

Toxicological Evaluation and Food Safety Assessment of Xylooligosaccharides Derived from Rice Straw Xylan Hydrolysis using Recombinant Endo- β -1,4-D-Xylanase: An Acute Oral Toxicity Study

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Abstract

Xylooligosaccharides (XOS) are recognized as prebiotics that stimulate the proliferation of probiotic bacteria and enhance short-chain fatty acid (SCFA) production. Due to their physicochemical stability, XOS have potential applications in functional foods; however, safety evaluation is essential, particularly when produced from lignocellulosic biomass without purification. This study evaluated the acute oral toxicity of a crude hydrolysate containing XOS obtained from rice straw xylan hydrolyzed using recombinant endo- β -1,4-D-xylanase expressed in *Saccharomyces cerevisiae*. The product was administered without further purification. Male Wistar rats received a single oral dose of 2000 mg/kg body weight and were observed for 14 days following OECD Guideline 420 (Fixed Dose Method). Parameters toxicity evaluated included body weight, clinical signs, hematological and biochemical indices, as well as macroscopic and histopathological alterations. Signs of toxicity were observed as changes in stool consistency within the first 4 to 24 hours. The estimated oral LD₅₀ was 2000 mg/kg body weight, with treatment-related mortality observed in 3 of 5 mice at this dose level. Based on these results, the test substance was classified into Category 4 according to the OECD Globally Harmonized System (GHS). Histopathological examination revealed mild to moderate degenerative changes in the liver and kidney tissues, while serum biochemical parameters remained within normal physiological ranges. These findings indicate moderate acute toxicity of the unpurified XOS-containing hydrolysate at high doses. Therefore, the observed effects cannot be attributed solely to XOS but may also arise from other hydrolysis by-products. Further purification and sub-chronic toxicity studies are required to confirm the intrinsic safety of XOS for food applications.

Keywords: Hematological, histological, recombinant xylanase, toxicity, wistar rats

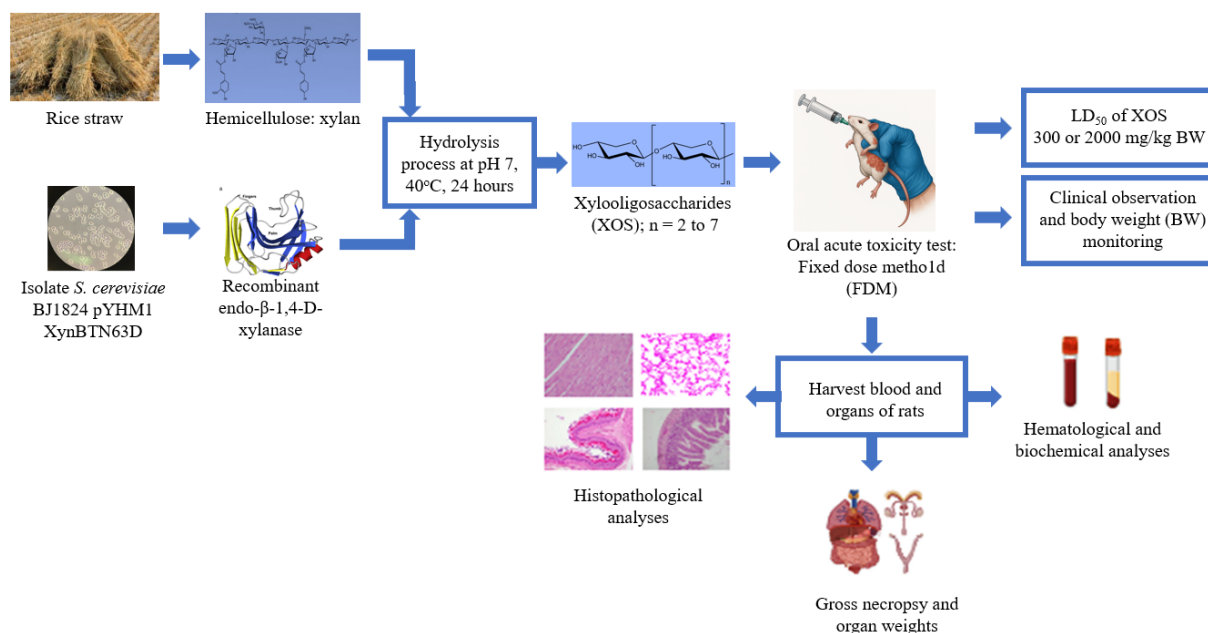
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Graphical Abstract



Introduction

In agricultural biotechnology, genetically engineered products have raised significant social impacts and ethical concerns. Public awareness regarding the potential effects of biotechnological products on human health has increased, highlighting the importance of consumer protection. Food safety analyses must be grounded in scientific evidence, and products should be rigorously evaluated for their suitability for human consumption. According to the guidelines of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), all biotechnology-derived foods must undergo assessments for allergenicity and toxicity. In Indonesia, Regulation Number 27 of 2018 issued by the Badan Pengawas Obat dan Makanan (BPOM) outlines the standards for the safety evaluation of food raw materials. The introduction of new technologies, including genetically engineered products, may influence the microbiological, toxicological, allergenic, and nutritional properties of raw materials. Consequently, comprehensive food safety assessments are essential to ensure that

such products meet regulatory standards and are safe for public consumption [1,2]

Xylooligosaccharides (XOS) are sugar oligomers composed of xylose units linked by β-(1→4) xylosidic bonds, with the molecular formula $C_{5n}H_{8n+2}O_{4n+1}$ and a degree of polymerization (DP) ≤ 20 [3,4,5]. As prebiotics, XOS are indigestible but exert beneficial effects on host health by stimulating the growth and activity of intestinal microbiota [6]. XOS naturally occur in fruits and vegetables; however, their amounts are generally insufficient to provide measurable health benefits. Therefore, to meet daily requirements, XOS must be consumed as a dietary supplement. In healthy adults, the minimum intake of XOS required to confer health and prebiotic benefits ranges from 1.4 to 5.0 g per day [7,8].

