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Acute Oral Toxicity and Organ Safety Assessment of Rutin in Sprague Dawley Rats in Accordance with OECD Test Guideline 425

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ABSTRACT

The study was conducted during the month of June, 2025 at College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India to investigate the acute oral toxicity profile of rutin, a naturally occurring flavonol glycoside, in female Sprague Dawley rats in accordance with OECD Guideline 425 at a limit dose of 2000 mg kg^{-1} body weight. Animals were monitored for fourteen days for mortality, clinical signs, general systemic parameters, biochemical changes, antioxidant status, relative organ weights, and histopathological alterations. No mortality or overt signs of toxicity were observed. Rutin treated rats exhibited comparatively lower body weight gain than controls, more evident during the second week, while feed and water intake remained unaffected and within normal physiological limits. No significant variation in serum ALT or AST was not observed. Renal function indices, like BUN and creatinine did not differ significantly from control values. A significant increase in relative liver weight was noted in the treated group, with no significant changes in other organs. Antioxidant assessment demonstrated a significant increase in superoxide dismutase activity in cardiac tissue and a significant reduction in hepatic lipid peroxidation, while catalase activity and reduced glutathione levels in heart and liver remained comparable to controls. Histopathological evaluation revealed no hepatic lesions and no marked pathological changes in other examined organs. Collectively, these findings indicated that oral administration of rutin at 2000 mg kg^{-1} did not produce acute systemic toxicity and was associated with measurable antioxidant effects, supporting its relative safety following single dose oral exposure.

KEYWORDS: Rutin, acute oral toxicity, OECD 425, antioxidant status

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1. INTRODUCTION

Phytochemicals are plant-derived bioactive chemical substances that contribute to the colour, aroma, flavour, and defence mechanisms of plants and provide additional health benefits to humans. Primary metabolites are essential for plant growth and fundamental physiological functions, whereas secondary metabolites contribute to ecological adaptation by facilitating pollinator attraction and protecting plants against pests and diseases. Based on their chemical structure and biological activity, phytochemicals are further classified into major groups, including polyphenols, flavonoids, carotenoids, alkaloids, and glucosinolates. Although these compounds are primarily synthesised for plant self-defence, extensive research has demonstrated that many phytochemicals exert protective effects in humans against cancer, cardiovascular diseases, arthritis, diabetes, and age-related disorders (Mutha et al., 2025). In addition, phytochemicals are increasingly utilised in animal husbandry for health promotion and disease prevention (Pamei et al., 2025; Devasena et al., 2026). Despite their therapeutic potential, phytochemicals may pose toxicity risks arising from inherently toxic plant metabolites (Jordan et al., 2010). Although plant-derived products are often considered safe, it remains essential to evaluate their toxicological profiles using both in vitro and in vivo experimental systems (Poonghuzhali et al., 2025; John et al., 2025). Toxicological evaluation constitutes a fundamental step in the development of new therapeutic agents, and regulatory agencies mandate systematic assessment of toxicity and pharmacological activity using validated experimental models (Parasuraman, 2011). Such studies provide essential information regarding the toxicological characteristics of candidate compounds, thereby ensuring safety prior to their evaluation in clinical trials (Arome and Chinedu, 2013). Acute and subacute toxicity studies represent rapid and reliable approaches for the preliminary assessment of toxicological risk. Acute oral toxicity testing is a critical initial component of safety evaluation and is designed to identify immediate adverse effects following single-dose exposure while providing preliminary information on hazard potential and dose tolerance. Internationally harmonised guidelines established by the Organisation for Economic Co-operation and Development provide standardised protocols for toxicity testing while emphasising ethical considerations and reduction in animal usage. Among these, OECD Test Guideline 425 is widely employed for substances anticipated to exhibit low acute toxicity, enabling reliable hazard classification using minimal animal numbers (Anonymous, 2022). Flavonoids constitute plant-derived polyphenolic compounds characterised by a common phenylchromane skeleton that permits extensive structural variation, giving rise to subclasses such as flavanols, flavones, flavanones, anthocyanidins, isoflavones, dihydroflavonols, catechins, and chalcones (Middleton et al., 2000). Flavonoids exhibit diverse biological activities, including antioxidant, antiviral, antibacterial, anti-inflammatory, antidiabetic, antihypertensive, and anticarcinogenic effects. These biological actions are largely attributed to their capacity to scavenge free radicals, chelate metal ions, and modulate enzyme systems involved in reactive oxygen species generation (Dias et al., 2005). Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) is a naturally occurring flavonol glycoside widely distributed in buckwheat, passion flower, black tea, apple peels, sweet potato, carrots, and red wine. Also referred to as rutoside, sophorin, vitamin P, or quercetin-3-rutinoside, rutin comprises the flavonol aglycone quercetin conjugated to the disaccharide rutinose and undergoes metabolic conversion to quercetin following absorption (Fabjan et al., 2003). Rutin exhibits multiple pharmacological activities, including antioxidant (La Casa et al., 2000), hepatoprotective (Khan et al., 2012), vasoprotective (Ganga et al., 2019), anticarcinogenic (Alonso-Castro et al., 2013), neuroprotective (Javed et al., 2012), antihypercholesterolaemic (Da Silva et al., 2001), antidiabetic, and cardioprotective effects (Kamalakannan and Prince, 2006). The present study therefore aimed to investigate the acute oral toxicity of rutin at a single limit dose of 2000 mg kg^{-1} in female Sprague–Dawley rats, with particular emphasis on evaluating its effects on body weight, feed and water intake, hepatic and renal function, relative organ weights, and systemic antioxidant status.

