International Journal of Research and Review
Volume 11; Issue: 6; June 2024
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Acute Dermal Toxicity of Liquid Smoke (*Cocos nucifera* L.) Grade 1

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DOI: https://doi.org/10.52403/ijrr.20240624

ABSTRACT

Liquid smoke, a product from coconut shell waste, is a food preservative and antimicrobial substitute. It is produced through pyrolysis at 400-600°C, producing solid, liquid, and gas products. Toxicity studies need to be conducted to test the safety of medicinal products. This is because medicinal products that meet the requirements are those that have proven their efficacy and safety. Liquid smoke, especially grade 1, has not been studied further regarding its safety. This study aimed to determine the acute toxicity potential of grade 1 coconut shell liquid smoke. The experimental method involved collecting and processing samples, making liquid smoke, purifying them, and testing the effects of acute dermal toxicity. Results showed mild damage to liver and kidney organs, with microscopic liver tissue damage and kidney tissue damage.

Keywords: *Cocos nucifera* L., liquid smoke, toxicity, acute, subchronic

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an essential crop in many countries, including India, the Philippines, Brazil, Thailand, and Indonesia. Coconut shell is an agricultural by-product of coconut meat processing. Cellulose, hemicellulose, and lignin are the main components of the coconut shell. The lignin content of coconut shells is a good source of phenols for liquid smoke production (Rahmasari and Yemis, 2022).

The process of producing coconut shell charcoal generates a lot of smoke and can potentially disrupt the surrounding environment. The smoke generated during the production of coconut shell charcoal can pollute the environment, so a process is used to liquefy the smoke into a liquid known as liquid smoke. However, liquid smoke is mixed with fly ash. It is brown, has a pungent odor, and contains high tar levels. (Sukamta et al., 2023).

Liquid smoke is more environmentally friendly as it is biodegradable and renewable. Liquid smoke is made from various types of waste, but the commercialized types of liquid smoke come from coconut shells, teak, and falcata wood. Pyrolysis liquid smoke contains lignin, cellulose, hemicellulose, and other carbon compounds (Ristiani et al., 2022).

Toxicity studies are tests to demonstrate the toxic effects of a substance on biological systems and to obtain dose-response data that can be used as samples for test preparations. The data obtained can provide information on the risk of human exposure

to the test drug and determine the dose for human use. Toxicity test results cannot be used absolutely to show or prove the safety of material or preparation in humans. Still, they can provide indications or clues of relative toxicity and can be used to identify toxic effects when exposed to humans (BPOM RI, 2022).

Based on the description above, this study aimed to determine the acute toxicity potential of coconut shell liquid smoke. Toxicity studies need to be conducted to test the safety of medicinal products. This is because medicinal products that meet the requirements are those that have proven their efficacy and safety. Liquid smoke, especially grade 1, has not been studied further regarding its safety. Acute toxicity tests were conducted using BPOM Regulation No. 2022 and OECD (*Organization for Economic Co-operation and Development*) guidelines.

LITERATURE REVIEW

Liquid smoke is one of the products that can be developed from coconut shell waste through pyrolysis. Pyrolysis (destructive distillation) is the thermochemical decomposition of biomass at 400–600 °C without the addition of oxygen, which breaks down the constituent components of hardwood to produce three products: solid, liquid, and gas (Aini Nur et al., 2022). Fitra et al. (2023) stated that the pyrolysis temperature influences the composition of the liquid smoke produced. The highest phenol and acetic acid content are at 340 and 380°C, where lignin breaks down into phenolic compounds and cellulose breaks down to produce acetic acid.

The antimicrobial test showed the maximum inhibition zone at 420°C. In addition, coconut shell liquid smoke is a good food preservative and a substitute for formalin because it has antimicrobial and antibacterial activity. Therefore, it has an inhibitory effect against pathogens. The antimicrobial and antioxidant compounds in coconut shell liquid smoke are aldehydes, carboxylic acids, and phenols. The most acidic compounds contained in coconut shell liquid smoke are derivatives of carboxylic acids such as furfural, furan, acetic acid, propionic acid, butyric acid, and valeric acid (Mulyawati et al., 2020).

Ristiani et al. (2022) and Andi et al. (2021) have explored the potential of coconut shell as a raw material for liquid smoke, a preservative through pyrolysis. Coconut shell, a hardwood with high lignin and cellulose content, produces liquid smoke with high phenol content, producing organic acids, phenols, carbonyls, and other compounds. The liquid smoke from coconut shells has a high percentage yield of polyphenols, total acid, and flavonoids, making it a valuable resource for various applications.