XOS, as prebiotics derived from enzymatic hydrolysis, have been successfully obtained from biomass residues or agro-industrial by-products [9,10]. The prebiotic potential of cassava pulp-derived XOS has been demonstrated both in vitro and in vivo, particularly through its ability to stimulate

the growth of probiotic bacteria and enhance short-chain fatty acid (SCFA) production[11]. Compared with other prebiotics, XOS exhibit distinct advantages, including greater stability at elevated temperatures and tolerance across a relatively broad pH range. The XOS obtained from biomass residues in this study are classified as genetically engineered products, as they are produced via hydrolysis using recombinant endo- β -1,4-D-xylanase [4].

The enzyme was originally identified from microorganisms inhabiting the termite gut, known for their efficient lignocellulose-degrading capability. The xylanase-encoding gene was subsequently isolated and introduced into a heterologous expression system to produce a recombinant enzyme in a new host, *Saccharomyces cerevisiae*[12]. The selection of *S. cerevisiae* as the expression host was based on its long history of safe use in food biotechnology and its classification as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA). This strategy enables the production of xylanase in a food-grade host system while maintaining the catalytic properties of the original termite-derived enzyme. Nevertheless, despite the GRAS status of the host organism, the safety of the final XOS product generated through recombinant enzymatic processing must be independently demonstrated through toxicological evaluation.

Several previous studies have reported the prebiotic properties and general safety of XOS derived from various biomass sources [13]. However, toxicological data remain limited for XOS specifically produced from rice straw xylan using recombinant xylanase systems. Differences in substrate origin and production methodology may influence the physicochemical characteristics and biological responses of the resulting

oligosaccharides. To the best of our knowledge, no study has systematically evaluated the acute oral toxicity of rice straw derived XOS produced via recombinant endo- β -1,4-D-xylanase in a mammalian model. This represents an important scientific and regulatory gap in the development of functional food ingredients derived from agricultural residues.

Therefore, the present study aimed to evaluate the acute oral toxicity of rice straw derived XOS by determining the LD₅₀ value and assessing hematological parameters, clinical biochemical profiles, macroscopic pathology, and histopathological alterations. The findings of this study are expected to provide novel toxicological evidence supporting the safe application of recombinant enzyme produced XOS as a functional food ingredient [14,15,16].

Materials and Methods

Materials

The materials used in this study included phosphate-citrate buffer (pH 7), chloroform for animal anesthesia, and 10% neutral buffered formalin (NBF). Xylooligosaccharides (XOS) were prepared through enzymatic hydrolysis of xylan derived from rice straw using recombinant endo- β -1,4-D-xylanase expressed in a *Saccharomyces cerevisiae* host. Albino Wistar rats of either sex, aged 6–8 weeks and weighing 200–250 g, were obtained from Wistar Farm, Malang, East Java, Indonesia. The animals were housed in standard cages and maintained under controlled laboratory conditions. Experimental protocols were conducted in accordance with BPOM (2022) guidelines. The experimental use of animals was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Jember (Approval No. 2882/UN25.8/KEPK/DL/2024). Prior to experimentation, the animals were

acclimatized for one week and provided with a standard pellet diet and water ad libitum.

Crude XOS productions

Xylan extracted from rice straw was dissolved in phosphate–citrate buffer (pH 7) at a concentration of 0.8% (w/v). The substrate was hydrolyzed with endo- β -1,4-D-xylanase at a ratio of 1:1 in a water bath at 40 °C for 24 h [11]. The hydrolysate containing xylooligosaccharides (crude XOS) was centrifuged at 8,000 rpm for 15 min, and the supernatant was concentrated by freeze-drying.

Acute toxicity

Healthy male Wistar rats were used in accordance with the Regulation of the Indonesian National Agency of Drug and Food Control (BPOM) No. 10 of 2022 concerning acute oral toxicity testing. All animals were fasted overnight with free access to water and weighed prior to XOS administration. The acute oral toxicity study was conducted using the fixed-dose procedure. A total of 11 experimental animals were used in this acute oral toxicity study. Two animals were assigned to the sighting study (one at 300 mg/kg body weight and one at 2000 mg/kg body weight), while the remaining nine animals were allocated to the main study. In the main study, the animals were randomly divided into two groups: a control group (n = 5), which received distilled water, and a treatment group (n = 4), which received the limit dose of XOS. Animals were observed for mortality, acute toxicity signs, and behavioral changes (e.g., aggression, abnormal vocalization, agitation, sedation, somnolence, convulsions, tremors, ataxia, catatonia, paralysis, fasciculation, prostration, unusual locomotion, and asphyxia) during the first 30 min and the first hour, followed by hourly observations for 5

h, and then periodically up to 48 h. Daily observations were conducted for 14 days to monitor general behavior, body weight changes, adverse symptoms, and mortality. According to the guideline, the LD₅₀ is greater than 5 g/kg if three or more rats survive. At the end of the experimental period, all animals were weighed, sacrificed by cervical dislocation, and subjected to necropsy for organ collection.

