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Solid Preservative Preparation from Decolorized Ethanolic Extract of Ginggiang (*Leea aequata* L.): A 90-Day Subchronic Toxicity Study in Rats

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Abstract: Natural preservatives derived from medicinal plants require a thorough safety evaluation before they can be used in food. Ginggiang has traditionally been used by the Kuta indigenous community in Ciamis, West Java, Indonesia, to preserve palm sap; however, its safety profile has not been evaluated. In this study, a solid preservative preparation was developed from the decolorized ethanolic extract of ginggiang leaves (SPP-ED), and its safety profile was assessed after repeated oral administration. The study focused on subchronic oral toxicity and identified the target organs affected by repeated exposure to SPP-ED. In a 90-day experiment, both male and female Wistar rats received SPP-ED at doses of 45, 150, or 1500 mg/kg body weight per day. Satellite groups were included to evaluate recovery. Various parameters, including behavioral, hematological, biochemical, urinalysis, organ index, and histopathological evaluations, were systematically analyzed. No mortality was observed during the study. The results showed that subchronic exposure to SPP-ED had sex-specific effects: female rats experienced renal alterations, characterized by changes in urinalysis, elevated blood urea nitrogen and creatinine levels, and signs of tubular degeneration. In contrast, male rats exhibited pulmonary changes, including an increased lung-to-body weight ratio, alveolitis, and fibrosis. The D1 dose (45 mg/kg body weight/day) was the lowest dose at which potentially adverse histopathological changes were observed; however, a definitive NOAEL and LOAEL could not be established. Further studies using lower dose levels are warranted to better characterize the dose-response relationship and establish the NOAEL and LOAEL of SPP-ED.

Introduction

The potential for developing natural preservatives is growing alongside increasing scientific evidence regarding various natural substances that possess antimicrobial activity (1). Traditional preservative practices have also inspired research aimed at supporting their wider application (2). These natural preservatives are expected to serve as alternatives to synthetic preservatives, which have long been in use but carry potential adverse health effects, and the emergence of preservative-tolerant microorganisms (3, 4). However, a comprehensive toxicological evaluation is essential before these preservatives can be utilized on a broader scale.

Ginggiang (*Leea aequata* L.) is a shrub traditionally used as a preservative for palm sap, an important raw material in the production of brown sugar. This practice remains prevalent in the Kuta indigenous community in Ciamis, Indonesia. Preservation is achieved by soaking the plant's dry leaves in a container with its sap. This practice has proven effective in delaying spoilage and maintaining the quality of palm sap before processing. The use of ginggiang leaves has been passed down through generations without any documented adverse health effects. However, empirical evidence alone is insufficient, making comprehensive toxicity testing essential (5, 6). Such testing is particularly important given that the ginggiang leaves are being formulated into a solid product intended for broader application.

In addition, ginggiang has a variety of empirical applications in the community, including its use as an antiseptic for the treatment of leprosy wounds (7). In addition, it serves important functions as an anesthetic,

antipyretic, and anti-itch agent, as well as for the management of bronchitis and tuberculosis (8). Several studies have also been conducted to develop preservatives derived from ginggiang, as the plant possesses significant antimicrobial properties, demonstrating efficacy as both an antibacterial and antifungal agent (8, 9). Based on these findings, a solid preservative preparation derived from decolorized ethanolic extract of ginggiang leaves (SPP-ED) was developed to improve handling, storage stability, and its application. However, its antimicrobial activity has been proven (10). The systemic safety following repeated oral exposure has not been investigated.

A comprehensive toxicological evaluation is required to establish the safety profile. The evaluation is conducted to generate health-based guidance values (HBGVs) (5, 11). According to the Organization for Economic Co-Operation and Development (OECD), repeated-dose toxicity studies provide the basis for identifying the No-Observed-Adverse-Effect-Level (NOAEL) and the Lowest-Observed-Adverse-Effect-Level (LOAEL) (5). The NOAEL or LOAEL is subsequently used as the reference point for deriving the acceptable daily intake (ADI) through the application of appropriate uncertainty factors (11, 12). A 90-day oral toxicity study is considered the standard approach for detecting cumulative toxic effects, identifying target organs, and characterizing dose-response relationships after repeated exposure. All these stages are included in food safety assessments (5, 13).

Experimental Section

Materials and Apparatus

The materials used in this study were a solid preservative preparation derived from decolorized ethanolic extract of ginggiang leaves (SPP-ED) as the test extract, standard laboratory rodent feed, and distilled water for drinking. Reagents for hematological and biochemical analyses included commercially available diagnostic kits for glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, alkaline phosphatase, blood urea nitrogen (BUN), creatinine, total protein, albumin, potassium, glutamic-oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). Histological reagents comprised hematoxylin monohydrate and eosin Y (Merck KGaA, Darmstadt, Germany), paraffin wax, xylene, ethanol series, and 10% neutral buffered formalin.

The major equipments used were an oral gavage sonde (Instech Laboratories Inc., Plymouth Meeting, PA, USA), metabolic cages (Tecniplast, Buguggiate, Italy), analytical balance (Sartorius AG, Göttingen, Germany), centrifuge (Eppendorf AG, Hamburg, Germany), hematology analyzer (Exigo™, Boule Diagnostics AB, Stockholm, Sweden), microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), rotary microtome (Leica Biosystems, Wetzlar, Germany), and ultrasonic bath (Branson Ultrasonics, Danbury, CT, USA).

All experimental procedures involving animals were conducted in accordance with Indonesian regulations on animal experimentation and internationally accepted guidelines for the care and use of laboratory animals (14). The study protocol was reviewed and approved by the Ethics Committee for the Use of Experimental Animals at the Institut Teknologi Bandung (Approval No. KEP/I/2023/VII/H230623PP/TSEG). No unexpected hazards or unusual risks were encountered during the experimental procedures. Standard laboratory safety protocols were strictly adhered to in the study.

Experimental Animals

Healthy male and female Wistar rats aged 8 weeks were used in this study. The animals were obtained from a local rat breeder in Bandung, West Java, Indonesia. A total of 84 rats (42 males and 42 females) were used in this study. The animals were acclimated for 7 days before treatment and randomly assigned to six experimental groups for each sex: control (C), satellite control (CS), low-dose group (D1), medium-dose group (D2), high-dose group (D3), and high-dose satellite group (D3S). The initial body weight was 197.17 ± 13.14 g in males and 186.26 ± 17.56 g in females.

In Vivo Test

Extract Preparation and Formulation

Ginggiang leaves were obtained from Ciamis, Indonesia, on January 10, 2023. The plant material was taxonomically authenticated at the Herbarium Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia. The plant was identified as *L. aequata* L. (Identification No. 33/HB/01/2023). In addition, the extraction and formulation processes were carried out at the Laboratory of Pharmacognosy, Faculty of Pharmacy, Universitas Bhakti Kencana, Indonesia. The extract was prepared by maceration with 96% ethanol, followed by decolorization with powdered activated carbon (2 g/100 mL extract), then concentrated, oven-dried at 45 °C, and formulated with microcrystalline cellulose (1:1) to obtain SPP-ED.

Dose Selection and Study Design

Dose levels were selected based on previously published efficacy data of a solid preservative preparation from ginggiang leaves (9). The low (45 mg/kg bw/day; D1) and middle (150 mg/kg bw/day; D2) doses corresponded to approximately 50-fold multiples of the lower and upper effective dose range identified in preservative efficacy studies (9). In addition, the high dose (1500 mg/kg bw/day; D3) was set at 10 times the middle dose to provide an adequate safety margin and to allow identification of potential target organs following repeated exposure, in accordance with general principles of subchronic toxicity testing.

Treatment and Recovery Period

The respective treatments were administered once daily for 90 consecutive days. At the end of the 90-day treatment period, the C, D1, D2, and D3 groups underwent the scheduled toxicological evaluations. The CS and D3S were maintained for an additional 30-day treatment-free recovery period. The satellite animals were subsequently evaluated at the end of the recovery period to assess the persistence or reversibility of treatment-related effects.

