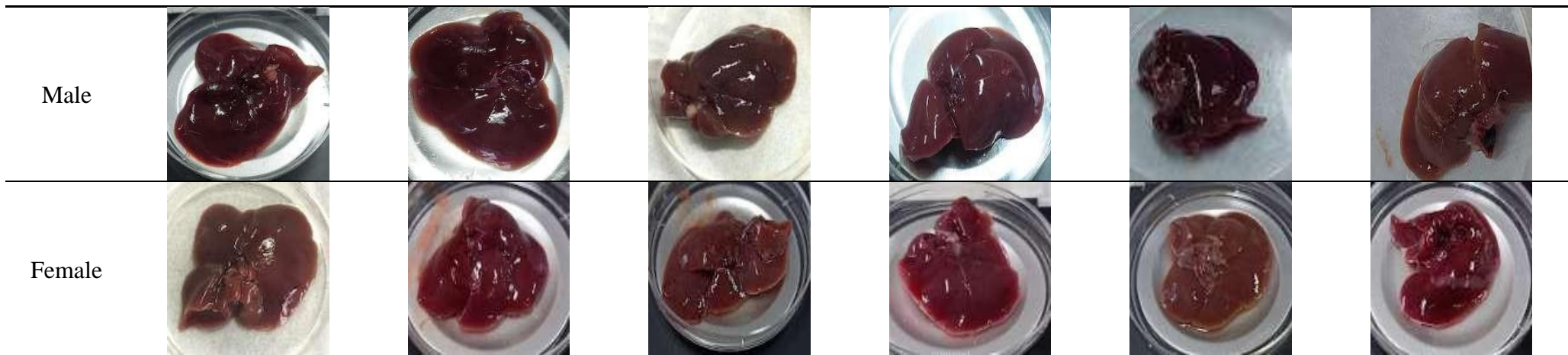
Criteria	Group					
	G1	G2	G3	G4	G5	G6
Skin	Normal	Normal	Normal	Normal	Normal	Normal
Fur	Normal	Normal	Normal	Normal	Normal	Normal
Mucosus	Normal	Normal	Normal	Normal	Normal	Normal
Secretion	Normal	Normal	Normal	Normal	Normal	Normal
Excretion	Normal	Normal	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal	Normal	Normal
Seizure	Normal	Normal	Normal	Normal	Normal	Normal

3.3. Organ Macropathology Analysis

In this study, the macropathology of the heart, liver, spleen, kidneys, lungs, and reproductive organs was assessed for both male and female rats following the 14-day administration of *Channa micropeltes*. The macroscopic examination of vital organs in treated animals showed no abnormalities in color or texture compared to the control group are shown in Table 3 [5].

Table 3. Organ macropathology Analysis 14 Days Subchronic Toxicity.

		HEART ORGAN <i>Rattus Norvegicus</i> Subchronic Toxicity <i>Chinna micropeltes</i>											
		G1		G2		G3		G4		G5		G6	
Male													
Female													
		LUNGS ORGAN <i>Rattus Norvegicus</i> Subchronic Toxicity <i>Chinna micropeltes</i>											
Male													
Female													
		LIVER ORGAN <i>Rattus Norvegicus</i> Subchronic Toxicity <i>Chinna micropeltes</i>											



SPLEEN ORGAN *Rattus Norvegicus* Subchronic Toxicity *Chinna micropeltes*

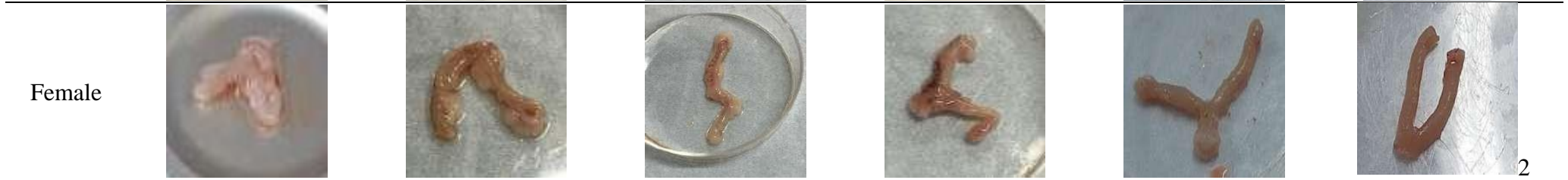


KIDNEY ORGAN *Rattus Norvegicus* Subchronic Toxicity *Chinna micropeltes*





TESTIS AND OVARIUM-UTERUS ORGAN *Rattus Norvegicus* Subchronic Toxicity *Chinna micropeltes*



3.4. Measurement of Relative Organ Weight

Relative organ weights of 14-day treated rats are shown in Table 4 for Male and Table 5 for Female. The relative organ weight analysis revealed no statistically significant differences between the control group and the treated groups in both male and female rats. In male rats, the heart's relative weight in the control group ($0.42 \pm 0.83\%$) remained comparable to that of the highest dose group ($0.32 \pm 0.07\%$), showing no signs of hypertrophy or atrophy. Similarly, the relative weights of other organs, including the lungs, liver (control: $3.48 \pm 0.83\%$, highest dose: $2.94 \pm 0.73\%$), spleen, kidneys, and testes remained stable across all groups. The satellite group (1000 mg/kg) also showed no significant changes, confirming no residual effects post-treatment. For female rats, the liver's relative weight in the control group ($3.70 \pm 0.57\%$) was comparable to that of the highest dose group ($3.27 \pm 0.30\%$), indicating no hepatotoxicity. Other organs, including the spleen, kidneys, heart, ovaries, and uterus, displayed no significant alterations in relative weights, further confirming the extract's non-toxic nature in females. The satellite group exhibited similar results, reinforcing the absence of long-term toxicity after the 14-day treatment period. The relative organ weight of each organ recorded at necropsy in the treatment groups did not show a significant difference ($P > 0.05$) compared to the control [12].

