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Research Paper

Comprehensive Toxicity Assessment of Coconut Shell Liquid Smoke: Acute and Subacute Oral Exposure in Wistar Rats

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ABSTRACT

Background: Coconut shells are abundant lignocellulosic by-products in Indonesia and, through pyrolysis and sequential purification, can be converted into grade 1 coconut shell liquid smoke. They contain phenolic and other oxygenated compounds associated with antioxidant and antimicrobial activities. However, its toxicological profile must be established before its use. This study evaluated the acute and 28-day repeated-dose oral toxicity of grade 1 coconut shell liquid smoke in Wistar rats.

Methods: A preliminary acute test was conducted at 300, 1000, and 2000 mg/kg BW (BW), followed by a main acute test at 1000 and 2000 mg/kg BW. For the repeated-dose study, rats received 250, 500, or 1000 mg/kg BW once daily for 28 days, while satellite groups were observed for an additional 14-day recovery period. Clinical signs, mortality, BW, relative organ weights, hematological and biochemical parameters, and histopathological changes were assessed.

Results: No mortality occurred during the preliminary acute test. In the main acute and repeated-dose studies, mortality and tissue alterations were recorded at moderate-to-high doses.

Conclusion: These findings indicate that grade 1 coconut shell liquid smoke may produce systemic and organ-specific toxicity only at higher exposure levels. However, further dose-response studies are required before a definitive safe exposure level can be established.

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Introduction

The coconut palm (Cocos nucifera L.), commonly known as the "tree of life," has substantial economic and cultural importance in tropical regions. Although most parts of the plant are widely used, coconut shells remain an underutilized lignocellulosic by-product containing cellulose, hemicellulose, and lignin, which can be thermally decomposed via pyrolysis to produce liquid smoke. This product contains phenolic compounds, organic acids, and carbonyl derivatives that contribute to its antioxidant, antimicrobial, and preservative properties, with phenolic compounds particularly associated with radical-scavenging activity and inhibition of microbial growth [1, 2]. However, crude liquid smoke may also contain tar and polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene, which are of toxicological concern due to their mutagenic and carcinogenic potential. Therefore, purification through sedimentation, filtration, adsorption, or sequential distillation is commonly performed to remove undesirable fractions and obtain different grades of liquid smoke, with grade 1 liquid smoke representing the most extensively purified fraction [3, 4].

Despite this purification process, safety cannot be assumed without toxicological evaluation, particularly when the product is intended for repeated oral exposure. Assessment of clinical signs, mortality, body-weight changes, relative organ weights, hematological and biochemical parameters, and histopathological alterations is necessary to characterize possible systemic and organ-specific toxicity [5]. Therefore, this study aimed to evaluate the acute and subacute oral toxicity of grade 1 coconut shell liquid smoke in Wistar rats in accordance with the Indonesian Food and Drug Authority guidelines. The findings are expected to provide a toxicological basis for determining whether further development of grade 1 coconut shell liquid smoke for food-related or biomedical applications is scientifically justified.

Materials and Methods

Materials and Animals

Coconut shells (Cocos nucifera L.) were sourced from a local market in Medan, Indonesia. Botanical authentication was performed at Herbarium Medanense, Universitas Sumatera Utara, under voucher number 207/MEDA/2022. The dried fruit material was milled to obtain nanoparticles measuring 731.4 ± 168.2 nm. A total of 114 two-month-old Wistar rats, comprising 57 males and 57 females and weighing 120–160 g, were housed under controlled conditions with unrestricted access to food and water. All experimental procedures were approved by the Ethics Committee of Universitas Sumatera Utara (Approval No. 00430/KEPH FMIPA/2023).

Pyrolysis, Distillation, and Characterization of Liquid Smoke

Coconut shells were cleaned, cut into small pieces, and pyrolyzed at 400°C for 255 min. The generated smoke was condensed through a cooling system to obtain grade 3 liquid smoke. Sequential distillation at 150°C and 100°C produced grade 2 and grade 1 liquid smoke, respectively, for the removal of tar and benzo[a]pyrene [6, 7]. Grade 1 liquid smoke was subsequently characterized using gas chromatography–mass spectrometry (GC–MS) and Fourier-transform infrared spectroscopy (FTIR).

Acute and Subacute Toxicity

The acute and subacute toxicity studies were conducted in accordance with Indonesian Food and Drug Authority Regulation No. 10 of 2022, which was used as the primary regulatory guideline. A preliminary acute toxicity evaluation was first conducted by orally exposing Wistar rats to grade 1 coconut shell liquid smoke at doses of 300–2000 mg/kg BW. The control group and each treatment group included three male and three female animals. Based on the preliminary observations, the definitive acute toxicity test was conducted at doses of 1000 and 2000 mg/kg BW, with five male and five female rats in the control group and each treatment group. A 0.5% Na-CMC suspension was administered to the control animals. Following a pre-administration fasting period, the rats were intensively examined for toxic manifestations during the initial 4 h and subsequently monitored once daily for 14 consecutive days. Clinical abnormalities, body-weight changes, and deaths were recorded to support the estimation of the median lethal dose (LD_{50}; Tables 1 and 2) [8–11].

