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Evaluation of Acute Oral Toxicity of Flavonoid Rutinoside on Liver Histopathology in Wistar Rats

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Abstract

This study aimed to evaluate the acute oral toxicity of the flavonoid rutinoside on liver histopathology in Wistar rats, following OECD Guideline No. 423. Twelve Wistar rats were divided into four groups: male control and female control receiving oral administration of 5% dimethyl sulfoxide (DMSO), and treatment groups of male and female rats receiving rutinoside at a dose of 5000 mg/kg body weight (BW) in 5% DMSO orally. Post-treatment, liver histopathological examinations were performed to assess potential toxicological effects. The results indicated no signs of acute toxicity, as no significant histopathological alterations were observed in the liver tissues of the treated groups compared to the control groups. This study suggests that oral administration of rutinoside at 5000 mg/kg BW does not induce acute liver toxicity in Wistar rats under experimental settings.

Keywords: acute oral toxicity, liver, rutinoside

Introduction

Rutinoside is a flavonoid glycoside primarily derived from the disaccharide rutinose bound to quercetin, and it is found in relatively small amounts in citrus fruits and various medicinal herbs such as *Sophora japonica*, *Ruta graveolens*, and *Capparis spinosa* (Ganeshpurkar & Saluja, 2017; Tungmunnithum et al., 2018). Although it is not abundantly present in commonly consumed foods, rutinoside has garnered attention due to its pharmacological potential, particularly for its antioxidant, anti-inflammatory, and vasoprotective effects. These properties have prompted its extraction, isolation, and incorporation into pharmaceutical and nutraceutical formulations in more concentrated forms, raising concerns about its safety profile with increased exposure (Dias et al., 2021).

Several studies have demonstrated the promising effects of rutinoside in various disease models, including osteoarthritis, through mechanisms involving the modulation of oxidative stress and inflammatory mediators. However, most research has focused on its efficacy rather than its toxicological impact. As natural compounds like rutinoside are increasingly formulated into dietary supplements or adjuvant therapies, their bioactive concentrations often exceed normal dietary intake levels. This shift necessitates a thorough evaluation of their safety, especially at higher doses that might be used therapeutically. The typical therapeutic dose of rutin or its glycosides, including rutinoside, in experimental studies ranges between 50–200 mg/kg body weight, far above what would be consumed in a standard diet, suggesting the importance of assessing potential toxicity under such conditions (Erhirhie et al., 2018; Hasnat et al., 2024; Krishnamurthy et al., 2017).

Toxicological evaluations are a critical component of early-phase pharmacological development, helping to identify potential adverse effects of bioactive compounds. The liver, being the central organ in xenobiotic metabolism, is particularly vulnerable to chemical-induced damage. Histopathological analysis of hepatic tissue remains a key endpoint in such studies, as it reveals structural and cellular alterations caused by toxic insults (Bassan et al., 2021; Datta et al., 2023; Fritsche et al., 2023).

Acute oral toxicity studies, guided by OECD protocols, are fundamental in determining the immediate effects of high-dose exposure to test substances. These protocols include well-defined methods for dose administration, observational periods, and tissue evaluations. Despite the favorable pharmacological

profile of rutinoside, there is still limited data regarding its acute toxicity, particularly its impact on hepatic histology. Given the increasing interest in its therapeutic applications and the possibility of supraphysiological intake through supplementation, a comprehensive toxicological assessment is warranted.

This study aims to investigate the histopathological effects of orally administered rutinoside on the liver tissue of Wistar rats. Wistar rats are widely used in toxicological research due to their well-characterized physiology and metabolism, making them an ideal model for early safety evaluations. By analyzing histological changes in liver tissue following acute exposure, this study seeks to contribute to the safety profiling of rutinoside and support its responsible therapeutic development.

Methods.

This research adhered to OECD Guideline No. 423 for assessing acute oral toxicity in Wistar rats, employing a genuine experimental design characterised by randomisation and a post-test-only control group structure. The population comprised 12 male and female Wistar strain albino rats categorised into four groups. The male control group (KJ) and the female control group (KB) were administered 5% DMSO orally through a gastric sonde. The male treatment group (PJ) and the female treatment group (PB) received a single oral dosage of 5000 mg/kg BW rutinoside mixed with 5% DMSO via gastric sonde.