Hematological and biochemical analyzes. At the end of the experimental period, blood samples were collected from the surviving animals via the abdominal aorta under chloroform anesthesia. Hematology samples were placed into EDTA-treated tubes and analyzed using a CA-500 counter (Drew Scientific Hemavet 950, USA). Parameters measured included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell count (WBC). For clinical chemistry, blood samples were collected into anticoagulant-free tubes, allowed to clot at room temperature, and centrifuged at 3000 rpm for 15 min to separate the serum. Serum biochemical analyses included total cholesterol (T.Cho), serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT).

Macroscopic and Histological examination

Five major organs—kidneys, spleen, liver, lungs, and heart—were carefully excised using sterile surgical instruments. Each organ was subjected to macroscopic examination, including assessment of size, color, texture, and the presence of visible lesions or abnormalities. Following macroscopic evaluation, the organs were placed into containers filled with 10% neutral

buffered formalin (NBF) for fixation. Histological preparation and microscopic examination of the organs were conducted at the Faculty of Pharmacy, University of Jember.

Statistical analyses

All values are expressed as mean $\pm$ SD (n = 5). Statistical analyses were performed using Jamovi statistical software (version 2.3.28). One-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant[17].

3. Result and Discussion

3.1. LD₅₀ Value and Weight Change

The results of the sighting study showed that Wistar rats administered crude XOS at doses of 300 and 2000 mg/kg body weight (BW) exhibited no mortality or moribund conditions; however, mild toxic signs were observed. Within the first 4 hours following oral administration of crude XOS, rats in both dose groups displayed signs of weakness and changes in stool consistency. In the 300 mg/kg BW group, stool consistency returned to normal within 24 hours. Based on the findings of the sighting study, a dose of 2000 mg/kg BW was selected for the main study. The results of the main study demonstrated changes in stool consistency in all rats within the first 4 hours after administration, followed by recovery to normal conditions. During the 14-day observation period, three Wistar rats in the main study were found dead. Accordingly, the oral LD₅₀ value of crude XOS was estimated to be <2000 mg/kg BW. According to Regulation No. 10 of 2022 issued by the Badan Pengawas Obat dan Makanan (BPOM), substances with an LD₅₀ in the

range of >300–2000 mg/kg BW are classified as moderately toxic. It should be noted that administration of XOS at an extremely high dose may induce marked osmotic effects in the gastrointestinal tract. As a non-digestible oligosaccharide, unabsorbed XOS can substantially increase luminal osmotic pressure, leading to excessive water retention, accelerated intestinal transit, and severe osmotic diarrhea. At such high dose levels, these effects may cause significant gastrointestinal imbalance and dehydration, indicating that XOS cannot be considered safe when administered at excessively high doses. Rapid fermentation of large quantities of XOS by intestinal microbiota may further generate short-chain fatty acids and gases, potentially resulting in gastrointestinal stress and systemic physiological burden. This result contrasts with the findings reported by Boonchuay et al., (2021) who demonstrated that purified corn-cob-derived XOS exhibited an LD₅₀ value greater than 5,000 mg/kg body weight, suggesting a practically non-toxic classification[3]. The primary distinction between the two studies lies in the degree of product refinement. While the previous study evaluated XOS that had undergone purification processes to remove residual impurities, the present study utilized crude XOS without purification.

Wistar rats that survived the main test after 14 days showed no other toxic symptoms. Changes in fecal consistency ceased on the second day of observation. Body weight gain occurred in line with the age increase of the test animals. Data on changes in body weight indicated that crude XOS at a dose of 2000 mg/kg BW had a considerable effect on the growth of Wistar rats. The graph of body weight changes in Wistar rats in the main test can be seen in Figure 1.

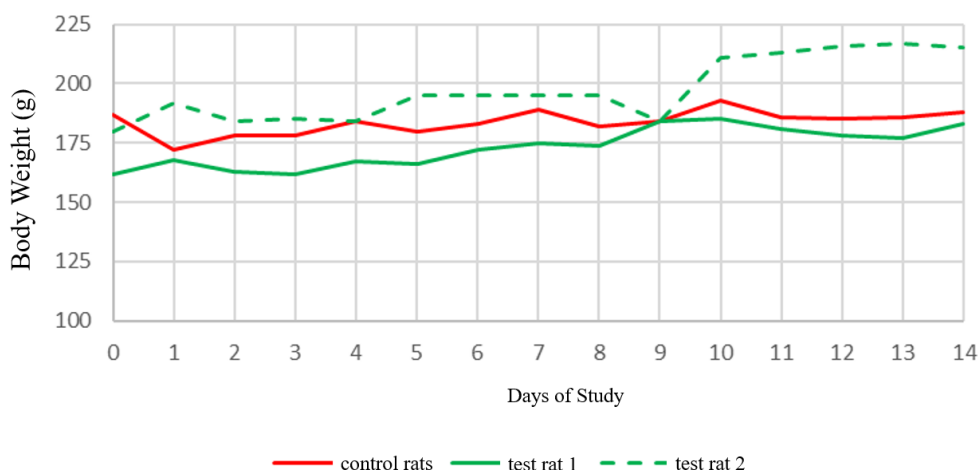


Figure 1. Body weight chart of male Wistar rats during the main test (administration of crude XOS at a dose of 2000 mg/kg BW resulted in an increase in the body weight gain of test rats)

Clinical biochemistry tests

Clinical biochemistry tests are a series of laboratory tests used to evaluate the chemical components in biological samples such as blood, urine, or serum. These tests provide information about organ function, enzyme levels, nutrient concentrations, electrolyte balance, and metabolic conditions in the body [18]. Blood sampling was carried out on the last day of the observation period. Blood samples obtained from the heart, or through cardiac puncture, are intended to collect a large quantity of high-quality blood at the final stage of the study [19]. Blood serum can provide information regarding organ function. The Clinical Biochemistry examination in this study aimed to evaluate the risk of cardiovascular disease in the liver using liver enzyme tests such as SGOT and SGPT, as well as total cholesterol measurement. The results of the clinical biochemistry examinations of Wistar rats administered the crude XOS are presented in Table 1.