Table 1. Preliminary Test Experimental Design.

Number of Rats		Treatment
Male	Female	
3	3	Provided a 0,5% sodium carboxymethyl cellulose (CMC Na) suspension
3	3	Given grade 1 liquid smoke dose of 300 mg/kg BW
3	3	Given grade 1 liquid smoke dose of 1000 mg/kg BW
3	3	Given grade 1 liquid smoke dose of 2000 mg/kg BW

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Table 2. Experimental Design for Primary Testing.

Number of Rats		Treatment	Description
Male	Female		
5	5	Provided a 0,5% sodium carboxymethyl cellulose (CMC Na) suspension	Control group
5	5	Given grade 1 liquid smoke dose of 1000 mg/kg BW	Testing group
5	5	Given grade 1 liquid smoke dose of 2000 mg/kg BW	

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For subacute toxicity, grade 1 coconut shell liquid smoke was administered orally at doses of 250, 500, and 1000 mg/kg BW for 28 days in accordance with Indonesian Food and Drug Authority Regulation No. 10 of 2022. Each control, treatment, and satellite group included five male and five female rats. Control animals received 0.5% Na-CMC, and satellite groups were observed for an additional 14 days to assess recovery. Clinical signs and mortality were monitored daily, while BW was recorded to adjust dosing and analyzed twice weekly. On day 28, animals were euthanized, and organs were collected for macroscopic examination, relative organ weight measurement, and histopathological analysis. The study design is presented in Table 3 [8–11].

Table 3. Experimental Design for Subacute Toxicity Test.

Number of Rats		Treatment	Description
Male	Female		
5	5	Provided a 0,5% sodium carboxymethyl cellulose (CMC Na) suspension	Control group
5	5	Given grade 1 liquid smoke dose of 250 mg/kg BW	Testing group
5	5	Given grade 1 liquid smoke dose of 500 mg/kg BW	
5	5	Given grade 1 liquid smoke dose of 1000 mg/kg BW	
5	5	Given grade 1 liquid smoke dose of 1000 mg/kg BW	Satellite group
5	5	Provided a 0,5% sodium carboxymethyl cellulose (CMC Na) suspension	

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Organ, Hematological, Biochemical, and Histopathological Examination

At the end of the acute and subacute studies, surviving animals were weighed and euthanized, while animals that died prematurely underwent immediate necropsy. The heart, liver, kidneys, spleen, lungs, brain, stomach, pancreas, testes, and ovaries were examined macroscopically, weighed, and normalized to body weight before histological processing [8–11].

Hematological parameters included white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Hepatic and renal profiles were assessed using aspartate aminotransferase, alanine aminotransferase, and creatinine. Tissues were fixed in 10% neutral-buffered formalin, sectioned, stained with hematoxylin and eosin, and examined for edema, necrosis, and hemorrhage [12–14].

Data Analysis

One-way ANOVA was used for parametric data, while the Kruskal-Wallis test was used for nonparametric data. Post-hoc analyses were performed using the Mann-Whitney U test (SPSS). Significance was set at $p < 0.05$. All data were expressed as mean ± standard deviation.

Results

Liquid Smoke Yield and Characterization

The yield of grade 3-1 coconut shell liquid smoke is shown in Table 4. The determination of grade 1 coconut shell liquid smoke using GC-MS is shown in Table 5. The FTIR analysis results for the Grade 1 liquid smoke sample are shown in Figure 1, which suggests the presence of phenolic and alcoholic compounds that confer antioxidant and antimicrobial properties.

Table 4. The Yield of Grade 3, Grade 2, and Grade 1 Coconut Shell Liquid Smoke.

Grade of the liquid smoke	Yield (%)
Grade 3	13.08% b/b
Grade 2	76.43% v/v
Grade 1	58.30% v/v

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Table 5. The GC-MS Result of Liquid Smoke Compounds in Coconut Shell.