2. MATERIALS AND METHODS

The study was conducted during the month of June, 2025 at College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

2.1. Chemicals and test material

Rutin trihydrate (95%, 18325) was purchased from Sisco Research Laboratories Pvt. Ltd. (SRL) Mumbai, India. All the reagents used were of analytical grade and were procured from SRL, Sigma-Aldrich USA, Merck and Himedia, India unless otherwise specified. Ready to use diagnostic kits procured from Agappe Diagnostics Ltd., Kerala, India were used for the estimation of serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine.

2.2. Experimental animals

The study was conducted in ten adult female Sprague Dawley (SD) rats weighing approximately 150 g body weight. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy with approval number:

CVAS/MTY/IAEC/25/89. The rats were obtained from the Small Animal Breeding Station (Reg. No.328/GO/ReBt-S/Re-L/01/CPCSEA dated 10/03/2022), College of Veterinary and Animal Sciences, Mannuthy and maintained at the experimental animal facility. All the animals were kept in well-ventilated polypropylene cages under standard laboratory conditions of 12h/12h light/dark cycle at 25±2°C temperature with relative humidity ranging around 50–70% and provided with free access to standard laboratory animal feed and clean reverse osmosed water *ad libitum*. A 14-day acclimation period was allowed before the commencement of the study.

2.3. Acute oral toxicity assay of rutin trihydrate

Acute oral toxicity study of rutin trihydrate was conducted in accordance with OECD Test Guideline 425 (Up-and-Down Procedure) (Anonymous, 2022). Healthy nulliparous and non-pregnant female SD rats (150–200 g, approximately 4 weeks of age) were randomly selected and acclimatized to standard laboratory conditions for 14 days prior to dosing. Animals were fasted for 12 h before administration of the test substance, with access to *ad libitum* water.

As per the OECD TG 425, limit test was conducted by administering rutin trihydrate suspended in 0.5 ml distilled water at a dose level of 2000 mg kg^{-1} body weight, orally as a single dose via 18-gauge orogastric needle. For the limit test, OECD 425 recommends starting with one animal at the test dose, followed by sequential dosing of up to four additional animals. Accordingly, rutin was administered to one female rat at a dose of 2,000 mg kg^{-1} based on individual body weight. Mortality and clinical symptoms were monitored at 10 min, 30 min, 1 h, 2 h, 4 h and 6 h following administration of the test compound and subsequently, animals were observed twice daily for mortality and once daily for any clinical signs, including behavioural changes and alterations in skin, mucous membranes, respiratory, cardiovascular, central nervous system and somatomotor functions. Food was provided 1–2 h after dosing. Following survival of the initially dosed animal, four additional rats were dosed under identical experimental conditions and monitored similarly. A corresponding group of five female rats received only distilled water at an equivalent volume and served as vehicle controls (Raj et al, 2023).