The biochemical analysis showed that total cholesterol levels in both the control group (26.07 mg/dL) and the crude XOS-treated group (32.71 mg/dL) were below the normal

reference range (37.0–85.0 mg/dL). Although a slight increase was observed in the treated group compared to the control, the values remained below physiological norms and did not indicate treatment-related lipid disturbances. Alterations in serum lipid parameters are commonly used to assess systemic metabolic effects in toxicity studies. Serum SGOT levels in both groups were within the normal reference range, with comparable values between the control (137.29 U/L) and treated animals (138.79 U/L), suggesting no evidence of hepatocellular injury. In toxicological evaluations, increases in SGOT and SGPT are considered sensitive indicators of liver cell damage. Although SGPT levels in both groups were above the stated normal range, the treated group exhibited lower SGPT levels (67.43 U/L) compared to the control group (92.47 U/L). Since hepatotoxicity is typically characterized by a treatment-related elevation in transaminases relative to controls, the findings do not indicate hepatic injury attributable to crude XOS. Overall, these biochemical findings suggest that crude XOS administered at 2000 mg/kg body weight did not produce significant alterations in liver function markers.

According to internationally accepted acute toxicity testing guidelines, biochemical parameters should be interpreted in

conjunction with clinical signs and mortality data to determine treatment-related effects [20].

Table 1. Clinical biochemistry results of male Wistar rats

Parameters	Control (n=5)	Crude XOS 2000 mg/kgBW (n=2*)	Normal Range
Total cholesterol (mg/dL)	26.07 ± 0.05	32.71 ± 11.47	37.0 – 85.0
SGOT (U/L)	137.29 ± 0.21	138.79 ± 6.05	85.71 – 213.33
SGPT (U/L)	92.47 ± 0.33	67.43 ± 6.26	22.68 – 45.64

*Values represent surviving animals in the 2000 mg/kg BW group

Hematological testing

Hematological testing in toxicity studies is crucial because it provides detailed information about the toxic effects of a substance on the blood system and body metabolism. Hematological examination involves analyzing leukocytes, erythrocytes, hemoglobin, platelets, and hematocrit, which can reveal abnormalities or changes that occur due to exposure to toxic substances. The results of the hematological analysis are presented in Table 2. In the hematological parameters of male rats, the

leukocyte and MCV values in test rat 2 showed a slight increase, while the MCHC value showed a slight decrease, all within the BPOM reference range. Meanwhile, all other hematological parameters showed no significant differences between the control and test rats, and all remained within the BPOM reference range. The slight increases and decreases in hematological parameters of the male rats were still within the normal range, indicating that there were no significant disturbances in the blood cell formation system [21].

Table 2. Hematological parameters of male Wistar rats.

Parameters	Control (n=5)	Crude XOS 2000 mg/kgBW (n=2*)	Normal Range
WBC ($10^3/\text{mm}^3$)	13.58 ± 0.39	13.46 ± 5.41	3.0 – 17.0
HGB (g/dL)	14.50 ± 0.10	13.90 ± 0.14	11.0 – 19.0
RBC ($10^6/\text{mm}^3$)	7.23 ± 0.16	7.30 ± 0.35	5.0 – 10
PLT ($10^3/\text{mm}^3$)	781 ± 0.50	929.5 ± 19.09	200 - 1500
HCT (%)	42.53 ± 0.35	42.20 ± 0.00	35.0 – 57.0
MCH (pg)	19.10 ± 0.05	19.00 ± 1.13	18.0 – 23.0
MCHC (g/dL)	32.53 ± 0.35	32.85 ± 0.21	31.0 – 40.0
MCV (fl)	57.9 ± 0.25	57.90 ± 2.82	46.0 – 65.0

Note: WBC, white blood cell; HGB, hemoglobin; RBC, red blood cell; PLT, platelet count; HCT, red blood cell specific volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume, *Values represent surviving animals in the 2000 mg/kg BW group

Macropathological testing

Macropathological observation aims to identify physical and morphological changes in organs due to exposure to the test compound. This observation provides an initial overview of the toxic effects that may occur [22]. Macropathological examination of the organs in rats was conducted by performing macroscopic observations and calculating the relative organ weights. The organs observed included the liver, kidneys, heart, spleen, and lungs. Relative organ weight is the ratio between the organ weight and the total body weight of the test animal,

usually expressed as a percentage. This parameter is commonly used in toxicological studies to assess the effects of the test substance on organ function and condition, as well as physiological disorders induced by exposure to chemicals or drugs [23]. The results of relative organ weight observations in rats are presented in Table 3. The changes in relative organ weight were not significant, indicating that administration of the crude XOS at a dose of 2000 mg/kg did not cause any meaningful changes in the liver. The macroscopic observations of each organ in the control group and test rat are shown in Figure 2.

Table 3. Relative organ weight of male Wistar rats.

Test Animals			Liver (%)	Kidney (%)	Heart (%)	Spleen (%)	Lung (%)
Control (n=5)			3.53 ± 0.81	0.53 ± 0.06	0.53 ± 0.06	0.53 ± 0.06	0.56 ± 0.10
Crude XOS	2000		4.10 ± 0.14	0.57 ± 0.05	0.57 ± 0.05	0.57 ± 0.13	0.63 ± 0.04
mg/kgBW (n=2*)							

*Values represent surviving animals in the 2000 mg/kg BW group

Assessment of relative organ weights revealed no statistically significant differences between the control and crude XOS-treated groups (2000 mg/kg BW) ($p > 0.05$). The relative liver weight in the treated group ($4.10 \pm 0.14\%$) was slightly higher than that of the control group ($3.53 \pm 0.81\%$); however, the difference was not statistically significant. Since alterations in liver weight may reflect hepatocellular hypertrophy, congestion, or inflammatory changes, the absence of significant variation suggests that crude XOS did not induce hepatotoxic effects at the tested dose [24].