In addition, research by Surboyo et al. (2019) showed that coconut shell liquid smoke can improve the healing of traumatic ulcers (wounds in the oral cavity) in people with diabetes. Saputra et al.'s (2021) research on wound dressings in the form of PVA, chitosan, or starch hydrogel patches combined with 12% liquid smoke and vitamin K mustard green extract showed that they were able to inhibit S. aureus bacteria optimally and accelerate animal wound healing significantly. This is in line with the research of Rayhan et al. (2023), which showed significant results on burn wound healing in rats after being given hydrogel combined with liquid smoke.

MATERIALS & METHODS

1. Type and Location of Research

The experimental method used included sample collection and processing, liquid smoke preparation, liquid smoke purification (grades 3, 2, and 1), and acute dermal toxicity effect testing on female rats. Observations included toxicological symptoms, body weight, mortality, relative organ weights, macropathology of the liver and kidney, histopathological examination of the liver and kidney, measurement of creatinine levels, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels. The research was

carried out at the *Approved Training Body* (ATB) Laboratory of Politeknik Medan as a place for the pyrolysis process and for acute dermal toxicity testing carried out at the Pharmacology and Toxicology Laboratory of the Universitas Sumatera Utara.

2. Tools and materials

2.1 Tools

The tools used in this study were a condenser, porcelain crucible, drying cabinet, electric microscope (Boeco Germany), analytical balance (AND GR-200, North Jakarta, Indonesia), animal balance, rotary evaporator (Heidolph Germany), set of surgical instruments, furnace (Nabertherm Germany).

2.2 Material

The material used in this study was a coconut shell. Researchers obtained the raw material from the traditional market located on Jl. Halat, Medan, North Sumatra, and the materials used were distilled water, ethanol 96% (Brataco, Semarang, Indonesia), and a 10% formalin solution (Smart Lab, Indonesia).

3. RESEARCH PROCEDURE

2.1. Coconut shell preparation

Coconut shells were cleaned of various impurities and then crushed using a hammer into small pieces so that the sample can enter the burner of the pyrolysis reactor.

2.2. Liquid Smoke Preparation Process

Liquid smoke was manufactured using a pyrolysis reactor assembled from a furnace and drum modified by adding a condenser made of stainless steel. The coconut shell was crushed into small pieces, then put into the burner until it was solid, and then tightly closed using a burner cover to prevent the release of smoke from the pyrolysis reactor during the pyrolysis process. Then, it was put into the furnace, where the smoke funnel that comes out was connected directly to the condenser. The combustion was carried out for 2,5 hours, reaching 400°C. The smoke generated from the combustion process in the furnace will pass through a series of condensers in a drum filled with ice water so that the smoke will condense, turn into liquid, and come out of the tap, then be collected in a storage container. The resulting liquid smoke was allowed to stand at room temperature to separate the liquid smoke. The top of the liquid smoke solution was pyroligneous liquor, while the bottom was tar sediment. At this stage, the liquid smoke produced is called crude smoke liquid (grade 3).

4. Liquid Smoke Purification Process

2.1. Grade 2

Purification of liquid smoke to obtain grade 2 liquid smoke that is better than grade 3 liquid smoke was done by distilling crude smoke liquid (grade 3 liquid smoke). 500 ml of liquid smoke was put into a distillation flask assembled with a Liebig condenser and then heated using a hot plate until it reached a maximum temperature of 200°C. This was ideal for getting a suitable distillate from liquid smoke (Hendra et al., 2014). In this study, distillation was carried out at a temperature of 125°C.

2.2. Grade 1

The purification of liquid smoke to obtain grade 1 liquid smoke was carried out by redestillation of the liquid smoke of grade 2, which was inserted into the distillation flask that had been coated with a Liebig condenser and then heated using a hotplate to reach a temperature of 100°C on the thermometer.

5. Acute dermal toxicity test

The study used rats weighing between 200 and 300 g, with two animals chosen for their sensitivity. Female animals were selected due to their sensitivity, while male animals were used if there was toxicological or toxicokinetic information about the test formulation. The animals were acclimatized in the laboratory for 5-7 days and grouped randomly to ensure uniform body weight distribution. They were placed in individual cages, and their fur was shaved to expose them to the test formulation. Shearing was done 24 hours before administration and repeated within a week (BPOM, 2022). The

study aimed to understand the effects of the test formulation on the skin.