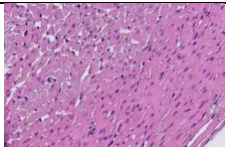
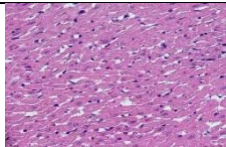
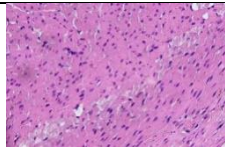
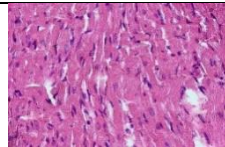
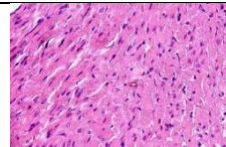
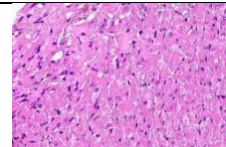
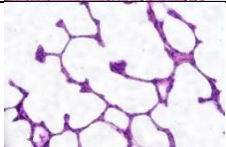
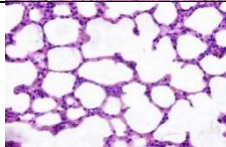
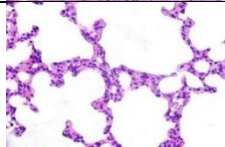
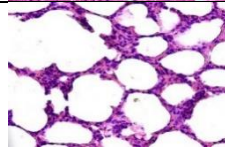
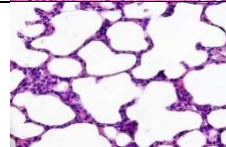
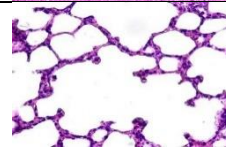
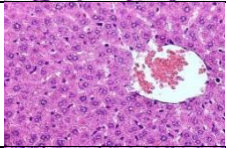
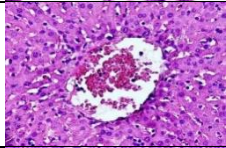
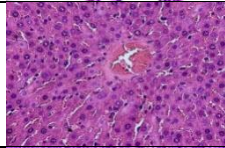
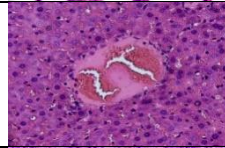
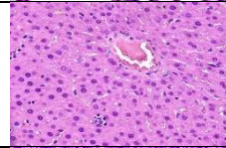
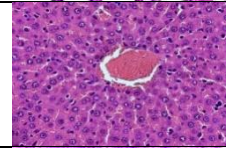
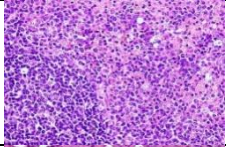
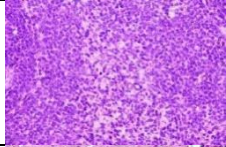
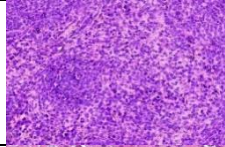
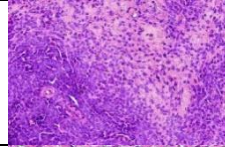
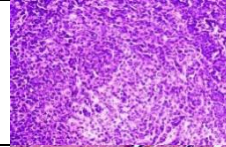
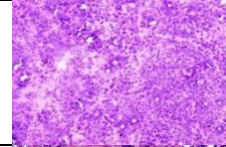
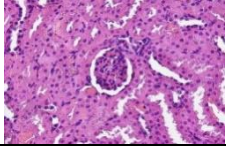
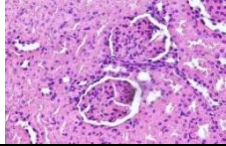
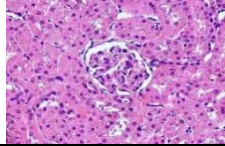