2.3.1. General systemic observations

The body weight, daily feed consumption and daily water intake of each rat were documented on Day 0 following an overnight fast and continued throughout the 14-day observation period. Daily feed consumption was calculated as the difference between the quantity of feed offered and the amount remaining after a 24 h period and water intake was determined similarly by subtracting the residual volume from the initial volume provided on the previous day.

2.3.2. Assessment of hepatic and renal functions

One mL blood was collected on day 7 and day 14 of the study through retro-orbital plexus puncture with heparinised capillary tubes into sterile centrifuge tubes without anticoagulant under isoflurane anaesthesia and kept at room temperature for clot formation. Serum was separated after centrifugation at 3000 rpm for 10 min and the serum AST, ALT, BUN and creatinine was estimated using a semi auto analyser (M/S Hospitex, Italy) with ready-to-use diagnostic kits from M/S Agappe Diagnostics Ltd. India (Paulose et al., 2016).

2.3.3. Relative organ weights of animals

The animals were euthanised using overdose of isoflurane at the end of the 14 day study after overnight fasting. The liver, heart, pancreas, kidney, spleen, stomach, lungs, ovary, uterus and brain were quickly harvested, washed in ice cold normal saline and weighed for calculating the relative organ weight as per the following formula (Tiwari et al., 2020).

Relative organ weight=(Organ weight/Body weight)×100

2.3.4. Antioxidant status of animals

Aliquots of heart and liver were collected in vials containing ice-cold 0.05 M phosphate buffer, 0.2 M phosphate buffer and Tris-HCl for the analyses of oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and lipid peroxidation (LPO/TBARS) respectively. SOD was determined by the method of Madesh and Balasubramanian (1998), level of catalase in organs were assessed by the method of Hadwan (2018). GSH level was determined as per the method described by Ellman (1959) and the levels of lipid peroxidation estimated using procedure employed by Fraga et al. (1988).

2.3.5. Histopathology

Representative samples of liver, heart, pancreas, kidney, spleen, stomach, lungs, ovary, uterus and brain were collected in 10% neutral buffered formalin for histopathological examination.

2.3.6. Statistical analysis

All the results were expressed as Mean±SEM with 'n' as the number of replicates. The statistical analysis was performed by Graphpad Prism software version 8.0.2. The data were analysed using two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test. Statistical significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of rutin on general systemic parameters

The effect of oral administration of rutin at the rate of 2000 mg kg^{-1} on body weight, feed intake and water intake was given in the Figure 1.

3.1.1. Body Weight

Weekly body weight gain was recorded in control and rutin-treated animals following oral administration of rutin at the limit dose of 2000 mg kg^{-1} body weight, in accordance with OECD Test Guideline 425. As presented in Figure 1, control animals showed a progressive and statistically