The test preparation involved applying the substance evenly over 10% of the skin surface, wrapping it with gauze, and covering it with elastic bandage and tape for 24 hours. Any residue was removed with water or a solvent. The dose range determination started with one test animal's initial 200 mg/kg body weight dose. If the animal dies, an additional dose is added. If no death or toxicity symptoms occur, an additional dose is given. The main study was continued based on determining the dose range (BPOM, 2022).

In this study, the initial dose for the main test was determined based on the results of a previous dose range determination study. The main test involved two groups of animals, with each group receiving different doses of liquid smoke. Group I received a 1000 mg/kg BW dose, while Group II received a 2000 mg/kg BW. Observations of clinical symptoms were made 24 hours after administration, and dead rats were counted up to 14 days after administration. On the 15th day, the rats were dissected, and their organs were weighed and histologized. Changes in body weight were analyzed weekly, and at the end of the test, the surviving animals were weighed and sacrificed. The relative organ weight of the liver and kidney was determined by weighing the dried organs and dividing them by body weight (BPOM, 2022). Macropathology study was done by dissecting and observing the liver and kidney, and organ histopathology was performed by staining the organs and examining them under a microscope.

STATISTICAL ANALYSIS

Acute dermal toxicity data from the test animals was statistically analyzed using SPSS with a one-way analysis of variance (ANOVA) method coupled with Tukey's post hoc test to determine significant treatment differences in each test.

RESULT AND DISCUSSION

1. Liquid Smoke Output

The grade 3 liquid smoke yield was 13.08% b/b obtained from 60 kg of coconut shell raw materials pyrolyzed at 400oC, producing 7.85 L of grade 3 liquid smoke. The grade 3 liquid smoke was then distilled at 150°C and obtained a yield of 76.43% v/v from 7.85 L of grade 3 liquid smoke and produced 6 L of grade 2 liquid smoke, while grade 1 liquid smoke distilled at 100°C obtained a yield of 58.30% v/v.

2. Acute Toxicity Test Results

2.1. Observation of toxic symptoms and death in preliminary tests

In the preliminary test, observations were made on the rats, such as tremors, seizures, diarrhea, salivation, swelling, hair and eye changes, mucous membrane changes, and animal movements, such as walking back and crawling over the abdomen. Observations in the control and treatment groups given liquid smoke doses of 200, 1000, and 2000 mg/kg BW indicated that the animals were moving normally and showed no symptoms of toxicity. It was the same as observing animal deaths after 24 hours and not seeing any mice die after administering liquid smoke. Therefore, it is necessary to conduct further tests in the form of the main test to see toxic symptoms and death of test animals, and observations were made on day 14 after the administration of liquid smoke.

2.2. Toxic symptom observation results in the primary test.

In the primary trial, animals were given liquid smoke doses of 1000 and 2000 mg/kg BW to see toxic symptoms and animal death. Table 1 shows that the animals had normal activities and did not show toxic symptoms in the control and treatment groups after administering the liquid smoke doses of 1000 and 2000 mg/kg BW. No animals died when the test preparation was within the 14-day observation period. Dosage determines the toxic properties of a compound. Increasing the dose will usually cause more organ systems to be affected and

will have very different working effects (Kruk et al., 2022).

Table 1. Observation of observation of toxic symptoms in the primary test

Toxic symptoms	Negative control	Dosage 1000 mg/Kg BW	Dosage 2000 mg/Kg BW
Tremors	-	-	-
Cramps	-	-	-
Salivation	-	-	-
Sweating	-	-	-
Diarrhea	-	-	-
Changes in the fur	-	-	-
Changes in the eyes	-	-	-
Change in the mucous membranes	-	-	-
Retreat pathway	-	-	-
Pathway using the stomach	-	-	-

Table 2. Results of animal death observations in the primary trial

Treatment	Number of mice	Number of dead mice
Negative control	2	0
Dosage 1000 mg/Kg BW	2	0
Dosage 2000 mg/Kg BW	2	0

The primary trial observed that administering liquid smoke through the dermal at doses less than 2,000 mg/kg BW is safe but not recommended due to exceeding the maximum human dose limit. The dose did not cause death or toxic symptoms in mice classified as category 5 or not classified at the 2000 mg/kg BW dose.

2.3. Observation of Average Weight in the Primary Test

The body weight of each rat was measured before and after the administration of liquid smoke to see its effect on the rat's body weight.