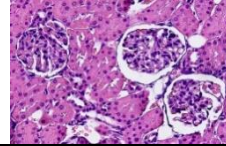
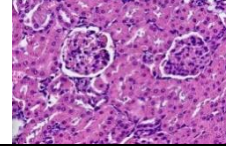
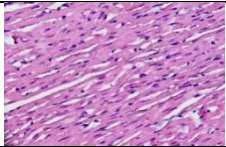
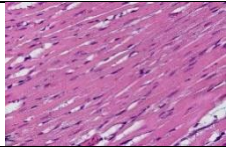
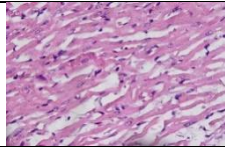
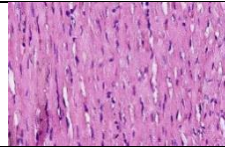
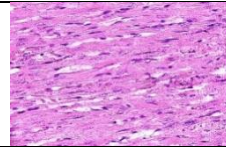
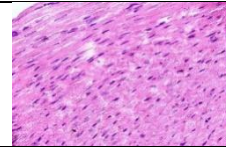
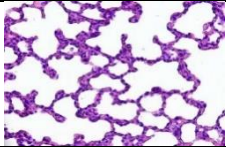
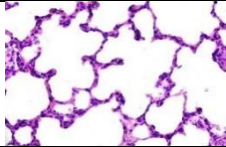
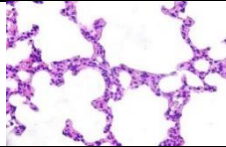
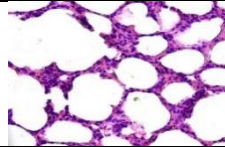
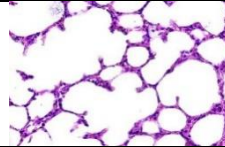
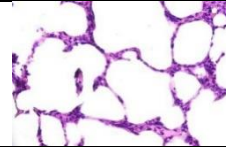
Table 4. Measurement of Relative Organ Weight 14 Days Subchronic Toxicity.

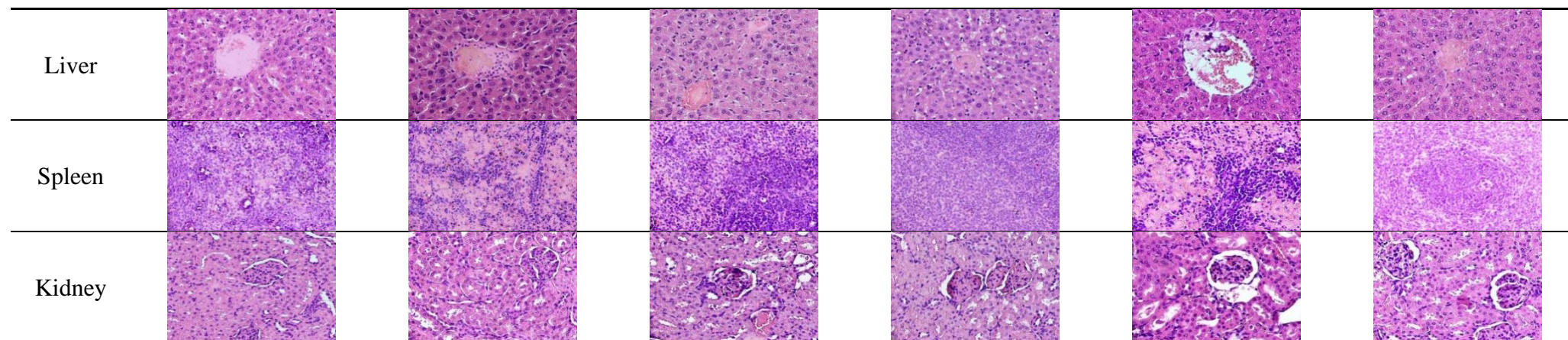
Male						
Organ	Group					
	G1	G2	G3	G4	G5	G6
Heart	0.42 ± 0.83	0.35 ± 0.04	0.28 ± 0.15	0.32 ± 0.07	0.31 ± 0.06	0.37 ± 0.07
Lung	1.12 ± 0.65	0.91 ± 0.26	0.76 ± 0.06	0.76 ± 0.14	2.03 ± 3.54	0.79 ± 0.55
Liver	3.48 ± 0.83	3.34 ± 1.15	3.26 ± 0.56	2.94 ± 0.73	2.93 ± 0.58	3.47 ± 0.68
Spleen	0.32 ± 0.11	0.43 ± 0.33	0.44 ± 0.29	0.47 ± 0.31	0.35 ± 0.22	0.43 ± 0.28
Kidney	0.81 ± 0.22	0.76 ± 0.17	0.74 ± 0.16	0.74 ± 0.16	0.74 ± 0.24	0.69 ± 0.16
Testis	2.11 ± 1.43	1.37 ± 0.98	1.43 ± 0.24	1.26 ± 0.47	1.43 ± 0.35	1.37 ± 0.47
Female						
Organ	Group					
	G1	G2	G3	G4	G5	G6
Heart	0.37 ± 0.10	0.35 ± 0.05	0.34 ± 0.05	0.36 ± 0.04	0.04 ± 0.12	0.38 ± 0.10
Lung	0.93 ± 0.28	0.81 ± 0.08	1.10 ± 0.71	1.01 ± 0.46	0.94 ± 0.18	0.89 ± 0.36
Liver	3.70 ± 0.57	3.06 ± 0.87	3.72 ± 0.62	3.27 ± 0.30	3.51 ± 0.59	3.78 ± 1.04
Spleen	0.61 ± 0.16	0.82 ± 0.31	0.55 ± 0.34	0.50 ± 0.42	0.75 ± 0.30	0.75 ± 0.23
Kidney	0.84 ± 0.13	0.67 ± 0.14	0.86 ± 0.19	0.82 ± 0.05	0.78 ± 0.16	0.79 ± 0.11
Ovary	0.12 ± 0.04	0.06 ± 0.06	0.28 ± 0.53	0.25 ± 0.36	0.06 ± 0.04	0.06 ± 0.02
Uterus	0.15 ± 0.04	0.22 ± 0.10	0.27 ± 0.14	0.18 ± 0.10	0.33 ± 0.21	0.15 ± 0.07