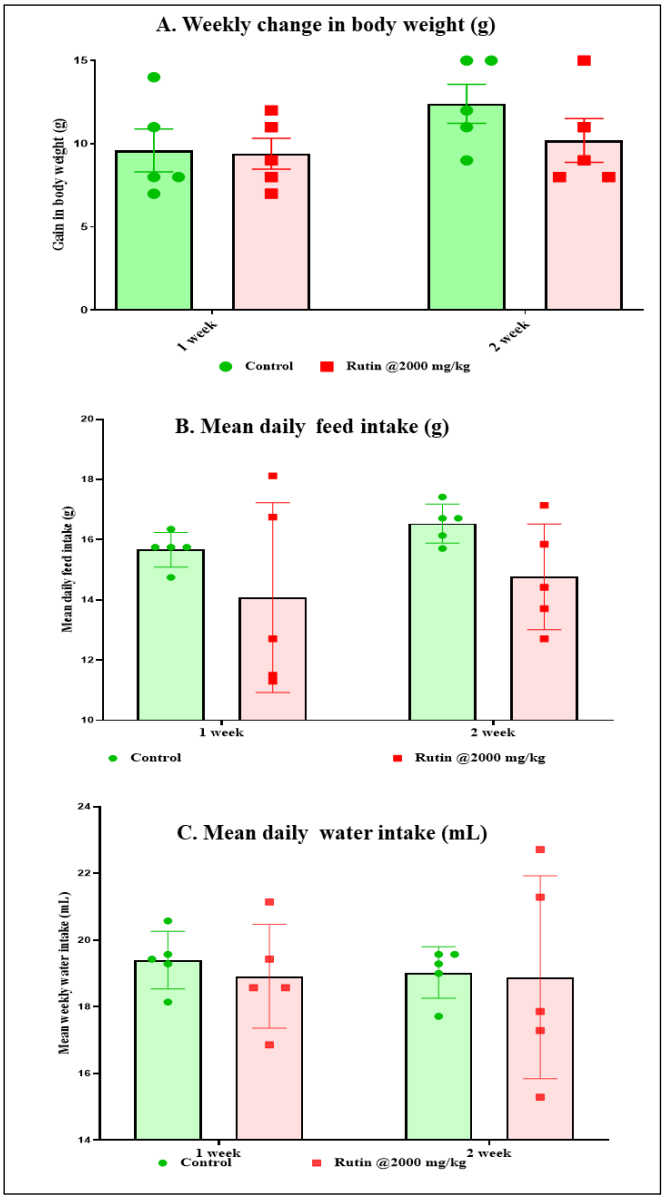

Figure 1: Mean weekly body weight gain in g (A), mean daily feed intake in g (B) and mean daily water intake in ml (C) of control and rutin treated groups at the rate of 2,000 mg kg^{-1}

significant increase in body weight gain from week 1 (9.6±1.29 mg kg^{-1}) to week 2 (12.4±1.17 mg kg^{-1}) (p<0.05). In contrast, rats treated with rutin exhibited comparatively lower body weight gain values of 9.4±0.93 mg kg^{-1} in week one and 10.2±1.32 mg kg^{-1} in week two, with no statistically significant difference between the two weeks. Between-group comparison demonstrated a lower magnitude of weight gain in the rutin-treated group at both time points, with the difference being more evident during week two.

Body weight change was regarded as a sensitive indicator of systemic toxicity, particularly in acute toxicity studies. Although a modest reduction in weight gain was observed in rutin-treated animals, the values remained within physiological limits and were not associated with mortality, abnormal clinical signs, or statistically significant intra-group differences. This pattern suggested the absence of overt systemic toxicity and supports the classification of rutin as a substance with low acute oral toxicity at 2000 mg kg^{-1}. Similar findings were reported by Hasumura et al. (2004), who observed slight numerical reductions in body weight gain in rats administered enzymatically decomposed rutin for thirteen weeks, without evidence of adverse toxicological outcomes.

3.1.2. Feed intake

Mean daily feed intake data for control and rutin-treated groups were shown in Figure 1. The control group demonstrated a slight increase in feed intake from week one (15.67±0.26 g) to week two (16.54±0.29 g). Animals treated with rutin at 2000 mg kg^{-1} consumed marginally lower amounts of feed, with values of 14.08±1.41 g during week one and 14.77±0.78 g during week two. Between-group comparison revealed numerically lower feed intake in the treated group at both observation points; however, these differences were not statistically significant (p>0.05).

Maintenance of normal feed intake following exposure to a test substance was indicative of preserved appetite, gastrointestinal function, and general well-being. The absence of significant alterations in feed consumption in rutin-treated animals suggested that high-dose acute exposure did not induce anorexia or metabolic distress, further supporting the lack of systemic toxicity under the experimental conditions.

3.1.3. Water intake

Weekly water intake values were presented in Figure 1. Control animals exhibited stable water consumption, recording 19.4±0.35 ml in week one and 19.03±0.31 ml in week two. Similarly, rutin-treated animals showed comparable intake values of 18.91±0.62 ml and 18.89±1.22 ml during week one and week two, respectively. No statistically significant differences were observed between control and treated groups at either time point (p>0.05).

Water intake is a sensitive marker of hydration status, renal function, and overall physiological balance. The comparable consumption patterns observed in both groups indicated that rutin administration at the limit dose did not disrupt normal drinking behaviour or fluid homeostasis. When considered together with stable feed intake, physiological body weight gain, and absence of abnormal clinical signs,

these findings collectively confirmed that rutin does not adversely affect general systemic parameters following acute oral exposure, in agreement with OECD TG 425 criteria for low toxicity classification.

3.2. Effect of rutin on hepatic and renal functions

The effect of rutin administration on hepatic and renal function, as assessed by ALT, AST, BUN and creatinine values, compared with the control was given in Figure 2.