Toxicological Evaluation

Throughout the 90-day treatment period and the

subsequent recovery period, the animals were observed daily for mortality, clinical signs, and behavioral changes. Body weight, feed consumption, and water consumption were recorded weekly. Urinalysis was performed at the end of the treatment and recovery periods. Hematological and biochemical examinations were conducted on the day following completion of the respective treatment or recovery period. At the end of each observation period, the animals were euthanized and subjected to necropsy, organ-weight assessment, and histopathological examination.

Histopathological Examination

At the end of the testing period, the animals were dissected. The liver, heart, lungs, kidneys, and spleen were collected. The organs were fixed in 10% neutral buffered formalin (NBF). The tissues were then processed according to routine histopathology procedures, including dehydration, clearing, paraffin infiltration, and paraffin embedding. The paraffin-embedded tissues were sectioned using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (H& E). The stained sections were examined microscopically for histopathological alterations.

Histopathological evaluation was performed by a veterinarian with expertise in veterinary pathology at the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Histopathological alterations were assessed semi-quantitatively using an ordinal scoring system. Lesions in the liver, heart, and kidneys were scored on a 0–3 scale, whereas lesions in the spleen were scored on a 0–4 scale. Alveolitis and pulmonary fibrosis were scored based on the percentage of affected lung area using a scale of 0–4 (0 = no lesion, 1 = lesion affecting ≤25% of the area, 2 = lesion affecting 26–50% of the area, 3 = lesion affecting 51–75% of the area, and 4 = lesion affecting ≥76% of the area). Four animals were randomly selected from each experimental group for histopathological evaluation.

Statistical Analysis

Data were expressed as mean ± SD and analyzed using one-way Analysis of Variance (ANOVA) followed by post hoc test (LSD) when applicable. LSD was applied as an exploratory post hoc test following a significant ANOVA result. Statistical significance was set at $p < 0.05$. For descriptive interpretation of the magnitude of statistical evidence, $p < 0.001$, $p < 0.05$, and $p < 0.1$ were indicated separately in the table using different superscript symbols. $P < 0.1$ interpreted as a statistical trend rather than statistical significance.

Results

Behavioral Observation

Subchronic oral exposure to SPP-ED for 90 days caused no mortality or overt signs of systemic toxicity during either the treatment or recovery periods. No abnormal secretions or gross behavioral disturbances were observed. However, at the highest dose (D3), male rats exhibited a significant reduction in motor activity ($p < 0.05$), whereas females showed a non-significant decrease. Female rats receiving D1 and D2 showed significantly increased exploratory behavior, as indicated by a higher number of head dips ($p < 0.05$). Increased head-dip frequency was commonly associated with enhanced exploratory activity and reduced anxiety-related behavior (15, 16). Constipation was observed in male rats at high doses but was absent in females.

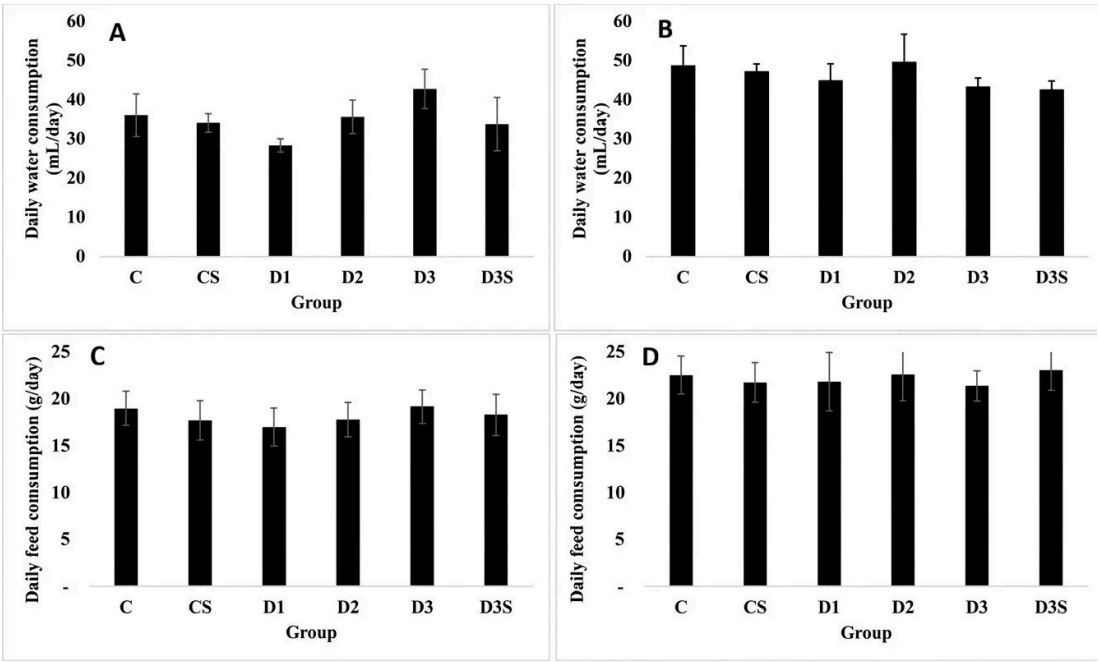


Figure 1. Average daily feed and water consumption of Wistar rats during 90-day oral administration of SPP-ED. (A) Average daily water consumption of female rats; (B) Average daily water consumption of male rats; (C) Average daily feed consumption of female rats; (D) Average daily feed consumption of male rats. Data are presented as mean ± SD (n=7).

Body Weight, Water, and Feed Consumption

In male rats, feed consumption and body weight gain were not significantly affected by SPP-ED treatment (**Figure 1**). However, water intake was significantly higher at D2 than in the controls. In female rats, D1 significantly reduced feed and water intake ($p < 0.05$), whereas D2 showed a similar, non-significant trend. D3 significantly increased water consumption ($p < 0.05$). No significant differences in body weight gain were observed in either sex during the treatment period, although females showed a slight, non-significant reduction compared with controls. During the recovery phase, satellite groups showed a general trend of decreased body weight, while male satellite controls continued to gain weight, though the increase was not statistically significant (**Figure 2**).

Urinalysis

Urinalysis showed abnormalities in urine color, odor, turbidity, volume, and specific gravity in female rats D2 and D3 (**Table 1**). Statistical analysis of the quantitative parameters showed no significant difference in pH among the groups in either sex. In male rats, urine volume and specific gravity did not differ significantly among groups. In female rats, urine volume and specific gravity differed significantly among groups, with the D3 group showing a significantly lower urine volume and a higher specific gravity than the C group ($p < 0.05$).

Hematology

Hematological parameters showed dose-and

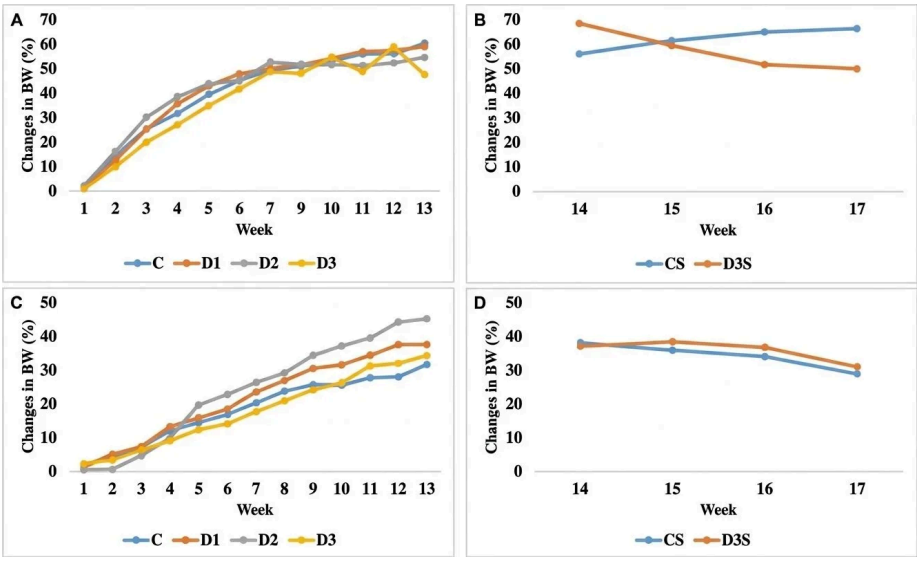


Figure 2. Changes in body weight of male and female Wistar rats during the treatment and recovery periods following repeated oral administration of SPP-ED. (A) Male rats during the treatment period; (B) male rats during the recovery period; (C) female rats during the treatment period; (D) female rats during the recovery period. Values are presented as mean ± SD (n=7).