Table 3. Observation of average weight of rats

Group	Day		
	0	7	14
Negative control	162±2.00	166.5±1.50	171.5±0.50
1000 mg/Kg BW	163±3.00	174.5±3.50	177.5±4.50
2000 mg/Kg BW	175±2.25	180 ± 1.00	186.1±2.50

Based on the observation of the average body weight of rats on the first, 7th, and 14th days, it showed that there was no significant difference ($p>0.05$) between weight gain in the control group and the liquid smoke group, i.e., $p = 0.413$ on day 1, $p = 0.682$ on day 7, and $p = 0.543$ on day 14. So, it can be concluded that the administration of liquid smoke on the first, 7th, and 14th days has no significant effect on the body weight of rats. Body weight is a sensitive indicator of toxic symptoms and is said to be toxic if there is a change in body weight of up to 10% (Hidayat et al., 2022). Based on statistical tests, there was no real change in body weight growth. The rat's body weight did not fluctuate more than 10%, either up or down, indicating that administration of liquid smoke at doses of 1000 and 2000 mg/Kg BW did not affect the rat's body weight growth (OECD 2001).

2.4. Results of Organ Macropathology Observations

Organ observations based on Table 4 and Table 5 that have been carried out include observing the color, surface, and consistency of the liver and kidneys.

Table 4. Observation of color, surface, and consistency of liver

Treatment	Observation		
	Color	Surface	Consistency
Negative control	Deep red	Slippery	chewy
Dosage 1000 mg/Kg BW	Deep red	Slippery	chewy
Dosage 2000 mg/Kg BW	Deep red	Slippery	chewy

Table 5. Observation of color, surface, and consistency of the kidneys

Treatment	Observation		
	Color	Surface	Consistency
Negative control	Red brown	Slippery	chewy
Dosage 1000 mg/Kg BW	Red brown	Slippery	chewy
Dosage 2000 mg/Kg BW	Red brown	Slippery	chewy

Based on the color, surface, and consistency of the liver and kidney, it can be seen that the liver and kidney organs were normal. The kidneys were red-brown, the surface structures were slippery, and the consistency was chewy. The color change is one of the parameters of toxic effects aimed at obtaining information about the toxicity of the test substance to the target organ and its impact on the organ (Lu, 1995).

2.5. Relative Organ Weight Observation Results

To calculate the relative weight, the organs were dried and weighed, after which the organs were immersed in a 10% buffer formalin solution. Histopathological preparations were made with hematoxylin-eosin staining and examined under a microscope (Satria et al., 2022).

Table 6. Relative organ weight observation results

Group	Liver (%)	Right kidney (%)	Left kidney (%)
Negative control	3.77± 0.13	0.42± 0.01	0.40±0.02
Dosage 1000 mg/KgBW	4.28± 0.53	0.41±0.03	0.37±0.07
Dosage 2000 mg/KgBW	4.27± 0.32	0.43± 0.01	0.41±0.01

Based on Table 6, the relative weight of the liver and kidney organs was measured. No significant difference was found between the control group and the trial group after the administration of liquid smoke at the significance of $p = 0.718$ ($p > 0.05$) for the liver, $p = 0.679$ ($p > 0.05$) for the right kidney, and $p = 0.568$ ($p > 0.05$), for the left kidney. Therefore, it can be concluded that dermal administration of liquid smoke does not affect the weight ratio of liver organs and kidneys to body weight.

2.6. Result of Hematological Observations

Based on the hematological results of rats, almost all data showed no significant differences between the control group and the trial group ($p>0.05$), with each significant value being Hb $p = 0.425$ ($p>0.05$); HCT $p = 0.945$ ($p>0.05$), WBC $p = 0.615$ ($p>0.05$), RBC $p = 0.755$ ($p>0.05$), Trombosit $p = 0.425$ ($p>0.05$), MCV $p = 0.235$ ($p>0.05$), EOS $p = 0.156$ ($p>0.05$), MON $p = 0.450$ ($p>0.05$). On the other hand, the values of MCH $p = 0.025$ ($p < 0.05$), MCHC $p = 0.045$ ($p < 0.05$), and BAS $p = 0.038$ ($p < 0.05$) showed significant differences between the control and test groups. Therefore, it can be concluded that liquid smoke does not affect the hematological test values of the test animals between the control group and the test group.