3.2.1. Effect on hepatic functions

3.2.1.1. Effect of rutin on alanine aminotransferase (ALT)

Serum alanine aminotransferase levels measured on day seven and day fourteen are presented in Figure 2. In the control group, ALT activity remained stable between day seven (61.65±3.18 U l^{-1}) and day fourteen (62.59±2.68 U l^{-1}), indicating no temporal variation. In contrast, rats treated with rutin at 2000 mg kg^{-1} exhibited a higher ALT level on day seven (71.78±20.55 U l^{-1}) compared to the corresponding control; however, this increase was not sustained over time. On day fourteen, ALT activity in the rutin-treated group declined to 47.72±6.99 U l^{-1}, which differed significantly from both the day seven treated value and the corresponding control value, as seen in the Figure 2.

ALT is considered a relatively specific biomarker of hepatocellular integrity, as it was predominantly localised within hepatocytes and released into circulation following membrane disruption or cellular injury (Ramaiah, 2011). The transient elevation observed on day seven, followed by a reduction below control values on day fourteen, in the absence of progressive increases or parallel changes in other clinical parameters, suggested a reversible and adaptive hepatic response rather than sustained hepatocellular damage. Such temporal variability within physiological limits was often observed following acute xenobiotic exposure and might reflect enzyme induction or metabolic adaptation rather than toxicity.

3.2.1.2. Effect of rutin on aspartate aminotransferase (AST)

Serum aspartate aminotransferase activity in control and rutin-treated animals is summarised in Figure 2. The control group showed consistent AST values on day seven (86.77±1.12 U l^{-1}) and day fourteen (86.85±0.92 U l^{-1}), with no significant difference between time points. In contrast, rutin-treated animals exhibited significantly elevated AST activity on both day seven (113.08±3.99 U l^{-1}) and day fourteen (114.70±6.06 U l^{-1}) when compared with the corresponding control values (p<0.05). Two-way ANOVA indicated that this elevation was treatment-related, with no significant temporal variation within the treated group.

AST is widely distributed in hepatic, cardiac, and skeletal muscle tissues, and isolated elevations in AST without concordant increases in ALT were often indicative of non-

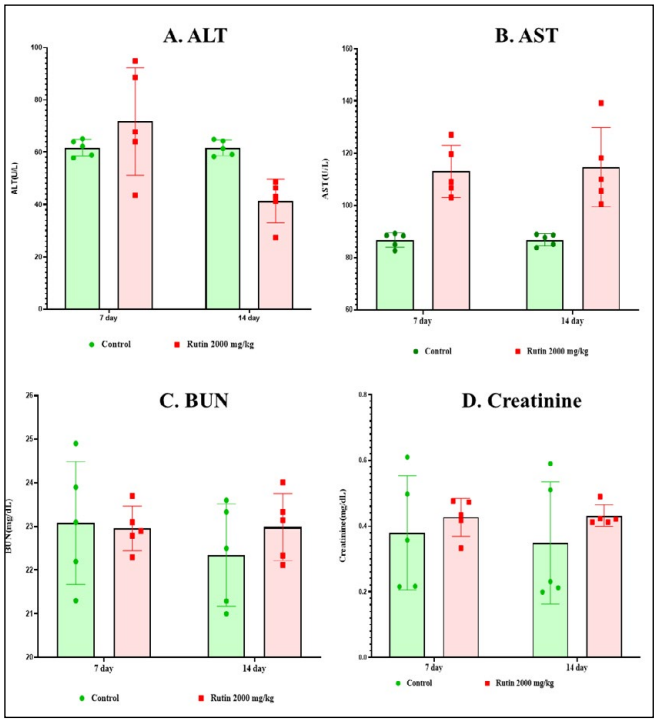

Figure 2: Serum biochemical parameters of hepatic and renal function of control and rutin treated groups at the rate of 2,000 mg kg^{-1}. A-Serum ALT (U l^{-1}), B- AST (U l^{-1}), C-serum creatinine (mg dl^{-1}), D- BUN (mg dl^{-1})

specific or adaptive metabolic responses rather than direct hepatocellular injury (Ramaiah, 2011). In the present study, the sustained elevation of AST in the absence of progressive ALT elevation, overt clinical signs, or histopathological lesions supported the interpretation that rutin induced adaptive hepatic enzyme modulation rather than clinically relevant hepatotoxicity. This interpretation was consistent with earlier reports demonstrating the hepatic safety of rutin at high oral doses (Sahib and Muhammad, 2021).