3.5. Histopathological Analyses

The light microscopy analysis of tissue sections from rats treated with *Channa micropeltes* extract, as well as from the control group, is shown in Table 5 and Table 6. Subchronic studies evaluate the potential adverse effects of prolonged or repeated exposure to plant extracts or compounds over a significant portion of the average lifespan of experimental animals, such as rodents. These studies specifically aim to provide information on target organ toxicity and are designed to determine the no-observed-adverse-effect level (NOAEL) [13]. The histopathological evaluation of man organs (heart, lungs, liver, spleen, kidneys, and reproductive organs) in both male and female rats treated with *Channa micropeltes* extract revealed no abnormalities or lesions. In this study, a histopathological score of 0 was assigned to all examined organs across treatment groups. Histopathological evaluation indicated that both the control and treated groups displayed normal tissue structure, with no signs of pathological lesions in any of the organs examined.

Table 5. Measurement of Relative Organ Weight 14 Days Subchronic Toxicity.

Organ	Histopatology Analysis in Male <i>Rattus Norvegicus</i> Subchronic Toxicity <i>Chinna micropeltes</i> (40x)					
	G1	G2	G3	G4	G5	G6
Heart						
Lungs						
Liver						
Spleen						
Kidney						
Organ	Histopatology Analysis in Female <i>Rattus Norvegicus</i> Subchronic Toxicity <i>Chinna micropeltes</i>					
	G1	G2	G3	G4	G5	G6
Heart						
Lungs						



3.6. Serum Biochemical Analyses

The administration of *Channa micropeltes* for 14 days resulted in no significant changes in ALT, AST, Creatinine, Urea, Glucose, Total Cholesterol, and Triglycerides levels compared to the control group for both male and female rats (Table 6 and 7). Likewise, the satellite group, which received 1000 mg/kg of *Channa micropeltes*, showed no significant differences in any biochemical parameters when compared to the control group in both male and female rats. Based on the blood biochemical analysis of *Channa micropeltes*, the results indicate no significant differences between the control and treatment groups. Key liver function indicators, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), showed no significant increase. The ALT levels in the control group were 25.3 ± 4.1 U/L, while in the group receiving the highest dose (1000 mg/kg), ALT was 26.1 ± 3.8 U/L. Similarly, the AST levels in the control group were 18.5 ± 3.2 U/L, compared to 19.2 ± 3.0 U/L in the highest-dose group. The reference range for ALT and AST is typically 35-85 U/L and 30-380 U/L. Nevertheless, the data can still be considered valid if they clearly show that the ALT values are not lower than 10% of the control, which would indicate no significant difference [14]. These results suggest that the extract did not cause significant liver damage. Additionally, kidney function parameters, such as creatinine and urea, remained stable. In the control group, creatinine levels were 0.67 ± 0.10 mg/dL, and in the highest-dose group, they were 0.71 ± 0.09 mg/dL. Urea levels were 17.4 ± 2.3 mg/dL in the control group and 18.0 ± 2.1 mg/dL in the highest-dose group. This is consistent with the reference data for creatinine and urea, which are 0.2-0.7 mg/dL and 12.33-77.6 mg/dL, respectively, indicating that the extract did not affect kidney function. The kidneys' ability to filter waste products remained intact, suggesting no nephrotoxic effects [15][16].

Glucose levels in the blood also remained within the normal range. The control group recorded a glucose level of 90.2 ± 12.4 mg/dL, while the highest-dose group had a level of 92.7 ± 11.8 mg/dL. Based on the reference glucose range of 50-160 mg/dL, the absence of significant fluctuations in glucose levels indicates that the extract did not disrupt metabolism in *Channa micropeltes*. Finally, the analysis of total cholesterol and triglycerides also showed no significant changes. Total cholesterol in the control group was 150.3 ± 14.6 mg/dL, and in the highest-dose group, it was 151.8 ± 13.9 mg/dL. Triglyceride levels were 80.7 ± 10.1 mg/dL in the control group and 79.5 ± 9.8 mg/dL in the highest-dose group. Based on the reference values for total cholesterol and triglycerides, which are 41-126 mg/dL and 20-114 mg/dL, respectively, the stability in cholesterol and triglyceride levels indicates that the extract did not impair lipid metabolism [16][17].

Table 7. Serum Biochemical Analyses in *Rattus norvegicus* 14 Days Subchronic Toxicity.