Table 1. Urinalysis parameters of male and female Wistar rats after 90-day oral administration of SPP-ED and after the recovery period (mean ± SD, n=7).

Urinalysis Parameter	Test group					
	C	D1	D2	D3	CS	D3S
			Male			
Organoleptic	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine
Volume (mL)	10.29 ± 5.59	10.86 ± 5.64	7.00 ± 3.52	8.86 ± 3.63	12.14 ± 7.67	6.86 ± 3.44
pH	6.57 ± 1.13	6.86 ± 0.90	6.17 ± 0.41	6.71 ± 0.95	7.00 ± 1.41	6.86 ± 1.46
SG (g/mL)	1.11 ± 0.03	1.11 ± 0.04	1.10 ± 0.02	1.08 ± 0.02	1.09 ± 0.05	1.09 ± 0.02
			Female			
Organoleptic	Clear to light yellow, distinctive odor of urine	Clear, distinctive odor of urine	Clear/yellow/brown, slightly cloudy, odorless	Yellow/brown, cloudy, odorless	Yellow, distinctive odor of urine	Yellow, distinctive odor of urine
Volume (mL)	14.00 ± 7.02	10.00 ± 5.93	12.67 ± 5.01	6.38 ± 4.06[b]	20.67 ± 8.16	12.00 ± 3.46
pH	6.57 ± 0.98	7.00 ± 1.10	6.50 ± 0.84	6.57 ± 0.98	7.33 ± 1.21	7.43 ± 0.98
SG (g/mL)	1.10 ± 0.06	1.10 ± 0.02	1.13 ± 0.03	1.15 ± 0.09[b]	1.05 ± 0.02	1.07 ± 0.03

Note: SG = specific gravity; C: control; D1-D3: SPP-ED doses at 45, 150, and 1500 mg/kg body weight/day; CS: satellite control; D3S: satellite D3. [b]Significantly different from the C ($p < 0.05$).

Table 2. Hematological parameters of male and female Wistar rats following 90-day repeated oral administration of SPP-ED and after the recovery period (mean ± SD, n=7).

Group	Testing periode				Reversibility period	
	C	D1	D2	D3	CS	D3S
Male						
WBC (x102/µL)	29.54 ± 9.56	19.49 ± 4.26	21.72 ± 5.20[a]	25.30 ± 6.58	35.97 ± 6.94	27.31 ± 6.71
LYM (x102/µL)	13.17 ± 3.91	10.03 ± 2.41[b]	10.80 ± 2.54[b]	11.63 ± 1.79	18.74 ± 2.95	16.89 ± 4.47
MON (x102/µL)	1.24 ± 0.53	2.09 ± 0.52[c]	2.08 ± 1.01[c]	1.89 ± 1.38[c]	1.63 ± 1.21	1.96 ± 0.49[c]
GRA (x102/µL)	15.13 ± 6.07	7.67 ± 1.68	8.83 ± 2.31	11.79 ± 4.50	14.59 ± 4.29	8.47 ± 2.80
HGB (g/dL)	14.26 ± 0.84	13.14 ± 1.00[b]	13.45 ± 0.97	12.89 ± 0.98[b]	13.56 ± 0.62	13.79 ± 0.69
MCH (pg)	16.71 ± 0.28	17.21 ± 0.89	17.05 ± 0.60	16.61 ± 0.43	16.76 ± 0.43	16.77 ± 0.47
MCHC (g/dL)	42.03 ± 0.95	41.76 ± 1.08	41.75 ± 0.57	41.27 ± 0.44	43.04 ± 0.54	42.74 ± 0.69
RBC (x106/µL)	8.52 ± 0.53	7.66 ± 0.79[b]	7.91 ± 0.75	7.75 ± 0.59[b]	8.10 ± 0.36	8.23 ± 0.53
MCV (fL)	39.89 ± 1.38	41.30 ± 2.73[a]	40.83 ± 1.88	40.31 ± 1.26	38.97 ± 1.06	39.30 ± 1.48
HCT (%)	33.94 ± 1.42	31.47 ± 1.94	32.23 ± 2.06	31.23 ± 2.08	31.56 ± 1.24	32.30 ± 1.35
RDW (%)	18.26 ± 0.60	18.24 ± 0.60	18.10 ± 0.39	18.74 ± 1.17	18.26 ± 0.35	17.90 ± 0.25
PLT (x103/µL)	361.14 ± 16.78	399.29 ± 13.06	339.17 ± 18.37	311.03 ± 17.70	348.14 ± 24.70	441.71 ± 18.75
MPV (fL)	5.74 ± 0.24	5.66 ± 0.38	5.85 ± 0.49	5.63 ± 0.24	5.51 ± 0.37	5.66 ± 0.52
Female						
WBC (x102/µL)	20.96 ± 5.24	28.13 ± 5.89[b]	23.30 ± 4.74	20.96 ± 2.80	19.19 ± 3.97	19.34 ± 5.20
LYM (x102/µL)	12.11 ± 2.92	15.83 ± 3.40[b]	11.97 ± 1.43	11.54 ± 1.89	10.86 ± 2.32	10.87 ± 3.49
MON (x102/µL)	1.66 ± 0.91	2.60 ± 0.83	1.75 ± 0.59	1.77 ± 0.53	1.56 ± 0.71	1.94 ± 0.40
GRA (x102/µL)	7.19 ± 1.90	9.70 ± 2.88	9.58 ± 3.72	7.64 ± 1.21	6.77 ± 1.66	6.53 ± 1.79
HGB (g/dL)	13.27 ± 0.38	13.88 ± 0.47	13.38 ± 0.84	13.00 ± 0.82	13.49 ± 0.34	13.20 ± 0.96
MCH (pg)	17.67 ± 0.54	17.85 ± 0.19	17.72 ± 0.50	17.79 ± 0.61	17.90 ± 0.26	17.77 ± 0.42
MCHC (g/dL)	41.89 ± 0.44	42.25 ± 0.35[b]	42.13 ± 0.24	41.30 ± 0.60	42.44 ± 0.12	41.41 ± 0.62
RBC (x106/µL)	7.53 ± 0.25	7.78 ± 0.23	7.56 ± 0.45	7.32 ± 0.48	7.55 ± 0.26	7.43 ± 0.61
MCV (fL)	41.73 ± 0.59	42.30 ± 0.70	42.07 ± 1.30	43.04 ± 1.90	42.20 ± 0.66	42.94 ± 1.52
HCT (%)	31.73 ± 0.91	32.90 ± 1.15	31.78 ± 1.84	31.47 ± 1.76	31.83 ± 0.79	31.84 ± 2.02
RDW (%)	17.34 ± 0.42	17.38 ± 0.58	17.17 ± 0.36	16.96 ± 0.28	16.84 ± 0.16	16.96 ± 0.28
PLT (x103/µL)	455.71 ± 15.50	417.83 ± 11.44	423.00 ± 18.98	332.14 ± 10.10	315.29 ± 11.56	377.86 ± 17.72
MPV (fL)	7.43 ± 4.21	5.73 ± 0.17	5.57 ± 0.24	5.74 ± 0.21	6.06 ± 0.18	5.93 ± 0.29

WBC: white blood cell; LYM: lymphocytes; MON: monocytes; GRA: granulocytes; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red distribution width; PLT: platelets; MPV: mean platelet volume. [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control (one-way ANOVA followed by post hoc test)

sex-dependent changes (**Table 2**). In male rats, total leukocyte counts were reduced at D1, and lymphocyte counts were significantly reduced at D1 and D2 ($p < 0.05$). Monocyte counts were increased in the D1, D2, D3, and D3S groups ($p < 0.001$). Hemoglobin and red blood cell counts were reduced at D1 and D2 ($p < 0.05$). In female rats, significant hematological changes were primarily observed at D1, including increased total leukocyte and lymphocyte counts and a higher mean corpuscular hemoglobin concentration ($P < 0.05$).