3.2.2. Effect of rutin on renal function

3.2.2.1. Effect of rutin on blood urea nitrogen (bun)

Blood urea nitrogen levels measured on day seven and day fourteen following oral administration of rutin at 2000 mg kg^{-1} are presented in Figure 2. Control animals showed BUN values of 23.08±0.56 mg dl^{-1} on day 7 and 22.34±0.47 mg dl^{-1} on day 14. Similarly, rutin-treated animals exhibited BUN levels of 22.96±0.20 mg dl^{-1} and 22.99±0.31 mg dl^{-1} on day seven and day fourteen, respectively. No statistically significant differences were observed between control and treated groups at either time point (p>0.05).

BUN is a sensitive indicator of renal excretory capacity and nitrogen metabolism, and elevations were commonly associated with impaired glomerular filtration or altered protein catabolism. The comparable BUN values observed in the present study indicated preserved renal function and

effective nitrogenous waste clearance following high-dose rutin administration.

3.2.2.2. Effect of rutin on serum creatinine

Serum creatinine levels are shown in Figure 2. The control group recorded creatinine concentrations of 0.38±0.07 mg dl^{-1} on day7 and 0.35±0.07 mg dl^{-1} on 14th day. In comparison, rutin-treated animals showed creatinine values of 0.43±0.02 mg dl^{-1} and 0.43±0.01 mg dl^{-1} on day 7 and day 14, respectively. No statistically significant differences were observed between groups at either time point, and all values remained within normal physiological limits.

Serum creatinine is a reliable marker of glomerular filtration rate, and stable creatinine concentrations, together with unchanged BUN levels, provide strong evidence for the absence of nephrotoxic effects. When considered alongside normal body weight, water intake, and kidney weights, these findings confirmed that acute oral exposure to rutin at 2000 mg kg^{-1} does not compromise renal function. Similar observations were reported by Sahib and Muhammad (2021), who demonstrated normal renal biochemical parameters following acute oral administration of rutin at doses up to 1.25 g kg^{-1} in mice.

3.3. Effect of rutin on relative organ weight of the rats

Relative organ weights of control and rutin-treated rats following oral administration of rutin at 2000 mg kg^{-1} body weight are presented in Table 1. A statistically significant increase in relative liver weight was observed in the rutin-treated group (4.09±0.10) compared to the control group (3.47±0.09), as indicated by different superscripts (p<0.05). In contrast, no statistically significant differences were detected between control and treated groups for the relative weights of the heart (0.36±0.01 vs 0.38±0.01), pancreas (0.37±0.01 vs 0.36±0.01), kidney (0.78±0.02 vs 0.79±0.03), spleen (0.29±0.03 vs 0.32±0.03), stomach (0.66±0.01 vs 0.70±0.01), lungs (0.87±0.05 vs 0.76±0.03), ovary (0.07±0.00 vs 0.09±0.00), uterus (0.15±0.06 vs 0.18±0.03), or brain (0.91±0.02 vs 0.91±0.01). The relative weights of these organs in the rutin-treated group remained comparable to control values, indicating the absence of treatment-related alterations in extrahepatic tissues.

Relative organ weight was a well-established and sensitive indicator of organ-specific toxicity, as it reflected changes independent of overall body weight and might reveal subtle toxic or adaptive responses to xenobiotic exposure (Piao et al., 2013). The isolated increase in relative liver weight observed in the present study, in the absence of mortality, abnormal clinical signs, or changes in the weights of other organs, suggested a physiological or adaptive hepatic response rather than overt toxicity. Such an increase might reflect enhanced metabolic activity associated with xenobiotic processing rather than hepatocellular injury.