Male						
Parameter	Group					
	G1	G2	G3	G4	G5	G6
Triglycerida	49.4 ± 18.17	48.2 ± 28.80	36 ± 8.15	39.2 ± 8.35	39.2 ± 19.77	33.4 ± 8.17
Glucose	68.4 ± 17.27	66.2 ± 26.21	75 ± 9.87	98.4 ± 2.35	122.4 ± 5.50	95.6 ± 0.45
Colesterol	76.4 ± 12.70	53.6 ± 12.10	62.4 ± 6.80	57.6 ± 5.77	59.8 ± 10.33	62.4 ± 10.24
Creatinin	0.64 ± 0.05	0.64 ± 0.11	0.64 ± 0.05	0.58 ± 0.04	0.54 ± 0.05	0.48 ± 0.04
Urea BUN	32.06 ± 3.06	29.54 ± 9.27	23.18 ± 1.30	26.96 ± 13.53	29.78 ± 2.90	25.38 ± 1.84
AST/SGOT	182.8 ± 37.41	192.6 ± 54.90	140.4 ± 25.37	157.2 ± 41.32	244.8 ± 22.48	190.2 ± 24.46
ALT/SGPT	50 ± 14.46	93 ± 43.92	43.2 ± 1.92	45.2 ± 14.32	65.2 ± 7.85	53 ± 14.62
Female						
Organ	Group					
	G1	G2	G3	G4	G5	G6
Triglycerida	47.2 ± 15.25	75.2 ± 28.67	41 ± 18.37	56.2 ± 31.58	56.6 ± 28.95	63.4 ± 19.50
Glucose	99.6 ± 17.85	76.6 ± 16.20	140.2 ± 36.48	98.8 ± 27.18	82.8 ± 13.20	89.2 ± 14.45
Colesterol	70.6 ± 14.70	59.4 ± 14.72	56.4 ± 9.07	55.4 ± 8.20	74.4 ± 11.63	63.6 ± 2.07
Creatinin	0.52 ± 0.04	0.64 ± 0.05	0.58 ± 0.08	0.58 ± 0.08	0.6 ± 0.2	0.54 ± 0.09

Urea BUN	35.48 ± 7.36	34.62 ± 6.40	36.46 ± 10.36	39.48 ± 6.05	32.46 ± 9.88	31.64 ± 9.66
AST/SGOT	245 ± 141.83	213 ± 63.59	224 ± 40.09	180.6 ± 23.20	171 ± 40.67	199.2 ± 144.53
ALT/SGPT	88.8 ± 48.90	53.8 ± 27.46	77.8 ± 25.96	69.2 ± 36.93	48.8 ± 33.03	81.6 ± 74.74

3.7. Hematological Analyses

Hematological analyses were performed using blood plasma to evaluate potential changes in blood chemistry and to detect any alterations related to *Channa micropeltes* extract treatment compared to the control group. The results of the hematological analysis did not show significant variations at any dose compared to the control group in both female and male rats, as presented in Table 8. Similarly, the satellite group showed no significant differences between the *Channa micropeltes* treated groups and the control group across all parameters [18]

Table 8. Measurement of Relative Organ Weight 14 Days Subchronic Toxicity.

Parameter	Male					
	Group					
	G1	G2	G3	G4	G5	G6
WBC	11.16 ± 3.82	7.56 ± 2.16	9.28 ± 4.66	4.95 ± 2.14	11.16 ± 3.83	6.2 ± 1.40
RBC	6.97 ± 0.67 ^a	7.94 ± 1.29	7.43 ± 0.56	7.69 ± 0.89	7.27 ± 0.70 ^a	6.80 ± 0.73
Hb	13.74 ± 2.02	14.74 ± 2.20	14.04 ± 1.14	13.9 ± 1.77	14.18 ± 1.74	14.58 ± 0.87
Hct	37.76 ± 3.89	41.66 ± 6.50	39.2 ± 3.12	39.92 ± 6.41	39.38 ± 5.16	40.5 ± 2.44
PLT	919.6 ± 31.67	865.2 ± 113.84	926 ± 104.86	853.5 ± 86.38	770.6 ± 138.09	779.6 ± 268.08
MCV	53 ± 2.12	51.2 ± 1.48	51 ± 2.94	51.8 ± 0.84	50 ± 1	52.6 ± 4.03
RDW-SD	18.24 ± 1.55	17.66 ± 1.22	17.98 ± 1.38	17.02 ± 0.96	17.32 ± 1.83	18.6 ± 2.73
MCH	19.6 ± 1.08	18.88 ± 0.70	18.62 ± 0.59	18.07 ± 0.99	19.48 ± 1.47	21.54 ± 1.72
Neutrophils	27.6 ± 16.34	20.8 ± 9.57	18.4 ± 7.92	28.5 ± 7.93	25.2 ± 6.14	28 ± 11.64 ^a
Lymphocytes	66.4 ± 16.02	73.8 ± 9.33	75.8 ± 9.09	65.5 ± 10.26	68.8 ± 5.76	65.4 ± 11.08
Monocytes	6 ± 1.22	5.4 ± 1.14	5.8 ± 1.78	6 ± 2.55	6 ± 0.82	6.4 ± 1.26
Organ	Female					
	Group					
	G1	G2	G3	G4	G5	G6
WBC	12.48 ± 7.20	11.4 ± 6.68	7.02 ± 4.49	4.26 ± 2.16	8.7 ± 5.29	10.34 ± 2.23
RBC	6.41 ± 1.32	6.91 ± 0.58	6.86 ± 0.54	7.47 ± 0.63	5.83 ± 0.62	5.21 ± 1.73
Hb	12.4 ± 3.38	13.57 ± 1.13	13.2 ± 1.43	13.96 ± 1.00	13.44 ± 1.65	12.08 ± 3.96
Hct	37.04 ± 3.82	37.84 ± 2.57	37.24 ± 2.65	39.44 ± 2.50	34.18 ± 8.96	33.56 ± 10.13
PLT	889.8 ± 425.98	667.83 ± 173.90	707.6 ± 190.97	704.4 ± 169.94	809.4 ± 370.46 ^a	713.4 ± 146.22
MCV	51.4 ± 3.71	54.6 ± 1.67	53.6 ± 1.81	52.6 ± 2.50	56.8 ± 5.80	57.8 ± 5.54
RDW-SD	37.04 ± 3.82	37.84 ± 1.58	37.24 ± 1.85	39.44 ± 1.11	19.02 ± 2.19	33.56 ± 1.93
MCH	19.94 ± 3.12	18.24 ± 1.58	18.46 ± 1.85	16.64 ± 1.11	19.02 ± 2.19	19.56 ± 1.93
Neutrophils	23.6 ± 7.02 ^a	17.2 ± 3.11	26.6 ± 9.72	24 ± 11.95	22 ± 4.63	16 ± 7.61
Lymphocytes	72.6 ± 7.12	76.6 ± 4.44	68.6 ± 10.18	70 ± 11.74	72.2 ± 5.63 ^a	78.6 ± 8.59
Monocytes	3.8 ± 1.30	6.2 ± 2.28	4.8 ± 0.83	6 ± 2.0	5.8 ± 2.38	5.4 ± 2.19