Table 7. Results of hematological observations

Group	Negative control	1000 mg/kg BW	2000 mg/kg BW
WBC (103/µL)	6.85 ± 0.45	24.81 ± 2.75	8.17 ± 7.68
RBC (106/µL)	8.06±0.57	5.83±0.39	3.55±2.96
Hb (g/dL)	13.20±2.8	12.05±0.55	6.45±5.45
HCT (%)	46.50±6.5	37.35±2.65	19.35±16.25
MCV (fL)	58.75±0.45	64.60±8.9	53.55±1.05
MCH (pg)	17.45±0.65*	20.85±2.35*	17.60±0.70*
MCHC (g %)	36.00±2.90*	32.3±0.8*	32.85±0.55*
Trombo (103/µL)	673.5±49.50	398.4±390.6	383.5±351.5
Neutrofil (%)	12.50±1.50	9.00±4.00	14.00±2.00
Limfosit (%)	62.00±2.00	79.00±8.00	66.00±9.00
MON (%)	1.10±0.10	4.50±0.50	12.50±5.50
EOS (%)	2.75±0.25	3.50±2.50	4.50±1.50
BAS (%)	0.55±0.15*	4.00±2.00*	2.50±0.50*

Description: *= there is a significant difference with the control group

2.7. Blood biochemical observation results

Blood samples from test animals were taken on the 15th day, and SGOT, SGPT, urea, and creatinine levels were observed. Biochemical blood observations of rats showed no significant difference ($p>0.05$) between the control and trial groups in the measurements of the levels. However, the mean values of the test group's SGPT, urea, and creatinine were above the normal range, according to Charles River Laboratories.

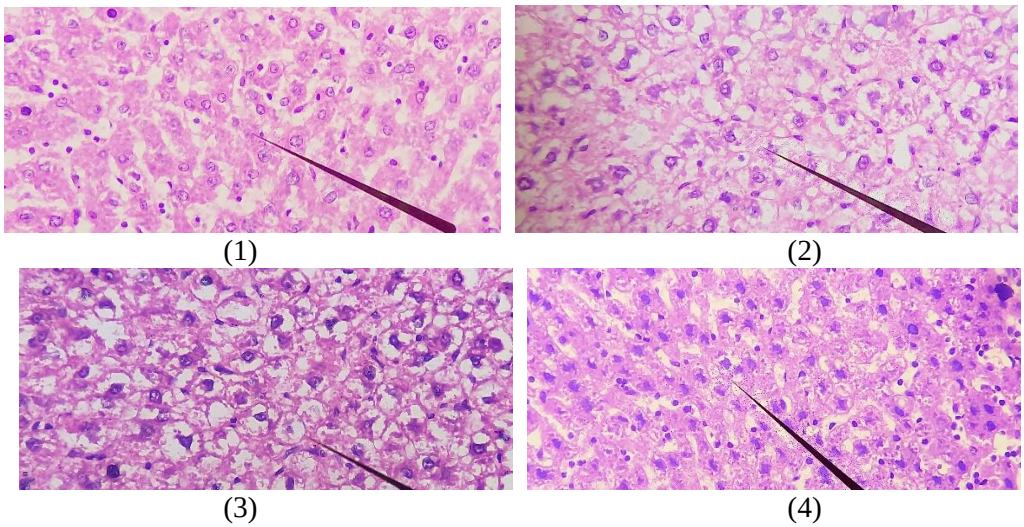
Table 8. Results of observations of biochemical blood parameters of mice

Group		Negative control	1000 mg/kgBW	2000 mg/kgBW
Liver	SGOT (U/L)/AST	180.0±1.00	195.0±3.00	191.0±2.00
	SGPT (U/L)/ALT	48.50 ±1.50	84.50±0.50	78.00±10.00
Kidney	Ureum (mg/dL)	27.00±3.00	57.00±5.00	57.50±3.50
	Creatinin (mg/dL)	0.64±0.04	0.84±0.11	0.71±0.14

The table above shows no significant differences in blood biochemical parameters. A good blood profile and its components within the normal range will indicate that the body is in good physiological health (Astuti et al., 2022). Damage to liver and kidney cells will affect blood biochemical values. The SGPT and SGOT enzyme values measure damage to liver cells or liver tissue. Damage that has occurred to liver cells causes increased levels of liver enzymes in the bloodstream, which are used to assess liver activity; this occurs due to disruption of the structure and function of the liver cell membrane. In cases where liver inflammation has resulted in damage, there is an increase in ALT activity earlier and more rapidly compared to AST levels (Fu et al., 2023).