Table 1: Relative organ weight (%) of vehicle control and rutin treated groups (Mean±SEM; n=5)

Organs	Control (Distilled water – 0.5 ml)	Rutin at 2000 mg kg^{-1}
Liver	3.47±0.09^{a}	4.09±0.1b
Heart	0.36±0.01	0.38±0.01
Pancreas	0.37±0.01	0.36±0.01
Kidney	0.78±0.02	0.79±0.03
Spleen	0.29±0.03	0.32±0.03
Stomach	0.66±0.01	0.7±0.01
Lungs	0.87±0.05	0.76±0.03
Ovary	0.07±0	0.09±0
Uterus	0.15±0.06	0.18±0.03
Brain	0.91±0.02	0.91±0.01

Values bearing different superscripts vary significantly at p<0.05 in the column

This interpretation was further supported by the lack of concurrent functional impairment or pathological alterations. Comparable findings were reported by Becho et al. (2015), who observed a mild increase in liver weight in rats administered rutin for ten days, which was considered biologically insignificant due to the absence of changes in serum ALT levels and the lack of persistence at longer exposure durations. Collectively, these findings indicated that acute oral administration of rutin at 2000 mg kg^{-1} did not induce adverse organ-specific toxicity, and the observed hepatic weight increase likely represented an adaptive, non-pathological response.

3.4. Effect of rutin on antioxidant status and lipid peroxidation

Effect of rutin administration on the antioxidant status when compared to the control was given in Figure 3.

3.4.1. Effect of rutin on superoxide dismutase (SOD)

Superoxide dismutase activity in heart and liver tissues of control and rutin-treated animals was presented in Figure 3. In the heart, SOD activity was significantly higher in the rutin-treated group (18.30±0.81 U mg^{-1} protein) compared with the control group (16.02±0.02 U mg^{-1}protein) (p<0.05). In the liver, SOD activity showed a numerical increase in the rutin-treated group (8.61±0.29) relative to controls (7.80±0.02), although this difference did not reach statistical significance.

SOD was a critical antioxidant enzyme responsible for catalysing the conversion of superoxide radicals into oxygen and hydrogen peroxide, thereby mitigating oxidative stress. The observed increase in cardiac SOD activity suggested an adaptive upregulation in response to potential reactive oxygen species generated during acute rutin exposure,

while the modest hepatic increase indicated maintained antioxidant defence without overt stress. These findings aligned with previous studies in which rutin enhanced SOD activity and reduced oxidative damage in tissues of diabetic rats (Tian et al., 2016), and in polyherbal formulations containing rutin, curcumin, and quercetin, normal enzymatic antioxidant mechanisms were maintained (Tiwari et al., 2020).

3.4.2. Effect of rutin on catalase activity

Catalase activity in heart and liver tissues following oral administration of rutin is summarised in Figure 3. In the heart, catalase activity remained comparable between control (19.10±4.16 U mg^{-1} protein) and treated animals (19.80±4.15 U mg^{-1} protein), with no statistically significant difference. In the liver, catalase activity was slightly lower in the rutin-treated group (33.78±3.10) compared with controls (39.19±4.50), although this reduction did not reach statistical significance.

Catalase acts as a secondary antioxidant defence enzyme, decomposing hydrogen peroxide into water and oxygen. The preservation of catalase activity in both heart and liver indicates that acute rutin exposure did not compromise enzymatic detoxification of hydrogen peroxide. This finding was consistent with previous reports demonstrating maintained catalase activity in tissues following acute administration of rutin or polyherbal formulations containing rutin (Tiwari et al., 2020; Tian et al., 2016).