4. Discussion

Repeated-dose toxicity studies are designed to reveal cumulative adverse effects, identify potential target organs, and provide a scientific basis for determining the NOAEL prior to longer-term exposure trials. Indonesian FDA Regulation PerBPOM No. 10/2022 emphasize integrated evaluation of clinical signs, growth, hematology, serum biochemistry, organ coefficients, and histopathology to capture both functional and structural toxicity across organ systems. In the context of natural-product development, subacute/subchronic safety data are especially important because bioactive extracts may exert subtle adaptive responses before overt lesions emerge [5][13].

However, this approach differs from subchronic testing outlined in the OECD Guidelines, primarily due to the nature of the product being tested, traditional medicine that will later be registered as a *fitofarmaka* (phytopharmaceutical product) [5][19]. The classification of traditional medicines in Indonesia is divided into three categories: *jamu* (traditional herbal medicine), *OHT* (standarisation traditional medicines), and *fitofarmaka* [19]. Each category is subject to different regulatory requirements and safety evaluations. BPOM RI provides guidelines indicating that subchronic toxicity testing can be conducted within a 14-day period, specifically for traditional medicines being developed for future *fitofarmaka* registration. This distinction highlights the regulatory framework's flexibility in accommodating traditional medicine products as they transition into more standardized health products, while ensuring thorough safety evaluation during development [5]

Oral administration in repeated-dose studies is particularly relevant because it mimics the real-world route of exposure for dietary supplements and functional foods. The clean safety profile observed here may reflect limited systemic accumulation and effective metabolic handling of the extract's constituents. Although pharmacokinetic data were not assessed in the present work, the lack of clinical signs, stable organ coefficients, and absent histopathologic lesions suggest that repeated oral exposure did not result in bioaccumulation or persistent tissue injury. Future studies incorporating ADME or PK profiling would be valuable to confirm gastrointestinal stability, absorption extent, and clearance kinetics, thereby strengthening mechanistic understanding of the extract's safety [20].

Body weight is a key indicator of systemic health in subchronic toxicity assessments. Throughout the 14-day oral administration, both male and female rats in all treated groups showed a progressive weight gain comparable to controls, consistent with normal physiological growth. The absence of statistically significant differences in body-weight change among dose groups indicates that *Channa micropeltes* extract did not interfere with growth or energy balance. Importantly, no animals exhibited weight loss or growth retardation, which are typical early signs of toxicity, and food-consumption patterns remained stable, suggesting no adverse effects on appetite, metabolism, or nutrient utilization. Given that a change of approximately $\geq 10\%$ body weight is often considered biologically relevant for subchronic toxicity [21], the normal weight trajectories observed here support the conclusion that the extract is non-toxic within the tested dose range, including 1000 mg/kg [22].

The macropathological examination of major organs, including the heart, liver, spleen, kidneys, lungs, and reproductive organs, revealed no visible abnormalities in either male or female rats across all treated and satellite groups. The heart, liver, spleen, kidneys, and lungs maintained normal size, color, and texture, with no signs of necrosis, lesions, or structural damage, indicating the absence of cardiotoxic, hepatotoxic, nephrotoxic, or pulmonary effects. Similarly, the reproductive organs (testes, ovaries, and uterus) showed no signs of toxicity or interference with reproductive health. These findings suggest that *Channa micropeltes* extract does not induce any organ-specific toxicity, even at the highest dose of 1000 mg/kg, confirming its overall safety. The macroscopic description of specimens constitutes a critical element of the pathology report, requiring precision and clinical relevance. Excessive or redundant details can unnecessarily extend the report, thereby elevating the risk of significant clinical information being overlooked by the attending clinician. Employing standardized templates for gross pathological reporting can enhance efficiency and mitigate these risks [23].