2.8. Results of Microscopic Organ Imaging Observations

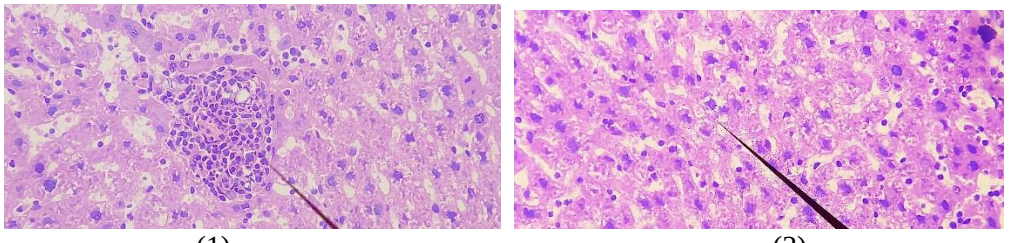
2.8.3. Microscopic result of liver tissue



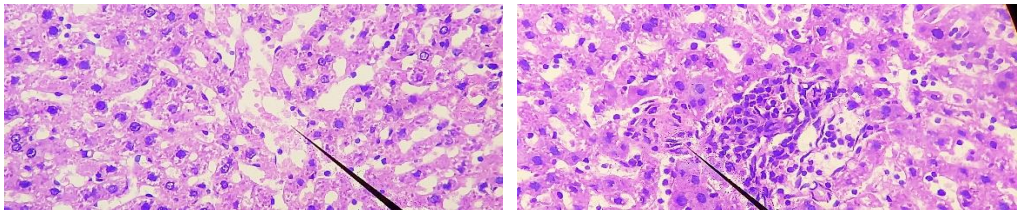
(1) (2)

(3) (4)

Figure 1. Microscopic observation of liver negative control group
Description: (1) Normal granuloma, (2) Normal sinusoid, (3) No inflammation, (4) No bleeding



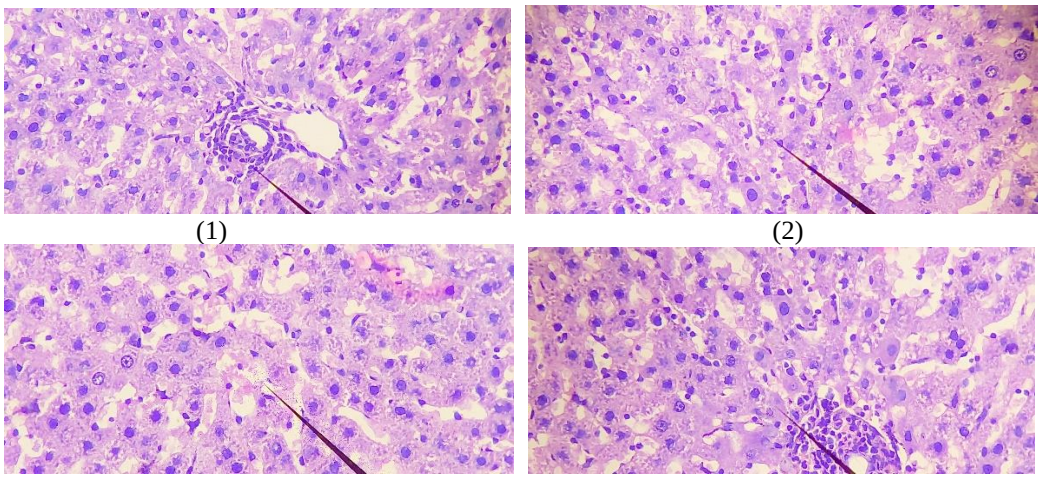
(1) (2)



(3) (4)

Figure 2. Microscopic observation of liver doses of 1000 mg/kgBW

Description: (1) Focal granuloma, (2) Sinusoid proliferation, (3) Moderate inflammation, (4) Hydropic degeneration



(1) (2)

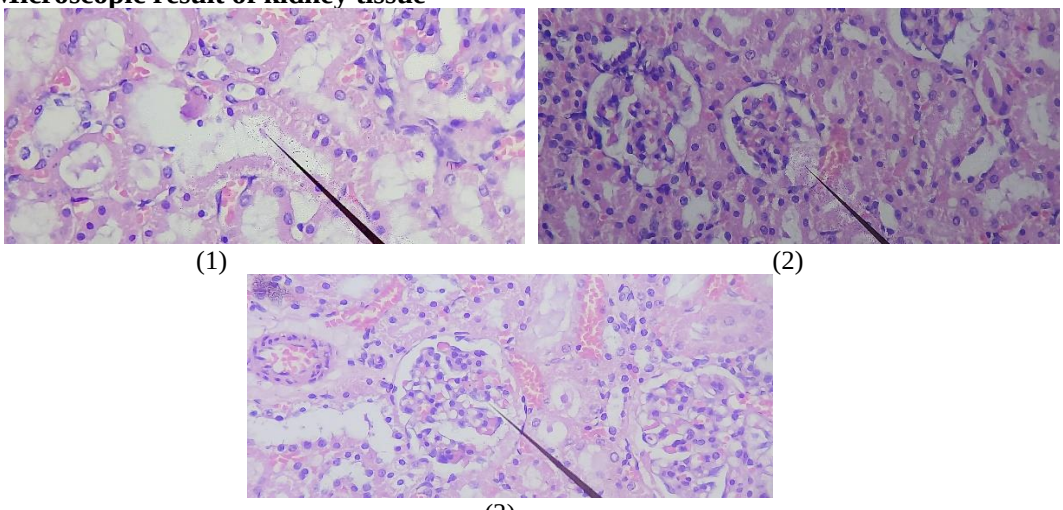
(3) (4)