No	Compounds	Similarity Index	Molecular Weight
1	Propylene Sulfide	97 %	74.15 g/mol
2	Diethyl Ether	96 %	74.12 g/mol
3	Urea	98 %	60.056 g/mol
4	Acetic Acid	98 %	60.05 g/mol
5	Propionic Acid	97 %	74.08 g/mol
6	1-Hidroxy-2-Butanone	97 %	88.11 g/mol
7	2-Cyclopenten-1-one	98 %	82.1 g/mol
8	Phenol	98 %	94.11 g/mol
9	Guaiacol	98 %	124.14 g/mol
10	4-Methoxyphenol	96 %	124.14 g/mol
11	2-Propanone	98 %	58.08 g/mol
12	Butyric Acid	95 %	88.11 g/mol
13	2-Methyl-2-cyclopentenone	96 %	96.13 g/mol

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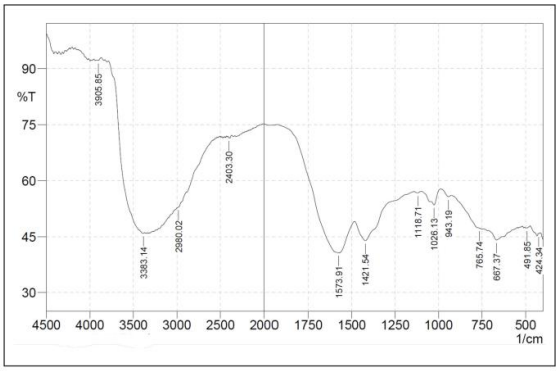

Figure 1. Results of the FTIR functional group analysis in grade 1 liquid smoke.

Acute Toxicity Result

Rats received grade 1 coconut shell liquid smoke at doses of 300–2000 mg/kg BW and were observed for 14 days. No mortality was recorded during the preliminary acute toxicity test (Table 6). In the definitive acute toxicity test, three deaths were recorded at 1000 mg/kg BW and two deaths at 2000 mg/kg BW (Table 7). Animals treated with 300 mg/kg BW showed no observable toxic symptoms, whereas mild behavioral changes, including backward walking and abdominal dragging, were observed at 1000 mg/kg BW. More pronounced clinical signs, including backward walking, walking on hind legs, and shivering, were observed at 2000 mg/kg BW (Tables 6 and 7). These findings indicate that no mortality occurred during the preliminary test, whereas mortality and dose-related clinical signs were observed during the definitive acute toxicity test at 1000 and 2000 mg/kg BW.

Table 6. Results of Toxic Symptoms and Rat Deaths Observation in Preliminary Tests.

Toxic Symptom	Normal Control	Dose 300	Dose 1000	Dose 2000
Seizures	-	-	-	-
Diarrhea	-	-	-	-
Shiver	-	-	-	*
Limp	-	-	-	-
Salivation	-	-	-	-
Walk backwards	-	-	*	*
Walk using the stomach.	-	-	*	*
Changes in the eyes	-	-	-	-
Changes in the mucous membrane	-	-	-	-
Number of dead rats	0	0	0	0

Note: (-) = does not show toxic symptoms; (*) = shows toxic symptoms.

Table 7. Results of Toxic Symptoms Observation in Primary Tests.

Toxic Symptom	Normal Control	Dose 1000	Dose 2000
Seizures	-	-	*
Diarrhea	-	-	*
Shiver	-	-	-
Limp	-	-	-
Salivation	-	-	-
Walk backwards	-	-	-
Walk using the stomach.	-	-	-
Changes in the eyes	-	-	*
Changes in the mucous membrane	-	*	*
Number of dead rats	0	3	2

Note: (-) = does not show toxic symptoms; (*) = shows toxic symptoms.

Body-weight changes were not significantly different among groups ($p > 0.05$; Table 8). The control group showed an increase from 128 ± 1.53 g on day 1 to 131 ± 3.67 g on day 14, while the 1000 and 2000 mg/kg BW groups changed from 136.6 ± 2.08 to 135±2.94 g and from 141 ± 5.65 to 141 ± 9.98 g, respectively [15]. Relative organ weights also showed no significant differences among groups ($p>0.05$; Table 8), suggesting that acute exposure did not markedly alter organ-weight profiles.

Table 8. Results of Body Weight and Relative Organ Weights.

Parameters	Units	Normal Control	Dose 1000	Dose 2000
BW (Day 1)	5	128±1,530	136,6±2,088	141±5,657
BW (Day 14)	5	131±3,67	135±2,94	141±9,98
Heart/100 g BW	5	0.482 ± 0.001	0.346 ± 0.001	0.463 ± 0.002
Liver/100 g BW	5	4.655 ± 0.001	4.734 ± 0.002	4.017 ± 0.001
Kidney/100 g BW	5	1.280 ± 0.003	1.096 ± 0.001	0.997 ± 0.0008
Limfa/100 g BW	5	1.177 ± 0.002	0.975 ± 0.002	0.563 ± 0.005
Lung/100 g BW	5	1.406 ± 0.44	1.658 ± 0.002	1.525 ± 0.04

Hematological and biochemical parameters showed no significant differences compared with the control group ($p>0.05$; Tables 9 and 10, respectively). These findings suggest that acute administration of grade 1 coconut shell liquid smoke did not induce marked

hematological, hepatic, or renal dysfunction under the present experimental conditions. However, this interpretation should be considered alongside histopathological findings, as tissue-level alterations were observed at higher doses [16, 17].