Relative organ weight is a sensitive endpoint in repeated-dose toxicity studies because organ mass can change before clear gross or microscopic lesions are detectable; altered organ-to-body-weight ratios may reflect hypertrophy, atrophy, congestion, or edema, especially in liver, kidneys, heart, and spleen [24][25]. Thus, a lack of significant organ-weight shifts is generally interpreted as no organ-specific toxic burden under the tested conditions. In this study, relative organ weights of male and female rats remained comparable between control and *Channa micropeltes* extract groups up to 1000 mg/kg, including the satellite cohort. The stability of major detoxification and target organs (liver, kidneys, heart, spleen, lungs) and reproductive organs suggests no adaptive enlargement or

degenerative shrinkage attributable to treatment, a pattern consistent with absence of subchronic cardiotoxicity, nephrotoxicity, hepatotoxicity, or reproductive toxicity [26]. The unchanged organ weights in the recovery (satellite) group further indicate no delayed or residual organ remodeling after cessation of dosing.

Organ-weight changes are often interpreted as early adaptive or adverse responses; however, their toxicological meaning becomes strongest when concordant with histopathologic findings. Large databases have shown that increased liver weight may accompany enzyme induction or hypertrophy, while decreases may indicate atrophy or chronic injury, and the presence or absence of histological correlate helps distinguish adaptation from toxicity. In the present study, the absence of organ-weight shifts together with uniformly normal histology indicates not only a lack of morphological injury but also an absence of subclinical adaptive stress responses in detoxification organs. This concordance across endpoints substantially reduces the likelihood of undetected target-organ toxicity at the tested doses [26].

Subchronic toxicity studies are intended to detect adverse effects arising from repeated exposure over a substantial portion of the rodent lifespan and to identify potential target-organ toxicity, thereby supporting the determination of the no-observed-adverse-effect level (NOAEL) [11][13]. In the present study, histopathological examination of major organs—including the heart, lungs, liver, spleen, kidneys, and reproductive organs showed normal tissue architecture in both male and female rats treated with *Channa micropeltes* extract. All organs received a histopathological score of 0, and no microscopic evidence of necrosis, inflammation, fibrosis, or cellular degeneration was observed, indicating preserved structural integrity and function [27]. The absence of lesions across detoxification, highly perfused, and reproductive organs provides strong support that the extract does not induce organ-specific toxicity under subchronic exposure. Evaluating both sexes is important because hormonal and metabolic differences may influence toxic responses to natural products; however, the comparable histological findings in males and females suggest no sex-related susceptibility within the tested dose range [28]. Collectively, these results contribute valuable safety data for selecting dose levels in longer-term studies and for refining safe exposure limits in future translational assessments [16]. The inclusion of a satellite (recovery) cohort enhances interpretation by assessing reversibility or delayed-onset toxicity after dosing cessation. In this work, recovery animals displayed organ weights, histology, and clinical profiles comparable to controls, suggesting no residual tissue remodeling or late-emerging toxicity. Such findings are important for natural extracts, where metabolite-driven effects might hypothetically appear after prolonged processing in vivo. The normal recovery profile therefore supports that any exposure-related changes, if present, are unlikely to persist or progress once dosing stops [29]

Serum biochemistry provides functional evidence of target-organ integrity during repeated-dose toxicity, particularly for the liver and kidneys. In this study, *Channa micropeltes* extract did not produce statistically significant changes in key hepatic enzymes (ALT, AST) or renal biomarkers (creatinine, urea) compared with controls, and all values remained within physiological limits. The absence of enzyme elevation indicates no hepatocellular injury or leakage, while stable creatinine and urea suggest preserved glomerular filtration and no nephrotoxic burden under subchronic exposure [30]. Metabolic parameters also remained unaffected. Blood glucose stayed within normal range, indicating that the extract did not disrupt systemic energy homeostasis. Likewise, total cholesterol and triglycerides showed no treatment-related shifts, supporting the conclusion that lipid metabolism was not impaired [16]. Taken together with the normal histopathology and organ-weight findings, the biochemical profile consistently demonstrates that repeated oral administration of *Channa micropeltes* extract up to 1000 mg/kg does not elicit liver, kidney, or metabolic toxicity in rats within the study duration.

Hematological parameters are sensitive indicators of systemic and immuno-hematopoietic toxicity in repeated-dose studies. In the present work, *Channa micropeltes* extract did not induce statistically significant alterations in major hematological indices in either sex, including WBC, RBC, hemoglobin, hematocrit, platelet count, erythrocyte indices (MCV, MCH, RDW-SD), and differential

leukocyte profiles. These findings indicate preserved hematopoiesis, oxygen-carrying capacity, and immune homeostasis under subchronic exposure [31]. Minor reductions in platelet and hematocrit values observed in a single female subgroup were not statistically significant, showed no dose-response pattern, and therefore are most consistent with normal biological variability rather than a treatment-related effect. Although reference intervals may vary slightly across sources, the overall pattern of values remaining within physiological limits supports the conclusion that the extract does not elicit hematological toxicity up to 1000 mg/kg [13]. When interpreted together with the normal organ weights, macropathology, histopathology, and serum biochemistry, the hematological profile corroborates the favorable safety of *Channa micropeltes* extract in this subchronic model.