Figure 3. Microscopic observation of liver doses of 2000 mg/kgBW

Description: (1) Focal granuloma, (2) Sinusoid proliferation, (3) Moderate inflammation, (4) Hydropic degeneration

Figures 1, 2, and 3 display the findings of a microscopic organ study. The control group had normal tissue conditions based on microscopic examination of liver tissue. There was no hydropic necrosis or degeneration, a normal state of granuloma, a normal sinusoid, no inflammation, and no bleeding. In contrast, the 1000 mg/kg BW test group showed atrophic degeneration, focal granulomas, enlarged sinusoids, and moderate liver organs.

2.8.3. Microscopic result of kidney tissue

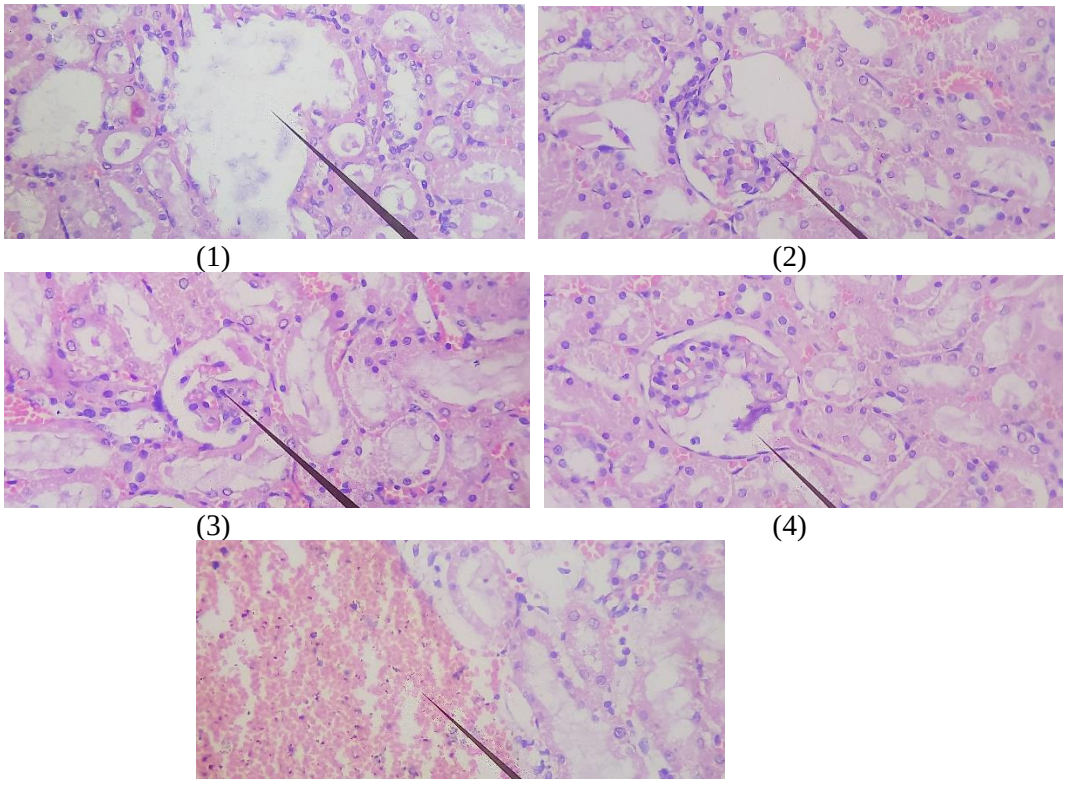


(1) (2)

(3)

Figure 4. Microscopic observation of kidney-negative control group

Description: (1) Normal tubules, (2) No endothelial damage, (3) No glomerulus atrophy