In male rats, WBC and lymphocyte counts decreased at selected dose levels, while monocyte counts increased. Increased monocyte counts may reflect inflammatory or immune responses. Although monocytosis is not specific to a particular inflammatory condition (17). The absence of a consistent dose-dependent pattern and the lack of corresponding clinical signs limit the interpretation of these changes as evidence of hematological toxicity.

The reduction in hemoglobin and RBC counts observed at D1 and D2 may indicate a mild alteration in erythroid parameters (18). However, the absence of a consistent dose-related response and the limited changes in other erythrocyte indices suggest that these findings should be interpreted with caution rather than as definitive evidence of anemia. Hematological changes should be interpreted based on the overall erythrocyte profile, including RBC count, hemoglobin, hematocrit, and erythrocyte indices, rather than on individual parameters alone (18, 19).

In female rats, the hematological changes were observed primarily on D1. This phenomenon was characterized by increases in WBC and lymphocyte counts, as well as in MCHC. These changes were not consistently observed at higher doses; a direct dose-related toxicological effect cannot be established from the hematological findings alone.

Serum Biochemical Parameters

Biochemical analysis showed significant alterations in renal-related parameters (**Table 3**). In male rats, glucose concentrations were significantly increased in D1, D2, and D3 ($p < 0.05$), while total cholesterol concentration was significantly decreased at D2 and D3 ($p < 0.05$). BUN was markedly increased at D1, D2, and D3 ($p < 0.001$), whereas creatinine was significantly decreased at the same dose levels ($p < 0.001$). Potassium concentrations were

Table 3. Serum biochemical parameters of male and female Wistar rats after 90-day exposure to SPP-ED and following the recovery period (mean ± SD, n=7).

Parameters	Testing period				Reversibility period	
	C	D1	D2	D3	CS	D3S
Male						
Glucose (mg/dL)	86.71 ± 2.71	101.14 ± 12.40[b]	109.00 ± 7.92[b]	102.86 ± 18.54[b]	98.86 ± 14.46	92.86 ± 6.77
Total cholesterol (mg/dL)	85.78 ± 22.43	83.58 ± 17.39	74.12 ± 10.98[b]	79.25 ± 22.06[b]	96.17 ± 8.64	97.24 ± 11.08
LDL (mg/dL)	34.03 ± 8.55	28.00 ± 11.04	33.78 ± 13.02	23.15 ± 5.56	36.74 ± 9.75	40.96 ± 9.58
HDL (mg/dL)	18.21 ± 7.71	34.20 ± 12.99	33.84 ± 17.26	32.43 ± 14.15	45.20 ± 7.20	39.31 ± 6.38
Triglyceride (mg/dL)	92.94 ± 6.09	97.27 ± 9.15	96.90 ± 7.37	97.12 ± 5.65	89.34 ± 6.77	93.78 ± 5.03
GPT (U/L)	19.86 ± 6.28	18.97 ± 4.06	23.41 ± 10.61	18.78 ± 4.08	22.25 ± 8.94	21.90 ± 3.26
GOT (U/L)	48.27 ± 10.75	45.92 ± 9.92	64.34 ± 38.49	64.80 ± 15.94	53.83 ± 20.83	48.83 ± 9.97
Alkaline phosphatase (U/L)	103.24 ± 19.45	73.98 ± 18.98[a]	89.80 ± 22.27	90.49 ± 20.79	109.59 ± 27.98	78.22 ± 14.26
BUN (mg/dL)	52.16 ± 18.41	101.68 ± 13.45[c]	101.27 ± 15.82[c]	104.46 ± 21.98[c]	37.50 ± 9.82	56.86 ± 9.90
Creatinine (mg/dL)	23.65 ± 4.82	14.97 ± 3.42[c]	16.99 ± 4.17[c]	16.44 ± 2.31[c]	22.18 ± 0.56	21.46 ± 0.39
Total protein (g/dL)	9.04 ± 0.95	7.83 ± 0.71	7.77 ± 0.96	8.63 ± 0.94	6.86 ± 0.63	7.13 ± 0.50
Albumin (g/dL)	4.40 ± 0.98	3.34 ± 0.22	3.75 ± 0.49	4.61 ± 0.76	2.72 ± 0.30	3.76 ± 0.32
Kalium (mmol/L)	9.52 ± 6.13	9.29 ± 4.06	11.33 ± 2.52[b]	11.18 ± 1.83[b]	9.46 ± 2.68	8.57 ± 4.87
Female						
Glucose (mg/dL)	83.00 ± 9.59	80.00 ± 7.00	83.17 ± 8.49	80.50 ± 2.50	98.29 ± 8.40	118.43 ± 13.05[b]
Total cholesterol (mg/dL)	75.55 ± 10.89	70.59 ± 8.48	66.70 ± 9.60	76.19 ± 9.00	78.28 ± 10.19	71.73 ± 15.05
LDL (mg/dL)	20.11 ± 8.35	12.52 ± 8.26	13.65 ± 8.42	23.83 ± 6.97	25.21 ± 9.39	13.89 ± 9.08
HDL (mg/dL)	35.89 ± 7.27	35.65 ± 6.58	32.62 ± 9.04	28.10 ± 12.64	27.55 ± 8.58	32.33 ± 6.95
Triglyceride (mg/dL)	107.10 ± 12.96	101.88 ± 9.80	102.13 ± 4.83	98.39 ± 8.53	110.06 ± 10.87	99.73 ± 10.09
GPT (U/L)	12.40 ± 6.00	13.71 ± 8.16	11.63 ± 3.18	12.69 ± 4.13	10.55 ± 3.60	11.53 ± 4.62
GOT (U/L)	25.47 ± 10.63	26.39 ± 9.19	31.54 ± 8.77	31.16 ± 4.80	24.00 ± 8.21	20.94 ± 5.23
Alkaline phosphatase (U/L)	38.74 ± 9.58	22.91 ± 4.46[b]	32.31 ± 8.50	44.05 ± 10.28	29.60 ± 6.32	53.01 ± 12.70[b]
BUN (mg/dL)	57.61 ± 11.44	54.92 ± 11.69	79.72 ± 25.37[b]	77.19 ± 11.71[b]	69.68 ± 12.17	60.14 ± 23.78
Creatinine (mg/dL)	8.46 ± 0.46	7.06 ± 0.69	7.02 ± 1.57	13.89 ± 5.76[b]	6.11 ± 2.19	12.55 ± 4.13[b]
Total protein (g/dL)	7.0 ± 2.35	6.31 ± 2.29	6.95 ± 2.24	7.76 ± 1.07	7.03 ± 1.43	7.94 ± 1.15
Albumin (g/dL)	3.77 ± 0.46	3.15 ± 0.32	3.47 ± 0.28	3.94 ± 0.54	3.80 ± 0.32	4.20 ± 0.43
Kalium (mmol/L)	6.25 ± 2.13	7.70 ± 4.70	8.64 ± 3.14	7.07 ± 3.27	5.26 ± 1.29	8.46 ± 2.22

Note: BUN: blood urea nitrogen; GPT: glutamic pyruvic transaminase (ALT); GOT: glutamic oxaloacetic transaminase (AST). [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control (one-way ANOVA followed by post hoc test).

Table 4. Organ index in male Wistar rats after 90-day oral administration of SPP-ED and after the recovery period (mean ± SD, n=7).