Prior to treatment, the animals were acclimatised for one week with unrestricted access to food and water. Observations were conducted at 30 minutes, 24 hours, and daily for 14 days to assess indicators of toxicity, such as tremors, convulsions, altered consciousness, aberrant gait, and respiratory distress. Termination occurred on day 14 for histological examination.

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. The experimental methods, encompassing the preparation of the rutinoside solution and its administration, were executed in the Pharmacology Laboratory at the University of Jember. Liver tissue collection and histological analysis were conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, University of Jember, using the Manja Roeinigk scoring model. Observations were performed using a double-blind methodology by two independent researchers under a light microscope at 400x magnification, examining five fields per sample and assessing hepatocyte damage in 20 hepatocytes in each field. Data were analyzed by the independent t-test. Tests for normality and homogeneity were performed via the Shapiro-Wilk and Levene tests.

Results.

This study aimed to investigate the acute oral toxicity of flavonoid rutinoside on the histology of the liver in Wistar rats via oral administration. Throughout the study, no experimental animals showed clinical signs of toxicity or died due to the compound. The absence of symptoms such as tremors, seizures, loss of consciousness, abnormal gait, or respiratory distress was observed in every group. In both the control and treatment groups, there was no evidence of a decrease in body weight from the baseline, that was more than ten percent, which indicates that the weight remained stable. Therefore, it can be deduced from these findings that the administration of rutinoside did not result in any significant systemic toxicity or physiological stress. These findings suggest that rutinoside administration did not induce systemic toxicity or physiological stress, as supported by the average scores presented in Table 1. Histopathological examinations of liver tissues were carried out, and the results are depicted in Figures 1, which collectively represent the various experimental groups. There were no significant differences in liver tissue architecture between the control and treatment groups, providing additional evidence that rutinoside is safe at the dose administered.

Discussion.

The findings of this study suggest that flavonoid rutinoside, administered at a high dose of 5000 mg/kg BW, did not induce acute toxicity in Wistar rats. No mortality or clinical symptoms of toxicity, including tremors, convulsions, altered gait, or respiratory distress, were detected during the research period, suggesting a positive safety profile for acute exposure. The absence of systemic toxicity is further supported by the stability of body weight in both the control and treatment groups, with no significant reduction observed. This is because body weight is a critical indicator of overall health and metabolic stability in experimental toxicity studies (Kale et al., 2022; Mah et al., 2022; Shayo et al., 2024).

Histopathological analysis of liver tissues revealed no significant structural damage or pathological alterations in the treatment groups compared to controls. The preserved hepatic architecture and absence of degenerative changes, such as necrosis or cellular swelling, underscore the non-toxic nature of rutinoside at the tested dosage. The findings of this study are consistent with the findings of Suzuki et al. (2015), who reported that an acute toxicity test revealed that food containing high dosages of rutinoside did not induce harm to the liver. The study's results, which investigated the acute toxicity of rutinoside at lower doses (5–20 mg/KgBB), add to the proof that rutinoside does not harm the liver. The findings of these trials collectively indicate that rutinoside does not cause liver toxicity, regardless of the dose, and this is true regardless of whether the drug is supplied in low or high doses.

Table 1. Manja Roenigk's average score for liver histopathology

Group	Score (mean ± SD)
KB	141,5 ± 2,500 (n=12)
KJ	136,83 ± 18,724 (n=12)
PB	135,17 ± 2,843 (n=12)
PJ	141 ± 9,849 (n=12)