An important element of toxicological interpretation is the safety margin between the highest tested dose and the expected efficacious or intended human-use dose. In this study, the top dose of 1000 mg/kg represents a multiple of the projected therapeutic exposure, thereby providing a conservative safety buffer. The absence of adverse findings at this level indicates a wide margin of safety, which is desirable for nutraceutical or biopharmaceutical candidates derived from food-based sources. Such a safety window strengthens the feasibility of advancing *Channa micropeltes* extract into longer duration studies and supports its practical use without requiring exposure near the toxicological threshold.

Overall, the findings of this study strengthen the translational positioning of *Channa micropeltes* extract as a safe bioactive candidate for further development in Ifalmin®. The consistent absence of toxic effects across systemic indicators (clinical signs and growth), functional parameters (hematology and serum biochemistry), and structural endpoints (macropathology, organ coefficients, and histopathology), including in the recovery cohort, provides a strong weight-of-evidence that short-term repeated exposure does not elicit target-organ toxicity. The uniform safety profile up to 1000 mg/kg bw/day offers a sound basis for selecting dose ranges in longer subchronic studies aligned with OECD/BPOM guidelines (14-day or 90-day studies) and for applying a more conservative approach to human-equivalent exposure estimation. From a product-development perspective, this early safety confirmation is essential to support longer-term efficacy testing and serves as a prerequisite for eventual clinical evaluation, particularly because Ifalmin® is intended as an orally administered natural-product preparation with potential for repeated use [5].

This study has several limitations, primarily related to the lack of literature on 14-day subchronic toxicity testing for traditional medicine products, which hinders the search for references and comparison with other studies. Additionally, *Ifalmin*, which contains *Channa micropeltes* (Toman fish), also has limited available literature, making it challenging to confirm safety and efficacy data in comparison with similar products. Further research will be conducted through clinical trials in humans to confirm these findings and assess the safety of the product for long-term use. It will also be registered as a *fitofarmaka* product in accordance with the guidelines set by BPOM RI.

5. Conclusions

This study demonstrates that a 14-day repeated-dose (subchronic) oral toxicity assessment of *Channa micropeltes* extract produced no treatment-related adverse effects at 270, 540, or 1000 mg/kg bw/day in either sex. No statistically significant changes were observed in body-weight gain, relative organ weights, serum biochemical parameters, or hematological indices compared with controls. Gross necropsy and histopathological evaluation of major organs showed normal architecture across control, treated, and recovery (satellite) cohorts, indicating no evidence of target-organ toxicity or delayed effects after dosing cessation. Therefore, the NOAEL is considered to be 1000 mg/kg bw/day for both males and females under the present 14-day exposure conditions. These findings provide robust preclinical safety support for the *Channa micropeltes* extract used in Ifalmin® and justify further development and longer-term studies to confirm chronic safety.

6. Patents

Ifalmin® is protected by Indonesian patent registration No. TR183313911, effective from 26 April 2023 to 26 April 2028. The patent is held by PT. Ismut Fitomedika Indonesia, with Dr. Mansur Ibrahim, M.Kes. listed as the inventor.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/doi/s1>, Figure S1: title; Table S1: title; Video S1: title.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee/Institutional Review Board of the Indonesian Food and Drug Authority (Badan POM RI). The study protocols were reviewed and obtained ethical clearance (Registration RG.02.05.42.423.01.8.2024.1450; Protocol No. A02/PUPK/A04/VIII/2024/Rev.3), with the date of approval 01 September 2024.

Informed Consent Statement: Not applicable

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to proprietary considerations related to product development.

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Conflicts of Interest: The authors declare a potential conflict of interest because this study was funded by PT. Ismut Fitomedika Indonesia (developer/patent holder of Ifalmin®) and Dr. Mansur Ibrahim, M.Kes. (inventor). The funders contributed to conceptualization, methodology, resources, data curation, validation, review/editing, visualization, and supervision. They had no role in data collection, primary statistical analysis, or the final decision to publish.

Abbreviations

The following abbreviations are used in this manuscript:

ADME	Absorption, Distribution, Metabolism, and Excretion
ALT	Alanine Aminotransferase.
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
BPOM RI	Badan Pengawas Obat dan Makanan (Indonesian Food and Drug Authority).
BW	Body Weight
EDTA	Ethylenediaminetetraacetic Acid.
FDA	Food and Drug Authority
g	gram
G1-G6	Group 1–Group 6
H&E	Hematoxylin and Eosin stain.
Hb	Hemoglobin.
Hct	Hematocrit.

PT.IFI	Perseroan Terbatas Ismut Fitomedika Indonesia (
IRB	Institutional Review Board.
Kg	kilogram.
MCV	Mean Corpuscular Volume.
MCH	Mean Corpuscular Hemoglobin.
mg	milligram.
mg/kg	milligram per kilogram.
µL	microliter.
µm	micrometer.
nm	nanometer.
NOAEL	No-Observed-Adverse-Effect Level.
OECD	Organisation for Economic Co-operation and Development.
PK	Pharmacokinetics.
PLT	Platelet Count.
RBC	Red Blood Cell count.
RDW-SD	Red Cell Distribution Width—Standard Deviation.
rpm	revolutions per minute.
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TG 407	Test Guideline 407
TR	Tanda Registrasi (Registration Code)
WBC	White Blood Cell count.
%	percent.

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