Group	Liver	Lung	Heart	Kidney	Adrenal	Spleen	Thymus	Seminal vesicle	Testicles
C	3.43 ± 0.25	0.63 ± 0.18	0.36 ± 0.04	0.64 ± 0.10	0.0262 ± 0.00	0.35 ± 0.06	0.09 ± 0.03	0.50 ± 0.07	1.03 ± 0.22
CS	3.45 ± 0.26	0.62 ± 0.11	0.34 ± 0.02	0.74 ± 0.12	0.0272 ± 0.00	0.32 ± 0.05	0.11 ± 0.03	0.49 ± 0.06	1.04 ± 0.14
D1	3.55 ± 0.34	0.80 ± 0.33	0.38 ± 0.03	0.71 ± 0.07	0.0267 ± 0.00	0.28 ± 0.06[a]	0.10 ± 0.04	0.47 ± 0.05	1.08 ± 0.13
D2	3.38 ± 0.22	0.54 ± 0.04	0.34 ± 0.01	0.64 ± 0.03	0.0223 ± 0.01	0.31 ± 0.04	0.09 ± 0.03	0.48 ± 0.06	1.02 ± 0.08
D3	3.58 ± 0.25	1.46 ± 1.48[c]	0.39 ± 0.04	0.72 ± 0.05	0.0277 ± 0.01	0.34 ± 0.05	0.10 ± 0.04	0.44 ± 0.09[a]	1.02 ± 0.06
D3S	3.52 ± 0.25	0.54 ± 0.10	0.30 ± 0.02[b]	0.67 ± 0.07	0.0223 ± 0.01	0.34 ± 0.07	0.09 ± 0.01	0.49 ± 0.05	0.98 ± 0.16

Note: Organ index = (organ weight/body weight) x 100%. [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

Table 5. Organ index in female Wistar rats after 90-day oral administration of SPP-ED and after the recovery period (mean ± SD, n=7).

Group	Liver	Lung	Heart	Kidney	Adrenal	Spleen	Thymus	Ovary	Uterus
C	3.09 ± 0.08	0.73 ± 0.21	0.38 ± 0.04	0.63 ± 0.04	0.0339 ± 0.01	0.36 ± 0.03	0.15 ± 0.03	0.0508 ± 0.01	0.29 ± 0.08
CS	3.11 ± 0.22	0.54 ± 0.06	0.35 ± 0.03	0.65 ± 0.04	0.0347 ± 0.01	0.32 ± 0.04	0.13 ± 0.03	0.0564 ± 0.01	0.37 ± 0.07[a]
D1	2.99 ± 0.31	0.69 ± 0.25	0.42 ± 0.04[b]	0.66 ± 0.09	0.0380 ± 0.01	0.37 ± 0.20	0.16 ± 0.03	0.0578 ± 0.01	0.29 ± 0.08
D2	2.77 ± 0.14[a]	0.58 ± 0.06	0.36 ± 0.02	0.58 ± 0.07	0.0386 ± 0.01	0.29 ± 0.04[a]	0.12 ± 0.01	0.0531 ± 0.01	0.28 ± 0.09
D3	3.10 ± 0.48	0.62 ± 0.19	0.39 ± 0.06	0.62 ± 0.09	0.0376 ± 0.01	0.34 ± 0.07	0.13 ± 0.03	0.0521 ± 0.01	0.24 ± 0.09
D3S	3.35 ± 0.24	0.60 ± 0.04	0.34 ± 0.02[a]	0.65 ± 0.04	0.0376 ± 0.01	0.35 ± 0.04	0.14 ± 0.04	0.0574 ± 0.01	0.40 ± 0.13[b]

Note: Organ index = (organ weight/body weight) x 100%. C: control; D1–D3: SPP-ED doses 45, 150, and 1500 mg/kg body weight/day; CS: satellite control; D3S: satellite D3. [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

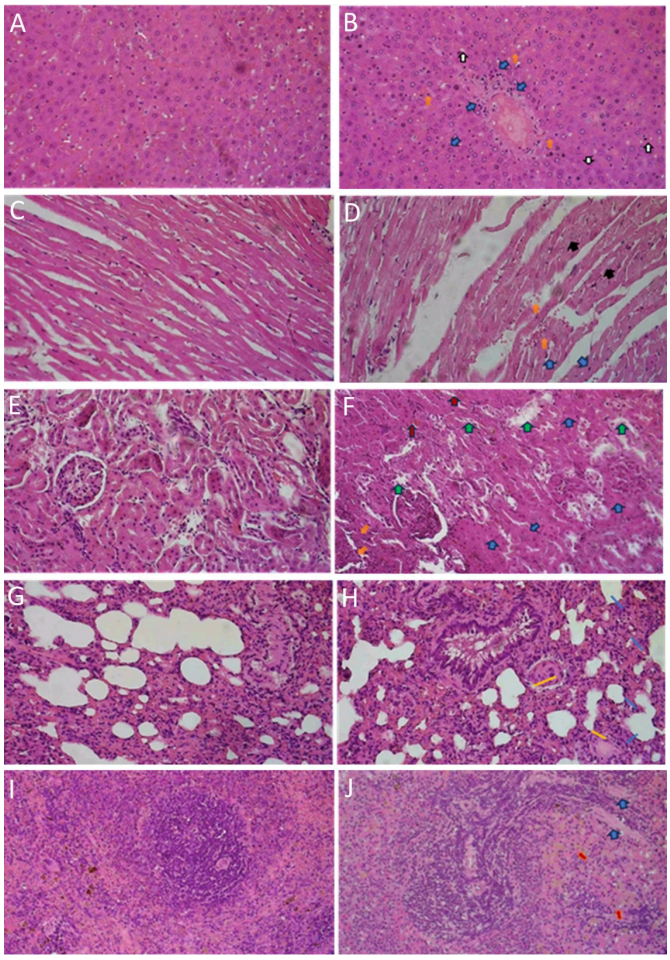


Figure 3. Representative histopathological images of various organs from Wistar rats following 90-day oral administration of SPP-ED (H&E staining). (A) Normal liver architecture in control rats; (B) Liver tissue from SPP-ED-treated rats showing hepatocyte degeneration (white arrows), inflammatory cell infiltration (blue arrows), and congestion (orange arrows) (magnification x400); (C) Normal myocardial structure in control rats; (D) Heart tissue from SPP-ED-treated rats showing necrosis (black arrows), inflammatory cell infiltration (blue arrows), and vascular congestion (orange arrows) (magnification x400); (E) Normal renal architecture in control rats; (F) Kidney tissue from SPP-ED-treated rats showing tubular necrosis (orange arrow), tubular dilation and atrophy (blue arrow), hemorrhage (red arrow), and tubular degeneration (green arrow) (magnification x400); (G) Normal lung architecture in control rats; (H) Lung tissue from SPP-ED-treated rats showing alveolitis (blue line) and pulmonary fibrosis (yellow line) (magnification x200); (I) Normal splenic architecture in control rats; (J) Spleen tissue from SPP-ED-treated rats showing fibrosis (blue arrows) and necrosis (red arrows) (magnification x400).

significantly increased at D2 and D3 ($p < 0.05$). No statistically significant differences were observed in LDL, HDL, triglycerides, GPT, GOT, total protein, or albumin.

In female rats, BUN was significantly increased at D2 and D3 ($p < 0.05$), accompanied by increased creatinine concentrations at D3 and D3S ($p < 0.05$). Glucose concentrations were significantly increased at D3S ($p < 0.05$). Alkaline phosphatase concentrations showed a significant decrease at D1 and a significant increase at D3S ($p < 0.05$). No statistically significant differences were observed in total cholesterol, LDL, HDL, triglycerides, GPT, GOT, total protein, albumin, or potassium concentrations.

Organ Index

Significant changes in organ indices were observed in a sex-specific manner. In male rats (**Table 4**), the lung index was increased at D3 compared to the C ($p < 0.001$). A statistical trend toward a lower spleen index was observed in D1 ($p < 0.1$). No statistically significant differences were observed in the liver, heart, kidney, adrenal gland, thymus, seminal vesicles, or testes index.

In female rats (**Table 5**), a statistical trend toward a lower liver index was observed at D2 ($p < 0.1$), while the heart index was significantly increased at D1 ($p < 0.05$). The spleen index showed a trend toward a decrease at D2 ($p < 0.1$). The uterus index was significantly increased in the D3S ($p < 0.05$). No statistically significant differences were observed in the lung, kidney, adrenal gland, or ovary indices.