Table 9. Results of Hematological Analysis.

Parameters	Units	Normal Control	Dose 1000	Dose 2000
WBC	10^3/uL	17.18 ± 1.20	15.87 ± 1.10	0.05 ± 0.01
RBC	10^6/uL	6.54 ± 0.50	5.89 ± 0.45	0.06 ± 0.01
HGB	g/dL	13.2 ± 0.60	11.1 ± 0.55	0.1 ± 0.02
HCT	%	42.8 ± 1.50	32 ± 1.20	0.3 ± 0.05
MCV	fL	65.4 ± 2.50	54.3 ± 2.00	50 ± 1.80
MCH	Pg	20.2 ± 1.00	18.8 ± 0.90	16.7 ± 0.80
MCHC	g/dL	30.8 ± 1.10	34.7 ± 1.20	33.3 ± 1.00

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Table 10. Results of Biochemical Analysis.

Parameters	Units	Normal Control	Dose 1000	Dose 2000
AST	%	192.4 ± 2.33	300 ± 5.20	303 ± 5.50
ALT	g/dL	48.20 ± 3.66	100 ± 4.50	91 ± 4.20
Urea	10^6/uL	27.60 ± 6.22	57 ± 3.80	40 ± 3.00
Creatinine	10^3/uL	0.76 ± 0.12	1 ± 0.10	1.14 ± 0.15

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Histopathological examination revealed dose-related tissue alterations (Figure 2). At 1000 mg/kg BW, edema was observed in several organs, including the spleen, lungs, stomach, pancreas, and brain, suggesting mild tissue responses. At 2000 mg/kg BW, more evident swelling, hemorrhage, and necrosis were observed in the spleen, lungs, stomach, pancreas, and brain (Figure 2). The arrows in Figure 2 indicate the principal histopathological alterations observed in the corresponding tissues. These findings indicate that although no mortality occurred, higher acute doses of grade 1 coconut shell liquid smoke may induce organ-level histopathological changes, particularly at 2000 mg/kg BW [18, 19].

Subacute Toxicity Results

Clinical monitoring revealed no recorded clinical signs of toxicity among animals receiving 250 or 500 mg/kg BW. However, Table 11 shows that one male and one female rat died in the 500 mg/kg BW group. In contrast, rats exposed to 1000 mg/kg BW exhibited mild clinical abnormalities, primarily shivering and backward locomotion. These effects were observed in both sexes and were more apparent in the satellite group. Mortality was recorded in three male and two female rats in the 1000 mg/kg BW group and in two male and two female rats in the satellite 1000 mg/kg BW group. No deaths occurred in the normal control, 250 mg/kg BW, or satellite-control groups. The clinical observations and mortality data are summarized in Table 11.

Discussion

Evaluation of the satellite animals after a 14-day recovery period indicated substantial resolution of clinical signs, with most rats remaining in good condition. The continued presence of mild abnormalities in a few animals may reflect limited residual effects. In conjunction with the acute toxicity findings, these results indicate that grade 1 coconut shell liquid smoke did not produce observable clinical toxicity at doses of 500 mg/kg BW or lower under the present experimental conditions. Body-weight changes and relative organ weights during the subacute study are presented in Table 12.

Table 13 shows no signs of anemia, inflammation, or sepsis associated with systemic toxicity due to the entry of harmful compounds into the organism, as indicated by hematological parameters. The corresponding biochemical results are presented in Table 14. All treated groups showed normal levels of urea, creatinine, and other kidney health indicators. The hematological and biochemical findings did not show statistically significant evidence of marked hepatic or renal dysfunction under the present experimental conditions. However, these findings should be interpreted together with the mortality and histopathological alterations observed in some treated group [20, 21].

Histopathological examinations were performed on organs from 10 rats to assess hemorrhage, necrosis, edema, and tissue swelling. The organs of male rats exhibited swelling at a dose of 500 mg/kg BW or higher in the liver, lungs, heart, kidneys, brain, spleen, testes, and pancreas, and edema at 250 mg/kg BW in the stomach (Figures 3 and 4). Necrotic alterations were observed at 500 mg/kg BW. The arrows in Figures 3 and 4 indicate the principal histopathological alterations observed in the corresponding tissues. Meanwhile, the female rats tested showed that edema in each organ began at a dose of 250 mg/kg BW. This study's comprehensive analysis of BW, hematological, and biochemical data also showed no significant deviations from the control group. These findings should be interpreted cautiously because histopathological alterations were observed in several organs at the tested doses, despite the absence of statistically significant changes in selected hematological and biochemical parameters [22, 23].

