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Acute Oral Toxicity Assessment of Rutinoside on Renal Histopathology in Wistar Rats

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Abstract: This study aimed to evaluate the acute oral toxicity of rutinoside by assessing renal histopathological changes in Wistar rats. Twelve Wistar rats (*Rattus norvegicus*), consisting of equal numbers of males and females and aged approximately three months, were randomly assigned to control and treatment groups. After an acclimatization period under standard laboratory conditions, the treatment group received a single oral dose of rutinoside (5000 mg/kg body weight) via gastric gavage in accordance with OECD Guideline 423, while the control group received the vehicle only. Animals were observed daily for 14 days for mortality, behavioral changes, and clinical signs of toxicity. At the end of the observation period, rats were euthanized, and both kidneys were collected for histopathological evaluation. Kidney tissues were fixed in 10% buffered formalin, processed, and stained with hematoxylin and eosin. Histological examination was performed at 400× magnification using a standardized scoring system, and statistical analysis was conducted using the Mann–Whitney U test. No mortality or treatment-related clinical signs were observed during the study period. Histopathological findings demonstrated no significant differences between the control and rutinoside-treated groups. Renal structures, including glomeruli and tubules, remained intact, with no evidence of degeneration, inflammation, or other pathological alterations. In conclusion, acute oral administration of rutinoside at a high dose did not induce renal toxicity in Wistar rats, suggesting a favorable acute safety profile. Further studies are required to evaluate the safety of rutinoside following repeated or long-term exposure.

Keywords: Acute Oral Toxicity, Renal Histopathology, Rutinoside

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1. Introduction

Flavonoids are a broad class of naturally occurring polyphenolic compounds widely distributed in fruits, vegetables, and medicinal plants, and they have attracted considerable scientific interest due to their diverse biological activities. Rutinoside, also known as rutin, is a flavonoid glycoside that has been extensively investigated for its anti-inflammatory, antioxidant, vasoprotective, and cytoprotective properties. Among these properties, its antioxidant activity is considered particularly important, as it plays a role in scavenging reactive oxygen species (ROS) and protecting cells from oxidative damage [4] [7] [14] [22].

Despite their protective roles, antioxidants may exert pro-oxidant effects under certain conditions, such as at high concentrations or in the presence of metal ions, potentially leading to oxidative stress and cellular injury. This dual behavior raises concerns regarding the safety of potent antioxidant compounds when administered at high doses. As the use of flavonoid-based supplements and herbal products continues to

increase, understanding the balance between their beneficial and potentially harmful effects has become increasingly important [5] [6] [10] [17].

Natural products are often perceived as inherently safe; however, several studies have demonstrated that bioactive plant-derived compounds may induce toxic effects depending on dose, duration of exposure, and target organ susceptibility. The kidneys are particularly vulnerable to toxic insults due to their high blood flow, filtration capacity, and role in the excretion of xenobiotics and their metabolites. Consequently, renal tissue frequently serves as a primary target in toxicity studies, making histopathological evaluation of the kidneys essential for safety [12] [15] [20] [21].

Although numerous studies have highlighted the protective and therapeutic effects of rutinoside, investigations focusing on its acute toxicity remain limited, especially with regard to renal histopathological changes following high-dose exposure. Previous research has predominantly emphasized its nephroprotective and antioxidant mechanisms, leaving a gap in knowledge concerning potential adverse renal effects after acute oral administration at limit doses. Addressing this gap is crucial to establish a more comprehensive safety profile for rutinoside.

Therefore, the present study aimed to evaluate the acute oral toxicity of rutinoside in Wistar rats at a limit dose of 5000 mg/kg body weight, with a specific focus on histopathological changes in the kidneys, in accordance with OECD Guideline 423. The findings of this study are expected to provide important toxicological evidence regarding the renal safety of rutinoside and serve as a scientific basis for future sub-chronic and chronic toxicity studies, as well as for its safe application as a therapeutic agent or dietary supplement.

2. Materials and Methods

Twelve Wistar rats (Rattus norvegicus), consisting of an equal number of male and female rats, each approximately three months old, were used for this study. There were three males and three females in each group. The rats were provided unrestricted access to water and food for seven days prior to the experiment to acclimate them to the laboratory environment. The treatment group received a single oral dose of rutinoside at 5000 mg/kg body weight, dissolved in 5% dimethyl sulfoxide (DMSO), administered via gastric gavage, while the control group received only the solvent (5% DMSO) and distilled water. For 14 consecutive days, the animals were observed daily for behavioral changes and clinical signs of toxicity.