Histopathological Results

Histopathological examination revealed microscopic alterations in the liver, heart, kidney, lung, and spleen (Tables 6-10). In the liver, changes were assessed using scores for inflammatory cell infiltration and congestion. In male rats, inflammatory cell infiltration increased in groups D1, D2, and D3 ($p < 0.001$) and D3S ($p < 0.05$). Meanwhile, in female rats, inflammatory cell infiltration increased in groups D2 and D3 ($p < 0.001$) and D3S ($p < 0.05$). Histological findings of the liver are shown in **Figure 3A** and **3B**, and the scores are presented in **Table 6**.

In the heart, several treatment groups exhibited significant changes (**Table 7**). In the hearts of male rats, inflammatory cell infiltration and congestion increased at D1 ($p < 0.05$), D2 ($p < 0.001$), and D3 ($p < 0.05$), whereas in female rats, congestion and edema increased at D2 and D3 ($p < 0.001$).

Renal histopathological changes were more pronounced in female rats. As shown in **Figure 3E**, **3F**, and **Table 8**, tubular degeneration increased significantly at D1 ($p < 0.05$), D2 ($p < 0.001$), D3 ($p < 0.001$), and D3S ($p < 0.001$). Hyperemia/hemorrhage also increased at D3 ($p < 0.1$) and D3S ($p < 0.05$). These findings were consistent with reduced urine volume, increased specific gravity, and elevated BUN and creatinine levels in female rats, suggesting structural and functional alterations in the kidneys. In male rats, degeneration increased at D3 ($p < 0.001$) and necrosis at D2 ($p < 0.001$), but the changes were less consistent. Overall, the findings indicate a more

Table 6. Semi-quantitative histopathological scores of liver tissue in male and female Wistar rats after 90-day repeated oral administration of SPP-ED and after a recovery period (mean ± SD, n=4).

Group	Average score							
	Male				Female			
	D	N	I	C	D	N	I	C
C	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.58
CS	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.58
D1	0.00 ± 0.00	0.00 ± 0.00	1.75 ± 0.50[c]	1.25 ± 0.96[b]	0.75 ± 0.96[b]	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.82
D2	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.50[c]	1.75 ± 0.50[c]	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00[c]	0.75 ± 0.50
D3	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.58[c]	1.75 ± 0.50[c]	0.00 ± 0.00	0.00 ± 0.00	2.50 ± 0.58[c]	0.50 ± 0.58
D3S	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00[b]	1.00 ± 0.82[b]	1.00 ± 0.00[c]	0.00 ± 0.00	1.75 ± 0.50[b]	0.50 ± 0.58

Note: D = degeneration; N = necrosis; I = inflammatory cell infiltration; C = congestion. Histopathological changes were scored semi-quantitatively on a scale of 0–3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe). C: control; D1–D3: SPP-ED doses of 45, 150, and 1500 mg/kg body weight/day, respectively; CS: satellite control; D3S: high-dose recovery group. [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control (one-way ANOVA followed by post hoc test).

Table 7. Semi-quantitative histopathological scores of heart tissue in male and female Wistar rats following 90-day oral exposure to SPP-ED and after the recovery period (mean ± SD, n=4).

Group	Average score							
	Male				Female			
	N	I	C	E	N	I	C	E
C	0.00 ± 0.00	0.25 ± 0.50	0.50 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.58	0.00 ± 0.00
D1	0.00 ± 0.00	1.00 ± 0.00[b]	1.50 ± 1.00[b]	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00[c]	1.75 ± 0.50[c]	0.00 ± 0.00
D2	0.25 ± 0.50	2.00 ± 0.00[c]	2.75 ± 0.50[c]	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.58[c]	1.25 ± 0.50[c]	0.00 ± 0.00
D3	0.00 ± 0.00	1.00 ± 0.00[b]	2.00 ± 0.82[b]	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.50[c]	2.75 ± 0.50[c]	0.00 ± 0.00
D3S	0.00 ± 0.00	0.33 ± 0.58	0.67 ± 0.58	0.00 ± 0.00	0.50 ± 0.58[b]	2.00 ± 0.00[c]	2.50 ± 0.58[c]	0.00 ± 0.00

Note: N = necrosis; I = inflammation; C = congestion; E = edema. Histopathological changes were scored semi-quantitatively on a scale of 0–3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe). [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

pronounced renal response in female rats, particularly at the highest dose.

Lung histopathological changes were more pronounced in male rats. As shown in **Figure 3G**, **3H**, and **Table 9**, alveolitis and pulmonary fibrosis scores were significantly increased at D1 and D2 ($p < 0.05$) and at D3 ($p < 0.001$). In the D3S recovery group, both alveolitis and fibrosis remained significantly increased ($p < 0.001$), indicating that the changes were not completely reversed after the recovery period. In female rats, alveolitis was

Table 8. Semi-quantitative histopathological scores of kidney tissue in male and female Wistar rats after 90-day oral administration of SPP-ED and after the recovery period (mean ± SD, n = 4).

Group	Average score							
	Male				Female			
	De	N	H	Di	De	N	H	Di
C	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.58	1.75 ± 0.50	0.50 ± 0.58	0.00 ± 0.00	0.75 ± 0.50	0.75 ± 0.50
CS	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.58	1.00 ± 0.82	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.58	1.00 ± 0.00
D1	0.00 ± 0.00	0.50 ± 0.58	1.00 ± 0.82	0.75 ± 0.96[a]	1.75 ± 0.96[b]	0.00 ± 0.00	1.25 ± 0.50	1.00 ± 0.00
D2	0.00 ± 0.00	1.50 ± 1.00[c]	1.25 ± 0.50	1.75 ± 0.50	2.00 ± 0.82[b]	0.25 ± 0.50	1.00 ± 0.82	1.25 ± 0.96
D3	1.00 ± 0.82[c]	0.00 ± 0.00	1.00 ± 0.82	1.75 ± 0.50	3.00 ± 0.00[c]	0.00 ± 0.00	1.50 ± 0.58[a]	1.50 ± 1.00
D3S	0.00 ± 0.00	0.25 ± 0.50	0.75 ± 0.50	2.00 ± 0.82	2.50 ± 0.58[c]	0.00 ± 0.00	2.25 ± 0.50[b]	0.75 ± 0.96

Note: De: degeneration; N: necrosis; H: hyperemia/hemorrhage; Di: tubular dilatation. Histopathological changes were scored semi-quantitatively on a scale of 0–3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe). [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

Table 9. Semi-quantitative alveolitis and pulmonary fibrosis scores in lung tissue of male and female Wistar rats following 90-day oral administration of SPP-ED and after the recovery period (mean ± SD, n = 4).

Group	Parameter	Average score	
		Male	Female
C	Alveolitis	1.75 ± 0.50	1.75 ± 0.96
	Fibrosis	0.25 ± 0.50	0.50 ± 0.58
CS	Alveolitis	1.75 ± 0.50	1.25 ± 0.50
	Fibrosis	1.00 ± 1.15	0.25 ± 0.50
D1	Alveolitis	2.75 ± 0.50[b]	3.00 ± 0.00[b]
	Fibrosis	1.75 ± 0.50[b]	1.00 ± 0.82
D2	Alveolitis	2.75 ± 0.50[b]	2.00 ± 0.00
	Fibrosis	1.75 ± 0.96[b]	1.25 ± 0.50
D3	Alveolitis	3.00 ± 0.00[c]	2.25 ± 0.50
	Fibrosis	2.25 ± 0.50[c]	0.75 ± 0.96
D3S	Alveolitis	3.00 ± 0.00[c]	3.00 ± 0.00[b]
	Fibrosis	2.75 ± 0.50[c]	1.00 ± 0.00

Note: Alveolitis and pulmonary fibrosis were scored based on the percentage of affected lung area: 0 = no lesion, 1 = lesion ≤ 25%, 2 = lesion = 26–50%, 3 = lesion = 51–75%, and 4 = lesion ≥ 76%. [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

Table 10. Semi-quantitative histopathological scores of splenic necrosis and fibrosis in male and female Wistar rats after 90-day oral administration of SPP-ED and following the recovery period (mean ± SD, n = 4).