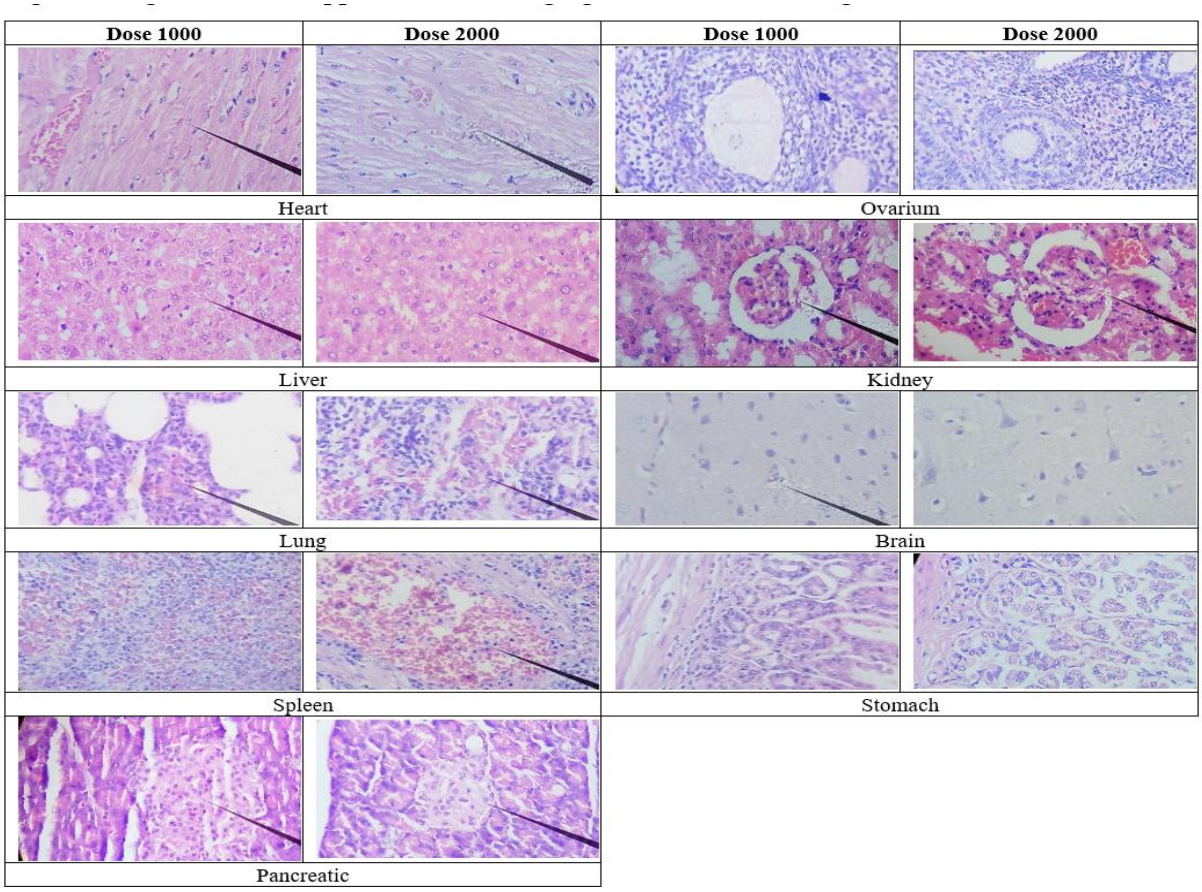

Figure 2. Histopathology of Organs for Acute Toxicity.

Table 11. Results of Toxic Symptoms Observation in Subacute Toxicity.

Toxic symptoms	Gender	Normal Control	Dose 250	Dose 500	Dose 1000	Satellite control	Satellite dose 1000
Shiver	Male	-	-	-	*	-	*
	Female	-	-	-	*	-	*
Salivation	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Limp	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Diarrhea	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Changes in fur and skin	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Changes in the eye	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Changes in the mucous membrane	Male	-	-	-	-	-	-
	Female	-	-	-	-	-	-
Walking backward/using the male stomach	Male	-	-	-	*	-	*
	Female	-	-	-	*	-	*
Number of dead Rats	Male	0	0	1	3	0	2
	Female	0	0	1	2	0	2

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Conclusion

Toxicity assessment results showed that the selected hematological and biochemical parameters did not differ significantly from those in the control under the present experimental conditions. Clinical signs, mortality, and histopathological alterations were observed in some treated groups, particularly at moderate-to-high doses. Histopathological examination revealed tissue alterations of varying severity across the tested groups. Therefore, the present findings should be interpreted in the context of the tested doses, 28-day exposure period, Wistar rat model, and measured toxicological endpoints. Further dose-

Table 12. Results of Subacute Body Weight and Relative Organ Weights.