At the end of the observation period, rats were euthanized using a combination of ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight). Both kidneys were then removed. After rinsing the kidneys with regular saline to eliminate any residual blood, they were preserved in 10% buffered formalin. Kidney tissues were processed into histological preparations and stained with hematoxylin-eosin (H&E). Histopathological evaluation was carried out in a double-blind manner using a scoring system that assessed parameters such as tubular lumen dilation, accumulation of cellular debris, vacuolization, Bowman's space widening, degeneration, hyperplasia, karyomegaly, and the presence of inclusion bodies [24].

For each slide, five fields of view were examined under a light microscope at 400× magnification. The ordinal histopathological scores were converted into interval data using the method of successive intervals (MSI). The data were then tested for normality using the Shapiro-Wilk test and for homogeneity using Levene’s test. Comparative analysis between groups was performed using the Mann-Whitney U test, while the reliability of the scoring system was assessed with Cronbach’s alpha. The research received ethical clearance from the Ethics Committee of the Faculty of Dentistry at the University of Jember (No. 2119/UN25.8/KEPK/DL/2023).

3. Results and Discussion

3.1. Results

None of the rats showed any symptoms of toxicity during the 14-day observation period that followed oral dosing. No physical changes, such as changes to the fur, eyes, or mucous membranes, were observed, and behavioral habits stayed the same. Neither the control group nor the treatment group experienced any long-term reductions in body weight, respiratory problems, tremors, seizures, or mortality. According to OECD Guideline 423, the estimated oral LD_{50} of rutinoside was found to be larger than 5000 mg/kg body weight, placing it under GHS category 5 (unclassified), as there were no clinical symptoms of toxicity or fatality at a single dose of 5000 mg/kg body weight.

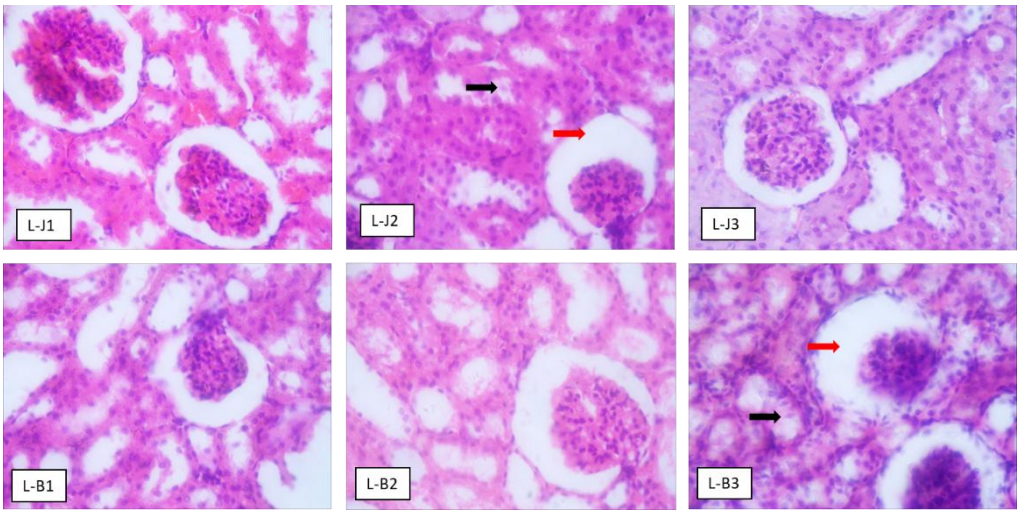

Figure 1. Histopathological features of Wistar rat kidneys at 400× magnification with H&E staining in the treatment group (J: male, B: female).

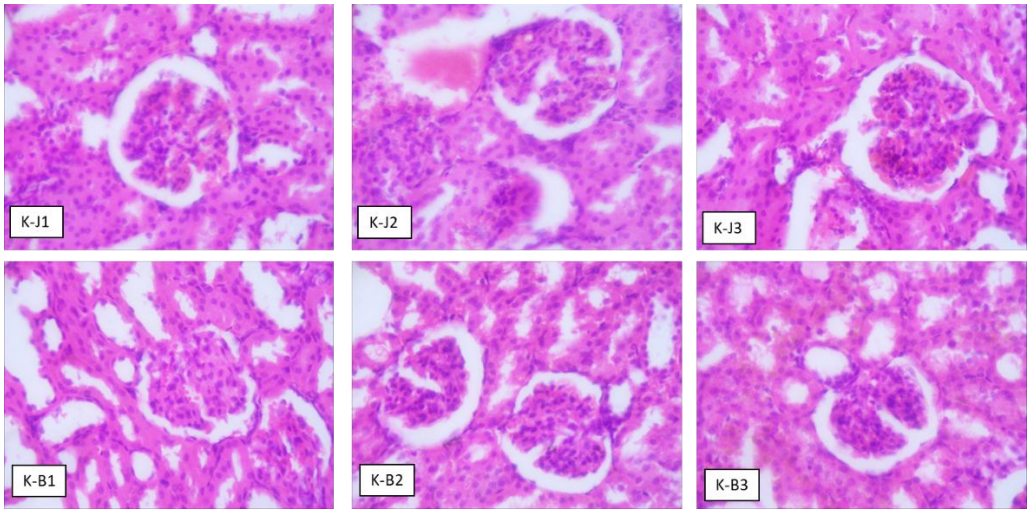

Figure 2. Histopathological features of Wistar rat kidneys at 400× magnification with H&E staining in the control group (J: male, B: female).