Group	Parameter	Average score ± SD	
		Male	Female
C	Necrosis	0.00 ± 0.00	0.00 ± 0.00
	Fibrosis	0.00 ± 0.00	0.00 ± 0.00
CS	Necrosis	0.00 ± 0.00	0.00 ± 0.00
	Fibrosis	0.00 ± 0.00	0.25 ± 0.50
D1	Necrosis	0.75 ± 0.50[b]	0.00 ± 0.00
	Fibrosis	0.00 ± 0.00	0.50 ± 0.58
D2	Necrosis	0.75 ± 0.50[b]	0.00 ± 0.00
	Fibrosis	0.00 ± 0.00	0.50 ± 0.58
D3	Necrosis	0.50 ± 0.58	0.25 ± 0.50
	Fibrosis	0.25 ± 0.50	0.50 ± 0.58
D3S	Necrosis	0.25 ± 0.50	0.00 ± 0.00
	Fibrosis	0.00 ± 0.00	1.00 ± 0.00[b]

Note: Splenic lesions were graded on a scale of 0–4 (0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe). [a] $p < 0.1$; [b] $p < 0.05$; [c] $p < 0.001$ vs control.

significantly increased at D1 and D3S ($p < 0.05$), while no significant difference was observed in pulmonary fibrosis. Overall, these findings indicate more pronounced pulmonary histopathological changes in male rats following SPP-ED administration.

Splenic histopathological changes were relatively limited compared with those observed in the kidneys and lungs. As shown in **Figure 3I**, **3J**, and **Table 10**, splenic necrosis was significantly increased in male rats at D1 and D2 ($p < 0.05$), while no significant increase was observed at D3 or D3S. In female rats, fibrosis increased significantly only in the D3S recovery group ($p < 0.05$). Overall, the changes were not consistently dose-dependent, indicating that the spleen was less consistently affected by SPP-ED administration.

Discussion

Repeated administration of SPP-ED for up to 90 days did not cause mortality. However, repeated exposure induces sex-specific biological and histopathological changes. The kidneys in female rats and the lungs in male rats showed the most consistent changes.

General Toxicity Profile and Dose-Related Effect

The lack of mortality, severe clinical signs, or marked body weight loss suggested that SPP-ED did not induce overt systemic toxicity under the conditions of this study (19). However, alterations in behavioral parameters, feed and water intake, hematological parameters, biochemical markers, organ indices, and histopathological parameters showed that repeated exposure elicited biologically relevant responses (20). Changes in feed and water intake were recognized as sensitive indicators of physiological stress and early toxicity in subchronic studies, often preceding overt pathological changes (21, 22).

Renal Histopathological Findings in Female Rats

Consistent changes were observed in the kidneys of female rats. Abnormal urinalysis parameters, including changes in urine color, turbidity, volume, and specific gravity, along with significantly elevated blood urea nitrogen and creatinine levels, indicated impaired renal function (20, 23, 24). These functional disturbances were corroborated by histopathological evidence of tubular degeneration, hyperemia, and tubular dilatation (23, 25, 26).

Renal tubular degeneration was considered an adverse outcome because it indicated structural damage that impaired the kidney's ability to excrete waste and maintain homeostasis (23, 25). In addition, the persistence of renal lesions in recovery groups showed incomplete reversibility at higher doses, reinforcing the classification of this effect as adverse rather than adaptive (19). From a regulatory toxicology perspective, renal tubular degeneration observed in female rats may represent an appropriate endpoint for dose-response analysis and HBGV derivation (19, 27–29).

Pulmonary Histopathological Findings in Male Rats

Repeated oral exposure to SPP-ED in rats resulted in pulmonary changes. The high-dose group showed an increased lung organ index and more severe alveolitis and fibrosis. Although the control group showed mild alveolitis and fibrosis, SPP-ED exposure tended to exacerbate these effects. The combination of an increased lung organ index and histopathological alterations, such as alveolitis and fibrosis, suggests that the lungs are likely affected by repeated exposure to SPP-ED (30, 31).

Alveolitis and fibrosis persisted in the recovery group. This suggests that cessation of exposure did not fully resolve the lung tissue lesions. Persistent lesions may indicate incomplete reversibility. Therefore, these changes in the lungs of male rats can be considered an endpoint for determining HBGV (19).

NOAEL and LOAEL

The NOAEL and LOAEL values for SPP-ED could not be definitively established in this study. However, the D1 dose represents the lowest dose at which potentially adverse histopathological changes were observed. Tubular degeneration in female rats, as well as alveolitis and pulmonary fibrosis, were observed in this study.

The D1 dose, the lowest tested dose, was substantially higher than the estimated human exposure to bread preserved with SPP-ED at the effective concentration. The effective preservative dose was 750–1500 mg SPP-ED per 750 g of bread. Based on an estimated daily bread consumption of 50 g in adults (32), the corresponding SPP-ED exposure would be approximately 0.83–1.67 mg/kg bw/day for an adult weighing 60 kg. Therefore, further studies at lower dose levels are warranted to establish the NOAEL and LOAEL for SPP-ED more accurately.

Conclusion

Subchronic exposure to SPP-ED for 90 days did not cause mortality in rats; however, it induced sex-specific biological and histopathological changes. In female rats, the most consistent changes were observed in the kidneys, characterized by urinalysis abnormalities, elevated blood urea nitrogen and creatinine levels, and renal histopathological lesions. In male rats, more prominent changes were observed in the lungs. These changes included an increase in the lung organ index and more severe pulmonary lesions. Although the D1 dose (45 mg/kg bw/day) was the lowest dose at which potentially adverse histopathological changes were observed, a definitive NOAEL and LOAEL could not be established in the present study. Further studies at lower dose levels are therefore warranted to characterize the dose-response relationship better and establish the NOAEL and LOAEL of SPP-ED.

Abbreviations

ADI – Acceptable Daily Intake; ALT – Alanine Aminotransferase (Glutamic Pyruvic Transaminase, GPT); ANOVA – Analysis of Variance; AST – Aspartate Aminotransferase (Glutamic Oxaloacetic Transaminase, GOT); BUN – Blood Urea Nitrogen; C – Control; CS – Satellite Control; D1 – Low-Dose Group (45 mg/kg body weight/day); D2 – Medium-Dose Group (150 mg/kg body weight/day); D3 – High-Dose Group (1500 mg/kg body weight/day); D3S – High-Dose Satellite Group; GPT – Glutamic Pyruvic Transaminase; GOT – Glutamic Oxaloacetic Transaminase; GRA – Granulocytes; H – Hyperemia/Hemorrhage; H& E – Hematoxylin and Eosin;

HBGV – Health-Based Guidance Value; HCT – Hematocrit; HDL – High-Density Lipoprotein; HGB – Hemoglobin; I – Inflammatory Cell Infiltration; LDL – Low-Density Lipoprotein; LOAEL – Lowest-Observed-Adverse-Effect Level; LSD – Least Significant Difference; LYM – Lymphocytes; MCH – Mean Corpuscular Hemoglobin; MCHC – Mean Corpuscular Hemoglobin Concentration; MCV – Mean Corpuscular Volume; MON – Monocytes; MPV – Mean Platelet Volume; N – Necrosis; NOAEL – No-Observed-Adverse-Effect Level; OECD – Organisation for Economic Co-operation and Development; PLT – Platelets; RBC – Red Blood Cells; RDW – Red Cell Distribution Width; SD – Standard Deviation; SG – Specific Gravity; SPP-ED – Solid Preservative Preparation from Decolorized Ethanolic Extract of *L. aequata* Leaves; WBC – White Blood Cells.

Declaration

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No AI tools or AI-assisted technologies were used in the preparation of this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics Statement

All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Bandung Institute of Technology (Approval No. KEP/I/2023/VII/H230623PP/TSEG).