Groups	BW (Day 1)	BW (Last Day)	Heart/100 g BW	Liver/100 g BW	Kidney/100 g BW	Limfa/100 g BW	Lung/100 g BW
Normal Control (Female)	162,2 ±1,83	177,0 ±2,10	0.348 ± 0.001	3.368 ± 0.001	0.925 ± 0.003	0.851 ± 0.002	1.017 ± 0.44
Normal Control (Male)	162,2 ±1,33	187,0 ±3,63	0.336 ± 0.001	1.877 ± 0.45	0.406 ± 0.01	0.594 ± 0.0021	0.968 ± 0.34
Satellite control (Female)	161,6 ±1,50	186,2 ±3,76	0.331 ± 0.001	3.201 ± 0.001	0.880 ± 0.003	0.809 ± 0.002	0.966 ± 0.44
Satellite control (Male)	162,0 ±1,67	195,0 ±2,45	0.323 ± 0.001	2.277 ± 0.06	0.446 ± 0.01	0.574 ± 0.0023	0.882 ± 0.32
Dose 250 (Female)	163,6 ± 3,5	182,4 ±6,34	0.338 ± 0.0014	3.431 ± 0.0039	0.639 ± 0.0016	0.628 ± 0.0046	0.839 ± 0.0015
Dose 250 (Male)	161,8 ±1,33	182,8 ±2,79	0.252 ± 0.0011	2.739 ± 0.0007	0.660 ± 0.0016	0.512 ± 0.0008	0.942 ± 0.0014
Dose 500 (Female)	160,4 ±3,07	182,4 ±4,27	0.305 ± 0.002	3.606 ± 0.1428	0.747 ± 0.0012	0.878 ± 0.0025	0.881 ± 0.0207
Dose 500 (Male)	163,4 ±2,42	186,6 ±3,12	0.317 ± 0.0017	3.433 ± 0.0026	0.910 ± 0.0012	0.884 ± 0.0021	0.926 ± 0.0005
Dose 1000 (Female)	182,6 ±4,32	185,8 ±2,99	0.254 ± 0.0015	3.480 ± 0.0023	0.806 ± 0.0010	0.717 ± 0.0023	1.219 ± 0.0027
Dose 1000 (Male)	160,4 ±3,07	206,6 ±4,41	0.642 ± 0.0055	3.504 ± 0.0031	0.298 ± 0.0002	0.520 ± 0.0028	0.561 ± 0.0050

Table 13. Results of Hematological Analysis.

Groups	WBC 10^3/uL	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL	MCH Pg	MCHC g/dL
Normal Control (Female)	7.40±0.42	8.15±0.84	14.00±0.88	47.30±2.63	56.82±3.10	17.78±1.00	34.00±2.92
Normal Control (Male)	9.48±3.89	7.54±0.52	14.18±0.73	41.43±1.11	57.58±4.02	18.80±1.01	33.16±1.75
Satellite control (Female)	7.12±0.21	7.53±0.35	14.28±0.92	43.94±3.02	58.36±1.93	18.42±0.61	33.12±1.71
Satellite control (Male)	8.22±0.58	7.09±0.58	13.24±0.41	36.04±0.70	51.76±1.06	18.62±0.83	33.92±0.13
Dose 250 (Female)	14.88±1.50	6.74±0.70	12.2±0.60	36.5±1.50	54.2±1.30	18.1±0.60	33.4±0.90
Dose 250 (Male)	4.91±0.90	6.91±0.80	12.5±0.70	36.2±1.40	52.4±1.20	18.1±0.60	34.5±1.20
Dose 500 (Female)	9.23±1.20	4.78±0.60	9.2±0.50	29.1±1.20	60.9±1.50	19.2±0.70	31.6±1.00
Dose 500 (Male)	7.82±1.10	5.34±0.60	10.4±0.60	33.6±1.30	63.9±1.60	19.5±0.70	31.0±0.80
Dose 1000 (Female)	2.73±0.80	4.36±0.50	8.6±0.40	26.2±1.00	60.1±1.40	19.7±0.80	32.8±1.10
Dose 1000 (Male)	9.30±1.30	7.05±0.80	13.1±0.80	38.4±1.60	54.5±1.30	18.6±0.60	34.1±1.10

Table 14. Results of Biochemical Analysis.