The relative kidney weights were $0.53 \pm 0.06\%$ in the control group and $0.57 \pm 0.05\%$ in the treated group, with no significant

difference observed ($p > 0.05$). Changes in kidney weight are commonly associated with nephrotoxicity, edema, or inflammatory processes. The lack of significant alteration indicates that crude XOS administration did not adversely affect renal integrity under acute exposure conditions [24].

Similarly, the relative heart weights in the control and treated groups were $0.53 \pm 0.06\%$ and $0.57 \pm 0.05\%$, respectively, showing no statistically significant difference ($p > 0.05$). Cardiac enlargement may indicate hypertrophy or toxic stress; therefore, the absence of significant changes suggests that crude XOS did not produce cardiotoxic effects [25].



Figure 2. Macroscopic examination of organs in control and test rats: (a) liver in control rat, (b) liver in test rat 1, (c) liver in test rat 2, (d) kidney in control rat, (e) kidney in test rat 1, (f) kidney in test rat 2, (g) heart in control rat, (h) heart in test rat 1, (i) heart in test rat 2, (j) spleen in control rat, (k) spleen in test rat 1, (l) spleen in test rat 2, (m) lung in control rat, (n) lung in test rat 1, (o) lung in test rat 2.

For the spleen and lungs, the relative organ weights were $0.53 \pm 0.06\%$ and $0.56 \pm 0.10\%$ in the control group, and $0.57 \pm 0.13\%$ and $0.63 \pm 0.04\%$ in the treated group,

respectively. Statistical analysis demonstrated no significant differences between groups ($p > 0.05$), indicating that crude XOS did not induce treatment-related

effects on immune or respiratory organs. Macroscopic examination corroborated these findings. All examined organs exhibited normal morphology, characterized by smooth surfaces, normal coloration, and absence of visible lesions, hemorrhage, congestion, or enlargement. No macroscopic damage was observed in the treated animals compared with the control group[26].

Histopathology

Histopathology is the analysis of organ or tissue conditions through microscopic observation of morphological and structural changes, as well as indications of damage, infection, or other mutagenic processes

caused by disease or toxic agents [27]. Based on the histopathological evaluation of the kidneys (Figure 3), differences were observed in test rat 1 (male) and test rat 2 (male) administered XOS at a dose of 2000 mg/kg BW compared with the control group. The kidneys of test rat 1 (male) and test rat 2 (male) were structurally preserved; however, mild abnormalities were observed in several glomeruli, characterized by glomerular swelling (black arrows). Normal glomeruli (green arrows) showed uniform size and a clearly defined Bowman's space. The swelling was limited to a small number of glomeruli, indicating a localized alteration that is unlikely to cause significant impairment of overall renal function.

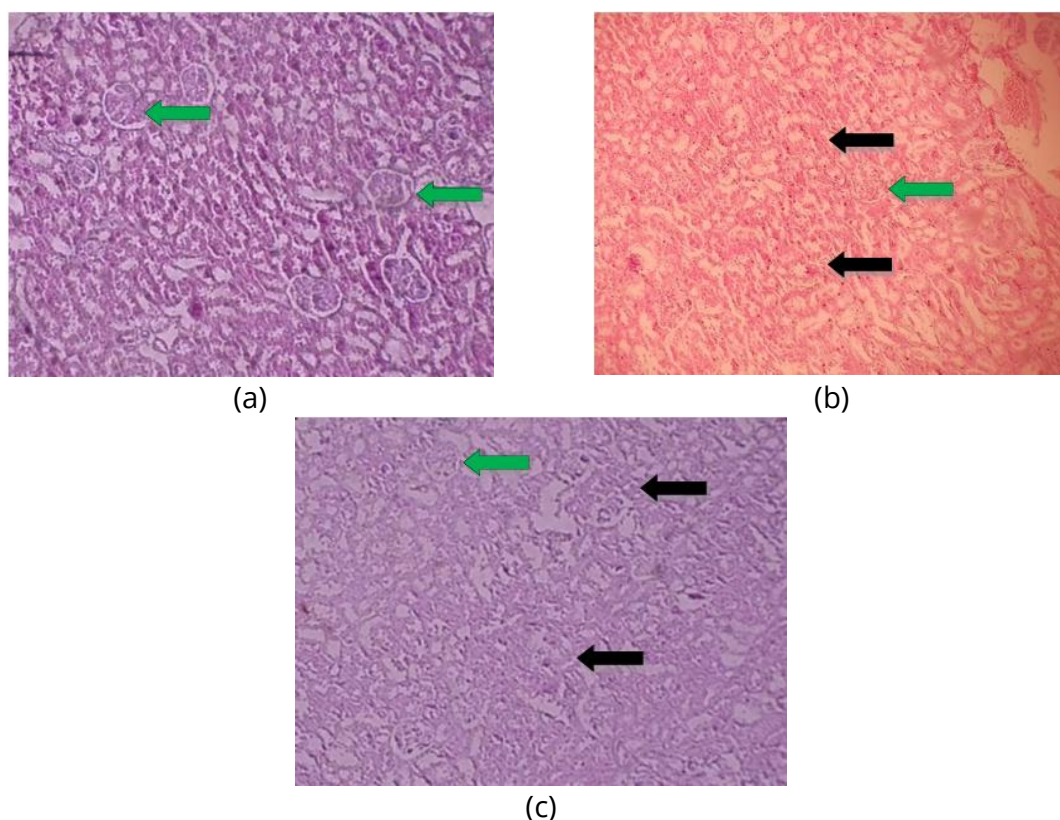


Figure 3. Histopathological observation of the kidney: (a) kidney in normal control; (b) kidney in test rat 1; (c) kidney in test rat 2. (Green arrows indicate normal glomeruli, black arrows indicate swollen glomeruli).