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References

1. Pinto L, Tapia-Rodríguez MR, Baruzzi F, Ayala-Zavala JF. Plant Antimicrobials for Food Quality and Safety: Recent Views and Future Challenges. Foods. 2023;12(12):2315. doi: https://doi.org/10.3390/foods12122315

2. Ahmadi N, Mosleh N, Yeganeh M, Ahmadi N, Malakouti S, Shahsavari S, *et al*. Procedures to evaluate potential of plants as natural food preservatives: Phytochemical characterization, novel extraction technology, and safety evaluation—A comprehensive review. Food Science & Nutrition. 2024;12(9):6142-6156. doi: https://doi.org/10.1002/fsn3.4303

3. Shi J, Xu J, Liu X, Goda AA, Salem SH, Deabes MM, *et al*. Evaluation of some artificial food preservatives and natural plant extracts as antimicrobial agents for safety. Discov Food. 2024;4(1):89. doi: https://doi.org/10.1007/s44187-024-00162-z

4. Erickson MC, Doyle MP. The Challenges of Eliminating or Substituting Antimicrobial Preservatives in Foods. Annu. Rev. Food Sci. Technol. 2017;8(1):371-390. doi: https://doi.org/10.1146/annurev-food-030216-025952

5. OECD. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Publishing; 2025. doi: https://doi.org/10.1787/9789264070707-en

6. Browne P, Paul Friedman K, Boekelheide K, Thomas RS. Adverse effects in traditional and alternative toxicity tests. Regulatory Toxicology and Pharmacology. 2024;148:105579. doi: https://doi.org/10.1016/j.yrtph.2024.105579

7. Bangun SR, Karo MB, Gulo BIC, Siburian AP, Manurung F, Sihotang LL. Perawatan Luka Penderita Kusta Dengan Daun Tetanus (*Leea aequata* L) Pengobatan Tradisonal Suku Karo. Creat J. Cumn Enga. 2021;4(5):1103-1108. doi: https://doi.org/10.33024/jkpm.v4i5.4212

8. Tun NL, Hu DB, Xia MY, Zhang DD, Yang J, Oo TN, *et al*. Chemical Constituents from Ethanoic Extracts of the Aerial Parts of *L. aequata* L., a Traditional Folk Medicine of Myanmar. Nat. Prod. Bioprospect. 2019;9(3):243-249. doi: https://doi.org/10.1007/s13659-019-0209-y

9. Purwaniati P, Kartasasmita RE, Iwo MI, Insanu M. Decolorized and Undecolorized Ethanol Extracts of Ginggiyang (Leea aequata L.) Leaves as Effective Preservatives of Edible Products. Food Technology and Biotechnology. 2025;63(4):493-501. doi: https://doi.org/10.17113/ftb.63.04.25.8838

10. Purwaniati P, Insanu M, Iwo MI, Kartasasmita RE. The Antimicrobial activity of Ginggiang (*L. aequata* L.) leaf extract as Preservative and Antiseptic. Research Journal of Pharmacy and Technology. 2025;18(3):1154-1164. doi: https://doi.org/10.52711/0974-360x.2025.00166

11. More S, Bampidis V, Benford D, Bragard C, Halldorsson T, Hougaard Bennekou S, *et al*. Statement on the derivation of Health-Based Guidance Values (HBGVs) for

regulated products that are also nutrients. EFSA Journal. 2021;19(3):e06479. doi: https://doi.org/10.2903/j.efsa.2021.6479

12. Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, et al. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal. 2017;15(10):e05022. doi: https://doi.org/10.2903/j.efsa.2017.5022

13. More S, Bampidis V, Benford D, Bragard C, Halldorsson T, Hernández-Jerez A, *et al*. Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health. EFSA Journal. 2021;19(8):e06768. doi: https://doi.org/10.2903/j.efsa.2021.6768

14. BPOM. Indonesia. Badan Pengawas Obat dan Makanan. Peraturan Badan Pengawas Obat dan Makanan Nomor 10 Tahun 2022 tentang Pedoman Uji Toksisitas Praklinik secara In Vivo. Berita Negara Republik Indonesia Tahun 2022 Nomor 490; 2022.

15. Casarrubea M, Di G, Aiello S, Crescimanno G. Physiology & Behavior The hole-board apparatus in the study of anxiety. Physiol Behav [Internet]. 2023;271(September):114346. doi: https://doi.org/10.1016/j.physbeh.2023.114346

16. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. European Journal of Pharmacology. 1998;350(1):21-29. doi: https://doi.org/10.1016/s0014-2999(98)00223-4

17. Dutta P, Nahrendorf M. Regulation and consequences of monocytosis. Immunological Reviews. 2014;262(1):167-178. doi: https://doi.org/10.1111/imr.12219

18. McClure DE. Clinical Pathology and Sample Collection in the Laboratory Rodent. Veterinary Clinics of North America: Exotic Animal Practice. 1999;2(3):565-590. doi: https://doi.org/10.1016/s1094-9194(17)30111-1

19. OECD. Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies. OECD; 2002. doi: https://doi.org/10.1787/9789264078482-en

20. Klaassen CD. Casarett and Doull's Toxicology. The Basic Science of Poisons. Postgraduate Medical Journal. 1981;57(666):271-271. doi: https://doi.org/10.1136/pgmj.57.666.271

21. Fuchs TC, Hewitt P. Biomarkers for Drug-Induced Renal Damage and Nephrotoxicity—An Overview for Applied Toxicology. Aaps J. 2011;13(4):615-631. doi: https://doi.org/10.1208/s12248-011-9301-x

22. Griffin BR, Faubel S, Edelstein CL. Biomarkers of Drug-Induced Kidney Toxicity. Therapeutic Drug Monitoring. 2019;41(2):213-226. doi: https://doi.org/10.1097/ftd.0000000000000589

23. Klaassen CD, editor. Casarett & Doull's Toxicology: The Basic Science of Poisons. 9th ed. New York: McGraw-Hill Education; 2019. 1620 p.

24. Greaves P. Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Studies. 4th ed. Amsterdam: Academic Press; 2012.

25. Al-Kuraishy H, Al-Naimi M, Rasheed H, Hussien N, Al-Gareeb A. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res. 2019;10(3):95. doi: https://doi.org/10.4103/japtr.japtr_336_18

26. Bosch E, Sommer I, Touw D. The influence of female sex and estrogens on drug pharmacokinetics: what is the evidence?. Expert Opinion on Drug Metabolism & Toxicology. 2025;21(6):637-647. doi: https://doi.org/10.1080/17425255.2025.2481891

27. Harvey BJ, Alvarez de la Rosa D. Sex Differences in Kidney Health and Disease. Nephron. 2025:149(2):77-103. doi: https://doi.org/10.1159/000541352

28. Thakur M, Wang B, Verma ML. Development and applications of nanobiosensors for sustainable agricultural and food industries: Recent developments, challenges, and perspectives. Environ Technol Innov [Internet]. 2022;26:102371. Available from: https://www.sciencedirect.com/science/article/pii/S2352186422000530

29. Renne RA, Dungworth DL, Keenan CM, Morgan KT, Hahn FF, Schwartz LW. Non-proliferative lesions of the respiratory tract in rats. Guides for Toxicologic Pathology. Washington, DC: STP/ARP/AFIP; 2003. Report No. R-1.

30. Malaviya R, Kipen HM, Businaro R, Laskin JD, Laskin DL. Pulmonary toxicants and fibrosis: innate and adaptive immune mechanisms. Toxicology and Applied Pharmacology. 2020;409:115272. doi: https://doi.org/10.1016/j.taap.2020.115272

31. Gondokesumo ME, Amir N. Peran Pengawasan Pemerintah dan Badan Pengawas Obat dan Makanan (BPOM) dalam Peredaran Obat Palsu di Negara Indonesia (Ditinjau dari Undang-Undang Nomor 36 Tahun 2009 dan Peraturan Kepala Badan Pengurus Obat dan Makanan). Perspektif Hukum. 2021;21(2):274-290. doi: https://doi.org/10.30649/ph.v21i2.16

Additional Information

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