Groups	AST %	ALT g/dL	Urea 10^6/uL	Creatinine 10^3/uL
Normal Control (Female)	192.4 ± 2.33	48.20 ± 3.66	27.60 ± 6.22	0.76 ± 0.12
Normal Control (Male)	141.0 ± 1.41	42.40 ± 2.87	24.12 ± 0.72	0.44 ± 0.04
Satellite control (Female)	131.4 ± 5.04	35.20 ± 3.97	27.80 ± 2.04	0.25 ± 0.01
Satellite control (Male)	139.8 ± 1.32	45.00 ± 1.26	24.80 ± 0.97	0.26 ± 0.10
Dose 250 (Female)	219 ± 4.50	49 ± 2.20	59 ± 3.00	1 ± 0.05
Dose 250 (Male)	192 ± 3.80	58 ± 2.80	47 ± 2.50	0.95 ± 0.04
Dose 500 (Female)	170 ± 3.50	54 ± 2.60	44 ± 2.20	1 ± 0.05
Dose 500 (Male)	280 ± 5.20	164 ± 4.80	37 ± 1.80	1.3 ± 0.06
Dose 1000 (Female)	196 ± 3.90	98 ± 3.50	37 ± 1.80	0.93 ± 0.04
Dose 1000 (Male)	19 ± 1.00	24 ± 1.50	37 ± 1.80	0.93 ± 0.04

response and longer-term studies are required before a definitive safe exposure level or its use in food or pharmaceutical applications can be established.

Ethical Approval

All experimental procedures were conducted in accordance with NIH guidelines and approved by the Ethical Clearance of the Animal Research Committee, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Medan (Ethics Code: 0430/KEPH-FMIPA/2023).

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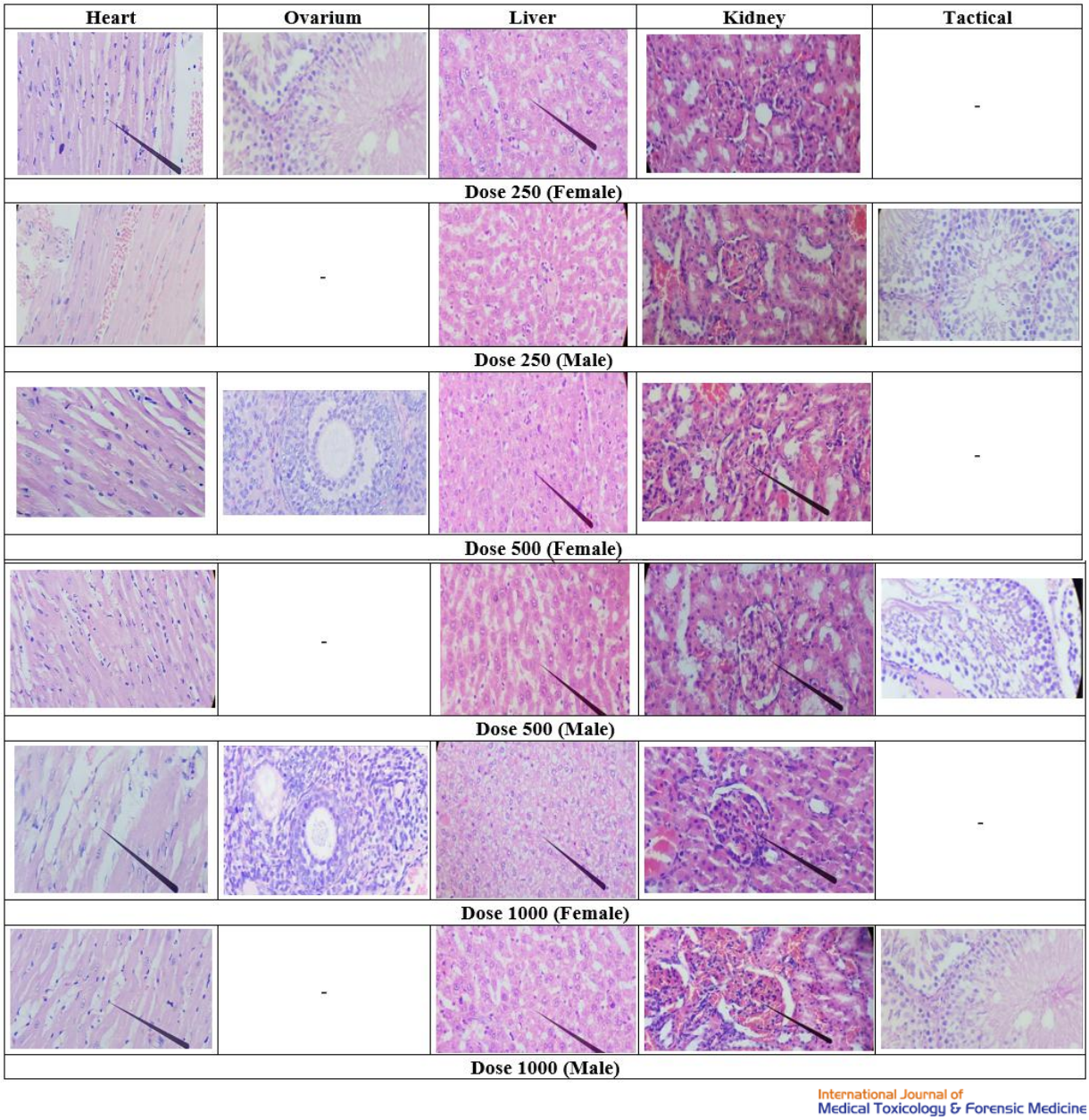