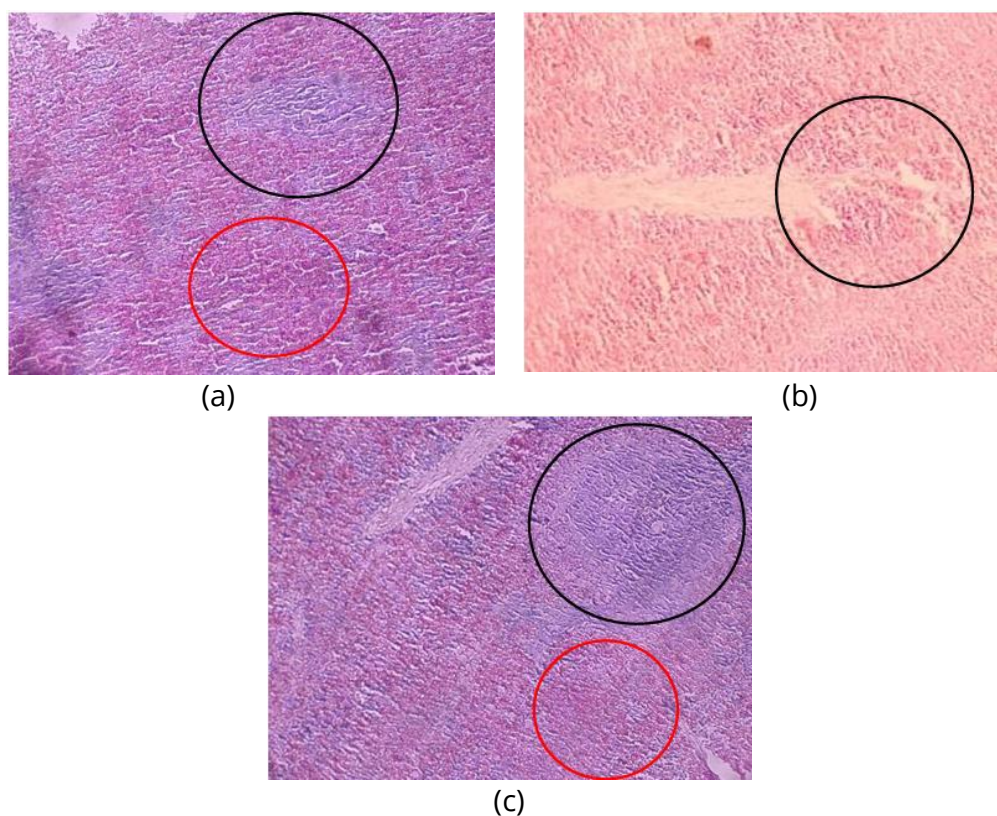


Figure 4. Histopathological observation of the spleen: (a) spleen in normal control; (b) spleen in test rat 1; (c) spleen in test rat 2. (Red circles indicate red pulp, black circles indicate white pulp).

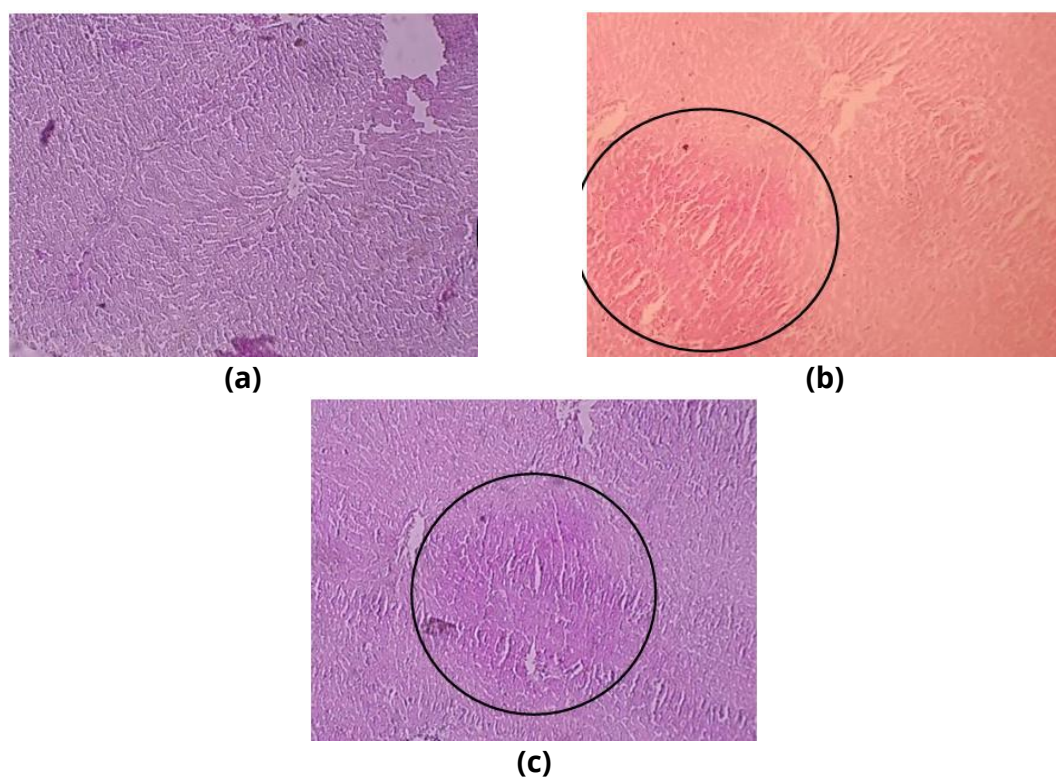


Figure 5. Histopathological observation of the liver: (a) liver in normal control; (b) liver in test rat 1; (c) liver in test rat 2. (Black circles indicate cytoplasmic condensation).