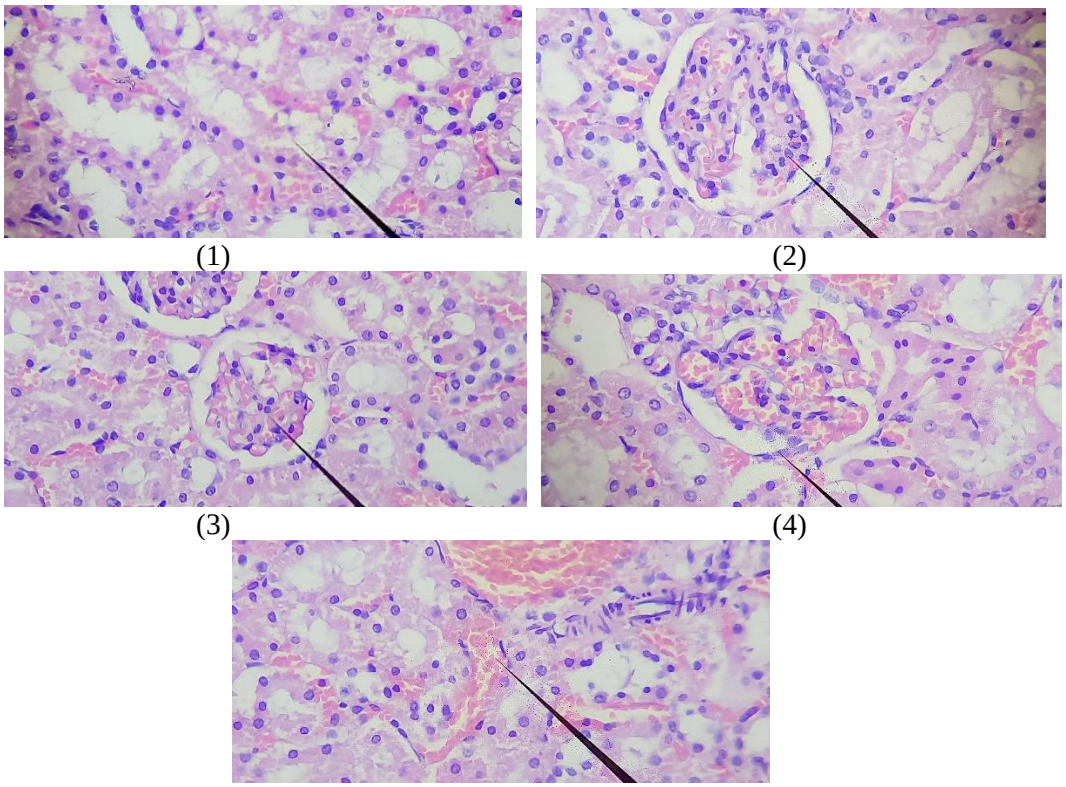
(1) (2)

(3) (4)

(5)

Figure 5. Microscopic observation of kidney doses of 1000 mg/kgBW

Description: (1) Tubular dilatation, (2) Loss of the endothelium, (3) Glomerular atrophy, (4) Retraction in the juxtaglomerular apparatus, (5) Inflammation, hemorrhage of less than 25% of the tissue



(1) (2)

(3) (4)

(5)

Figure 6. Microscopic observation of kidney doses of 2000 mg/kgBW

Description: (1) Tubular dilatation, (2) Swelling of the endothelium, (3) There is glomerular atrophy, (4) Thickening of the Bowman capsule, (5) Inflammation, hemorrhage of less than 25% of the tissue

Figures 4,5 and 6 show significant differences in the histological picture of the kidneys between the control animal group and the liquid smoke group, where the kidney organs in the control group were still in normal condition. In contrast, the test group that was administered liquid smoke for 14 days showed tubular dilatation, atrophy of the glomerulus, swelling or loss of part of the endothelium, thickening of the Bowman capsule, and the occurrence of inflammation and hemorrhage of less than 25% of the total tissue.

DISCUSSION

Based on observations in the acute toxicity test of coconut shell liquid smoke, the results showed that the 2000 mg/kg BW dose of liquid smoke did not show any death or toxic symptoms observed in rats and was included in category 5 or not classified according to the Globally Harmonized Classification System (GHS) category for chemicals and mixtures. So, it can be concluded that dermal administration of grade 1 coconut shell liquid smoke at a dose of 2000 mg/Kg BW is safe, although there is mild damage to organs.

CONCLUSION

Liquid smoke from coconut shell (*Cocos nucifera* L.) grade 1 has acute toxicity effects with minor observed damage to the liver organs and kidneys of rats.

Declaration by Authors

Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Hardiyanti Rukmana, Masfria, Emil Salim. Acute dermal toxicity of liquid smoke (*Cocos nucifera L.*) grade 1. *International Journal of Research and Review*. 2024; 11(6): 208-218. DOI: *https://doi.org/10.52403/ijrr.20240624*

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