Figure 3. Histopathology for Subacute Toxicity (Heart, Testis, Ovary, Liver, and Kidney.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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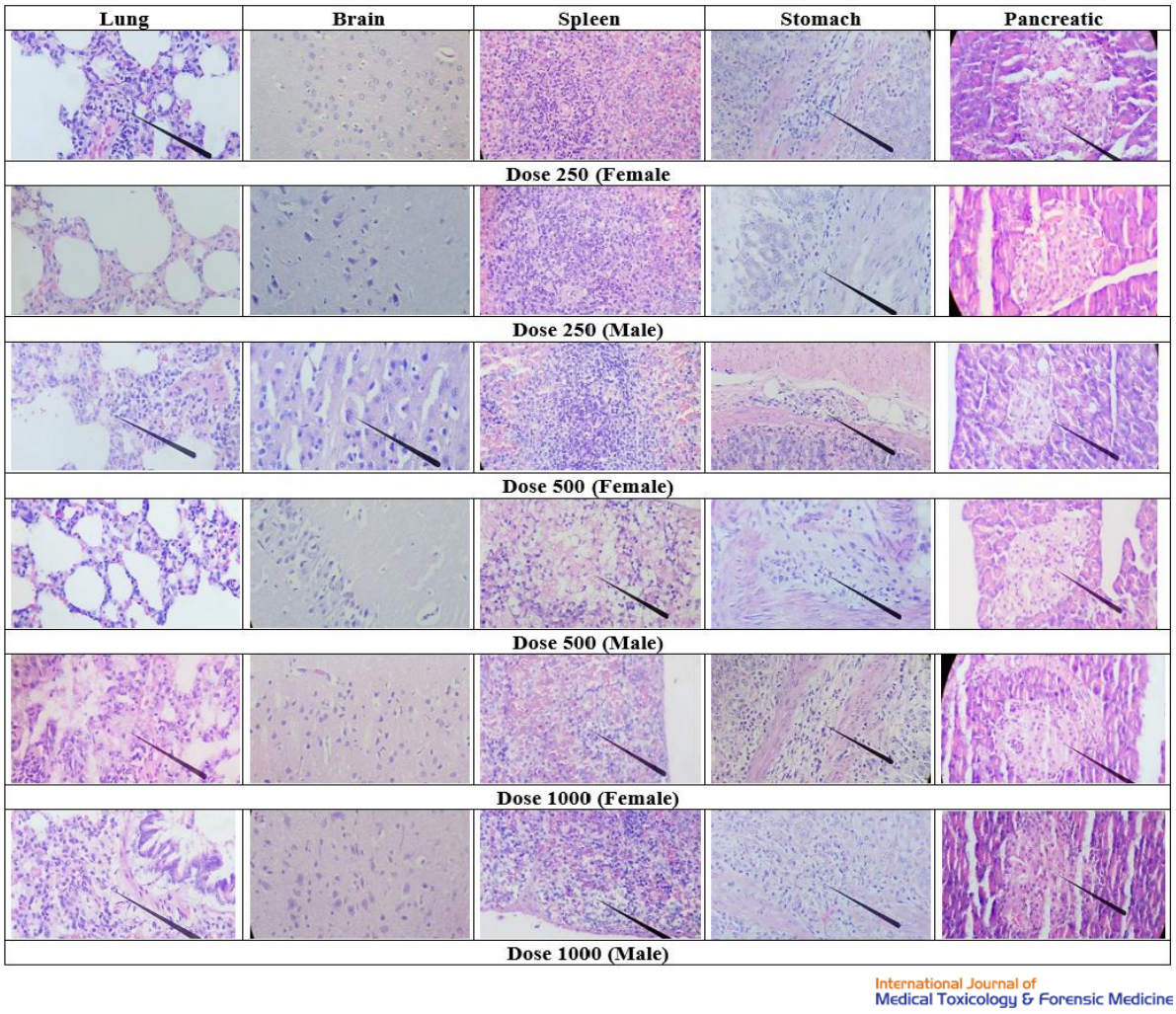

Figure 4. Histopathology for Subacute Toxicity (Spleen, Lung, Stomach, and Brain).

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