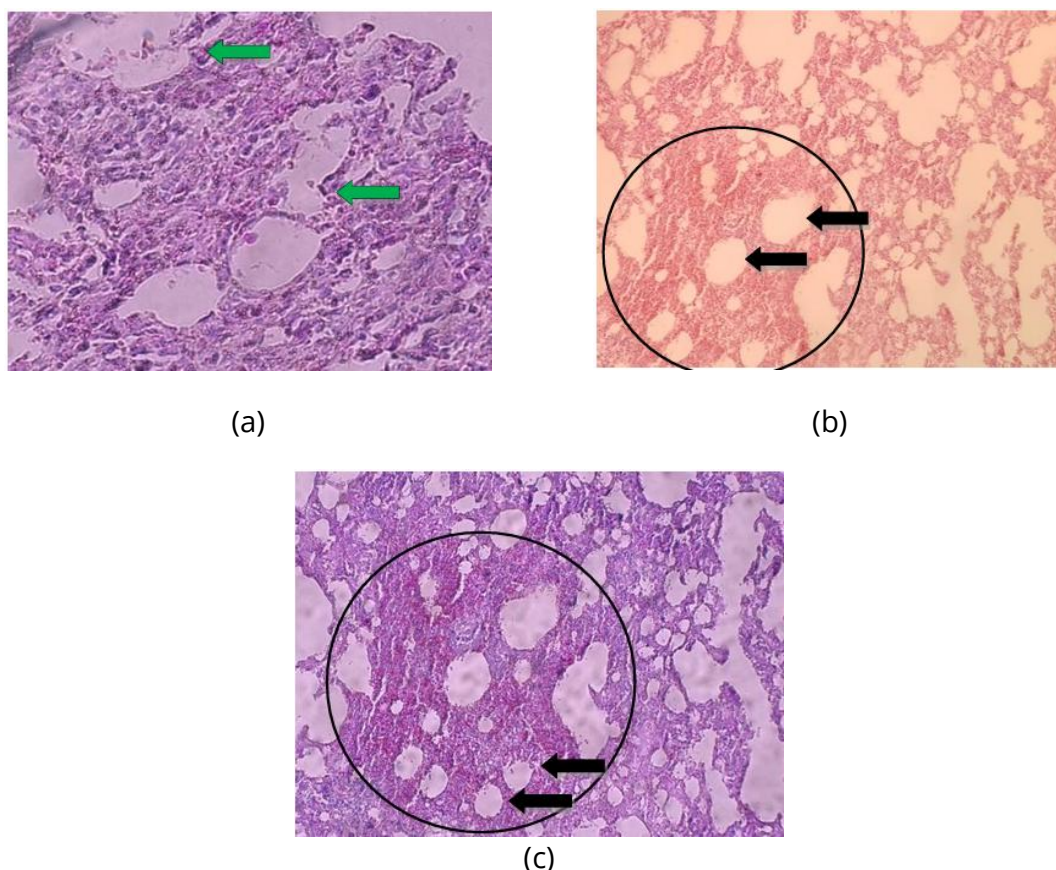


Figure 6. Histopathological observation of the lungs: (a) lung in normal control; (b) lung in test rat 1; (c) lung in test rat 2. (Green arrows indicate normal alveoli, black arrows indicate dilated alveoli, black circles indicate hemorrhage).

Histopathological examination of the spleen (Figure 4) revealed no significant differences between the control group and test rats 1 (male) and test rat 2 (male), which received XOS at a dose of 2000 mg/kg BW. The spleen tissue of both control and treated rats demonstrated typical architecture composed of red pulp and white pulp. The white pulp consisted of lymphocytes with a purplish hue, indicated by black circles, whereas the red pulp was composed of erythroblasts, indicated by red circles. In test rat 1 (male), the lymphoid tissue remained well preserved; however, some sections appeared less distinct due to artifacts caused by tissue slicing. Overall, no structural damage was detected in the spleens of treated rats, and their cellular morphology was comparable to that of the control group.

In contrast, histopathological examination of the liver (Figure 5) demonstrated mild alterations. Control rats demonstrated normal hepatic architecture. In contrast, liver sections from test rat 1 (male) and test rat 2 (male) revealed focal areas of increased staining intensity (black circles), corresponding to cytoplasmic condensation in hepatocytes. These alterations were localized predominantly in zone 1 (periportal region) of the hepatic lobule. Cytoplasmic condensation in the absence of necrosis or extensive cellular damage is generally considered indicative of mild, early-stage hepatocellular degeneration, representing the earliest detectable pathological change in hepatocytes [28].

Histopathological examination of the lungs (Figure 6) in test rat 1 (male) and test rat 2 (male) revealed focal inflammatory changes,

indicating structural alterations in the pulmonary tissue. The affected sections showed distended alveoli with widened interalveolar spaces (black arrows), whereas normal alveoli (green arrows) exhibited uniform architecture without apparent enlargement. The widening of alveolar spaces was associated with inflammatory

cell infiltration. In addition, hemorrhagic foci (black circles) were observed, characterized by the presence of erythrocytes within the interalveolar spaces. The hemorrhage likely resulted from vascular dilation and increased vascular permeability, allowing blood cells to extravasate into the surrounding alveolar tissue [29].