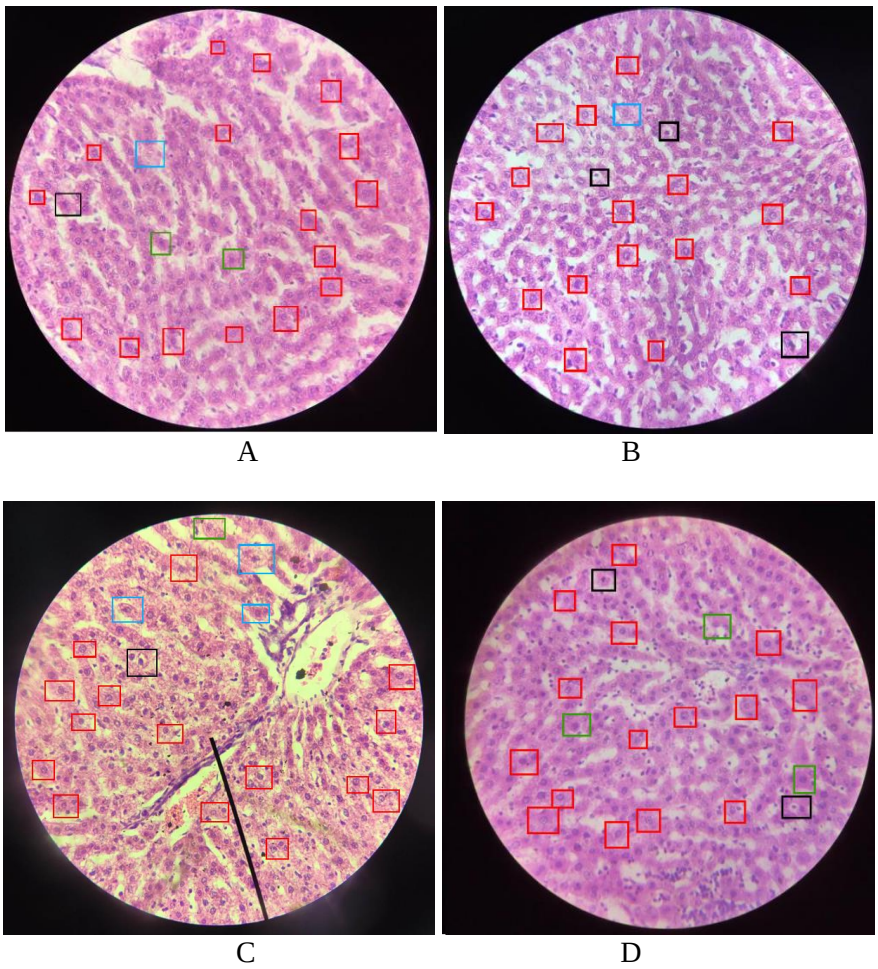

Figure 1. Liver histopathological features, light microscope, 400x; A. female control group (KB) B. male control group (KJ) C. female treatment group (PB) D. male treatment group (PJ). Description: (red box: normal hepatocytes; green box: parenchymatous degeneration hepatocytes; blue box: hydropic degeneration hepatocytes; black box: necrosis hepatocytes).

The absence of hepatotoxicity in this acute exposure study provides initial evidence of rutinoside's safety; however, further studies involving multiple dosages and extended observation periods are necessary to assess long-term safety comprehensively (Elrasoul et al., 2020; Saeed et al., 2024; Suzuki et al., 2015).

While acute toxicity studies are critical for preliminary safety assessments, chronic exposure research is essential to evaluate cumulative effects that may not be evident in short-term studies. Studies using different dosages and extended observation times are advised to completely characterize rutinoside's safety profile and long-term liver effects. Furthermore, research examining different organ systems would offer a more thorough comprehension of the compound's overall biological impact. Consistent with the toxicity classification systems used in this study, including OECD 423, GHS, and BPOM RI, the LD50 value of rutinoside exceeds 5000 mg/kg BW, classifying it as non-toxic. Consequently, even at high doses, rutinoside does not exhibit harmful effects.

Conclusion.

During an acute oral toxicity test, the administration of rutinoside to Wistar rats did not have any effect on the histological structure of the liver, according to the findings of the study. The researchers suggest that more study is required to evaluate the safety profile of rutinoside over a longer time frame in light of these facts. In order to evaluate the subchronic and chronic toxicity of rutinoside, as well as its potential effects on other organs, additional research might be carried out next. Prior to determining whether or not rutinoside has the potential to be used as a pharmacological agent, these investigations need to ensure that it is safe.

Conflict of Interest.

The authors disclosed no possible conflicts of interest.

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Author contribution.

Rena Normasari (Author 1) authored the article and led the research, with Ananda Maryam Jamila (Author 2) assisting in the research process and data analysis, while Azham Purwandhono (Author 3) supervised the manuscript.

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