Histopathological examination of kidney tissues stained with hematoxylin-eosin revealed no observable structural abnormalities in either the control or treatment groups at 400× magnification (Figures 1 and 2). All kidney lesions were scored using the Anggraini scoring system. Data analysis revealed that the ordinal histopathological scores were not normally distributed (Shapiro-Wilk test, $p = 0$), but they were homogeneous (Levene's test, $p = 0.234$). A comparative analysis using the Mann-Whitney U test revealed no significant difference between the control and treatment groups ($p = 0.523$). Reliability

analysis using Cronbach's alpha yielded a value of 0.897, indicating excellent internal consistency of the histopathological scoring method. This suggests that the scoring was highly reliable and consistent across different observations.

These results indicate that acute oral administration of rutinoside at the limit dose did not induce significant histopathological alterations in the kidneys of Wistar rats, and the histopathological assessment method used was robust and reproducible.

3.2. Discussion

The present study demonstrated that oral administration of rutinoside at doses of 2000 mg/kg and 5000 mg/kg body weight did not produce observable toxicity or mortality in normal Wistar rats. The Mann-Whitney comparison yielded a *p-value* of 0.523, indicating no significant differences between the control and treatment groups in terms of renal histopathological changes. These findings suggest that a single high dose of rutinoside does not induce significant microscopic alterations in the kidneys, and that its antioxidant properties are not converted into pro-oxidant effects under the conditions tested. The absence of mortality further supports that the 5000 mg/kg dose is not toxic, corresponding to an oral LD_{50} > 5000 mg/kg body weight, which falls into the GHS category 5 (unclassified) according to OECD Guideline 423 [8] [9] [18].

The lack of significant renal changes may be attributed to the diverse biological activities of rutinoside, including anti-inflammatory, antimicrobial, antitumor, and anti-asthmatic effects, as well as its potent free radical scavenging activity. Rutinoside has been shown to exert potent antioxidant effects in vitro, with efficacy dependent on concentration, including hydroxyl and superoxide radical-scavenging activities. Its chemical structure allows direct interaction with reactive oxygen species (ROS), while also enhancing cellular antioxidant defenses by increasing glutathione (GSH) levels and the activity of enzymes such as superoxide dismutase (SOD) and catalase (CAT). Additionally, rutinoside can inhibit xanthine oxidase, thereby reducing ROS production [1] [3] [15] [23].

The observed protective effects align with previous studies demonstrating that rutinoside or rutin mitigates oxidative stress and inflammation in various models of renal injury. For instance, rutin reduced inflammasome activation and lipid accumulation in fructose-fed hyperuricemic rats, and pretreatment with rutin protected against cisplatin-induced nephrotoxicity by restoring renal function and oxidative stress biomarkers. These findings support the notion that the antioxidant and anti-inflammatory mechanisms of rutinoside are likely responsible for the absence of significant renal damage in this study [2] [11] [13] [14] [25].

Limitations of this study include the use of only hematoxylin-eosin staining for histopathological evaluation, which has lower sensitivity compared to immunohistochemistry, and the focus on acute toxicity without assessment of sub-chronic or chronic effects. Nevertheless, the results provide strong preliminary evidence that high-dose oral administration of rutinoside is safe and does not adversely affect renal structure in Wistar rats.

4. Conclusions

Based on this study, oral administration of rutinoside at a dose of 5000 mg/kg body weight in Wistar rats did not cause mortality, behavioral changes, or physical signs of toxicity within 14 days of observation. Additionally, histopathological analysis of the kidneys revealed slight and non-specific changes, with no discernible differences between the treatment and control groups. These findings suggest that rutinoside has a high safety margin and can be categorized as practically non-toxic under OECD 423 guidelines.

Author Contributions: R.N. oversaw the conceptualization, resource allocation, supervision, project administration, and funding acquisition. The methodology was developed collaboratively by R.N. and Y.M.P. Y.M.P. undertook the formal analysis, investigation, data curation, and drafting

of the original manuscript. R.N. participated in the review and editing of the manuscript. All authors have read and approved the final version for publication.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Faculty of Dentistry, University of Jember (Protocol No. 2119/UN25.8/KEPK/DL/2023). The research received ethical clearance in accordance with institutional guidelines to ensure proper animal welfare and ethical conduct of the study.

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Conflicts of Interest: The authors declare no conflict of interest.

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