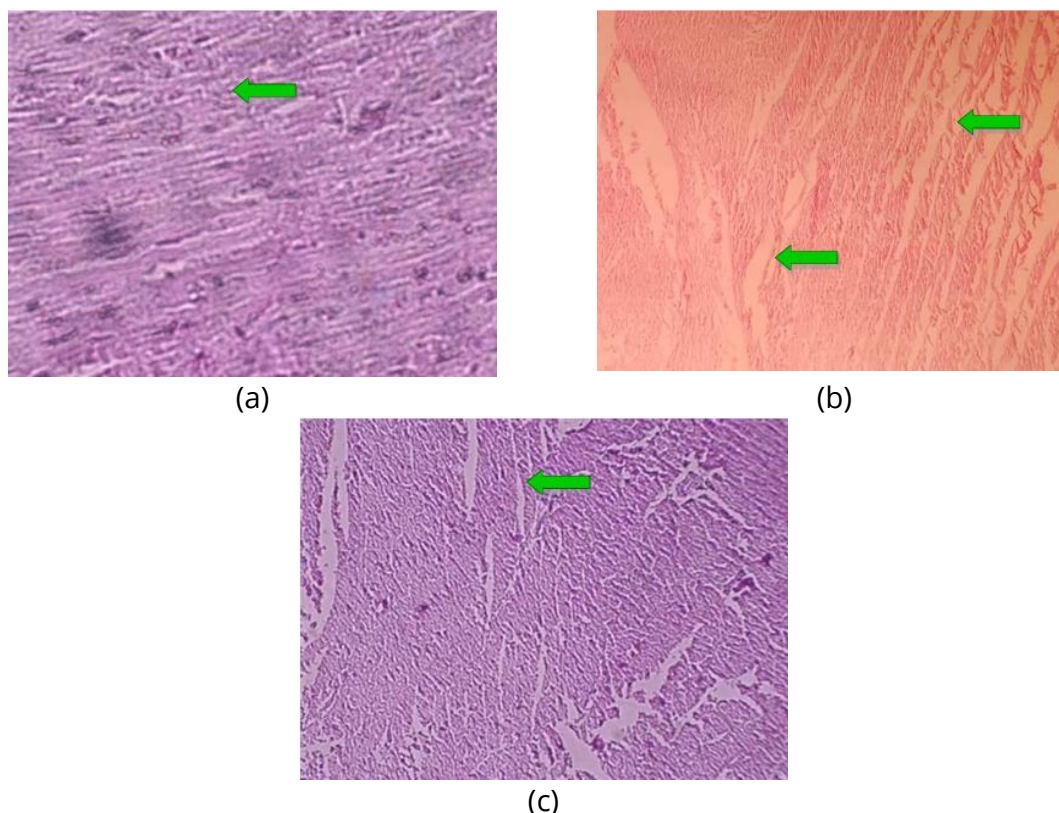


Figure 7. Histopathological observation of the heart: (a) heart in normal control; (b) heart in test rat 1; (c) heart in test rat 2. (Green arrows indicate normal heart wall).

Considering that the route of administration was oral, the pulmonary lesions observed are unlikely to be attributable to inhalation of XOS. The findings may instead reflect secondary systemic effects, aspiration-related changes during oral gavage, or nonspecific inflammatory responses.

Based on histopathological observation of the heart, no differences were observed between the control rats and test rat 1 (male) and test rat 2 (male) administered XOS at a dose of 2000 mg/kg BW. Histopathological evaluation of the heart (Figure 7) in control

rats and test rats 1 and 2 (male) showed well-preserved heart walls, with no evidence of damage or abnormalities.

Conclusion

The acute oral toxicity study demonstrated that crude XOS administered at a dose of 2000 mg/kg body weight resulted in mortality in 3 out of 5 rats during the 14-day observation period. Based on these findings, the oral LD₅₀ of crude XOS was estimated to be <2000 mg/kg body weight. According to the classification criteria of the United

Nations Globally Harmonized System (GHS), the substance falls under Category 4 for acute oral toxicity. Biochemical evaluation revealed no marked elevation of hepatic transaminases (SGOT and SGPT) relative to controls, suggesting the absence of significant hepatocellular injury. Histopathological examination showed mild and localized degenerative changes in the liver, focal glomerular swelling in the kidneys, and inflammatory and hemorrhagic foci in the lungs, without evidence of extensive necrosis or severe structural damage. These findings suggest that although mortality occurred at the tested dose, overt target-organ toxicity was not strongly demonstrated. Given that the test material was crude XOS, the observed toxicity may be associated not only with the oligosaccharides themselves but also with residual impurities, by-products, or endotoxin contamination remaining from the production process. Therefore, further purification and compositional characterization of XOS are recommended to reduce potential impurities and better evaluate its intrinsic safety profile. Additional studies using purified XOS preparations are necessary to clarify the relationship between purity level and acute toxicity outcomes.

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Author Contributions

Conceptualization, AAI Ratnadewi and Evi Umayah; Methodology, AAI Ratnadewi, Mei Syafriadi; Validation, Cahya, Reisma and Putra; Formal Analysis, Tri Agus Siswoyo and Putra; Investigation, AAI Ratnadewi; Data Curation, Cahya, Reisma and Putra; Writing –

Original Draft Preparation, Cahya, Reisma and AAI Ratnadewi; Writing – Review & Editing, Evi Umayah.

Conflict of Interest

The authors declare no conflict of interest

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