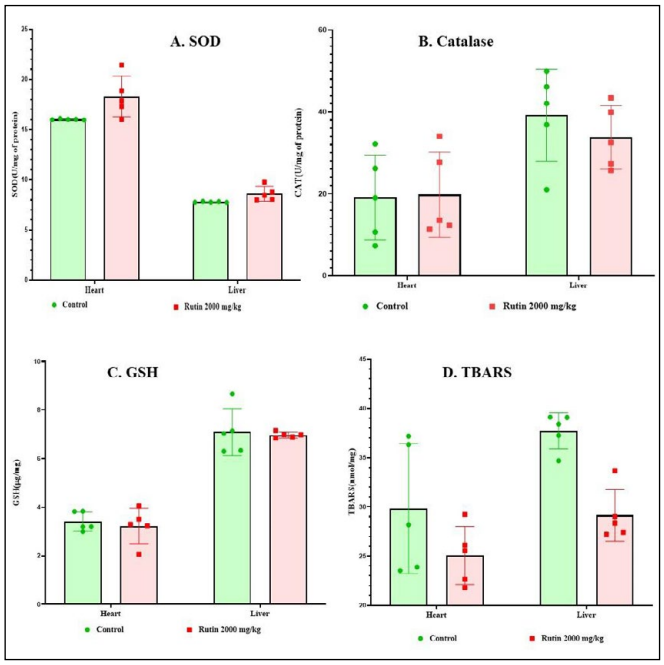

Figure 3: Effect of control and rutin treated at 2000 mg kg^{-1} treated groups on antioxidant parameters- A- SOD (U mg^{-1} of protein), B- Catalase (U mg^{-1} of protein), C- GSH (µg mg-1 of tissue) and D-effect on lipid peroxidation expressed as TBARS (nmol mg^{-1} of tissue)

3.4.3. Effect of rutin on reduced glutathione (gsh)

Levels of reduced glutathione in the heart and liver were shown in Figure 3. In the heart, GSH levels were comparable between control (3.41±0.16 µg mg^{-1} tissue) and rutin-treated animals (3.23±0.32 µg mg^{-1} tissue). Hepatic GSH concentrations also showed no appreciable difference between control (7.10±0.38) and treated animals (6.97±0.05). No statistically significant differences were observed between control and treated groups in either tissue.

GSH is a key intracellular antioxidant that neutralised superoxide radicals, peroxyl radicals, and singlet oxygen, maintaining redox homeostasis and preventing oxidative damage (Li et al., 2012). The preservation of both cardiac and hepatic GSH levels suggested that rutin administration at 2000 mg kg^{-1} does not deplete primary antioxidant defences, thereby protecting cellular structures from oxidative injury. This observation supported earlier findings that rutin either alone or in combination with other flavonoids preserves intracellular glutathione levels under acute exposure conditions (Tiwari et al., 2020; Tian et al., 2016).

3.4.4. Effect of rutin on lipid peroxidation (tbars)

Lipid peroxidation, assessed by thiobarbituric acid reactive substances (TBARS) is presented in Figure 3. In the heart, TBARS levels were numerically lower in the rutin-treated group (25.07±1.32 nmol mg^{-1} tissue) compared with the control group (29.83±2.64), although this difference was not statistically significant. In the liver, TBARS levels were significantly reduced in rutin-treated animals (29.14±1.19 nmol mg^{-1} tissue) compared with controls (37.71±0.74) (p<0.05), indicating a treatment-related decrease in lipid peroxidation.

Lipid peroxidation represented oxidative degradation of membrane polyunsaturated lipids and could compromise cellular integrity if unchecked. The significant reduction in hepatic TBARS and numerical decrease in the heart reflect decreased oxidative stress and preserved membrane stability. Together with maintained SOD, catalase, and GSH levels, these results indicated that rutin not only avoids inducing oxidative injury but might confer tissue-protective effects during acute exposure. These findings were consistent with Tian et al. (2016), who reported rutin-mediated attenuation of oxidative stress in diabetic rat tissues, and with Tiwari et al. (2020), who showed preserved antioxidant enzyme activity in polyherbal combinations containing rutin.

3.5. Histopathology of vital organs

Histopathological examination of major organs from animals administered distilled water at 0.5 ml as vehicle control revealed normal tissue architecture in the liver, heart, kidney, lung, spleen, pancreas, brain, uterus and

ovary. Similarly, animals treated orally with rutin at a dose of 2,000 mg kg^{-1} under OECD Test Guideline 425 exhibited preserved histoarchitecture in all examined organs, with no evidence of cellular degeneration, necrosis, inflammatory infiltrates, vascular congestion or structural abnormalities when compared with the control group. The liver showed intact hepatic cords and sinusoids, the heart displayed normal myocardial fibres, the kidneys exhibited well-defined glomeruli and tubules, and the lungs showed normal alveolar architecture. The spleen, pancreas and brain maintained normal cytoarchitecture, while reproductive organs, including the uterus and ovary, also appeared histologically normal. Overall, no treatment-related histopathological alterations were observed in any organ following acute oral administration of rutin at 2,000 mg kg^{-1}, indicating the absence of target organ toxicity under the

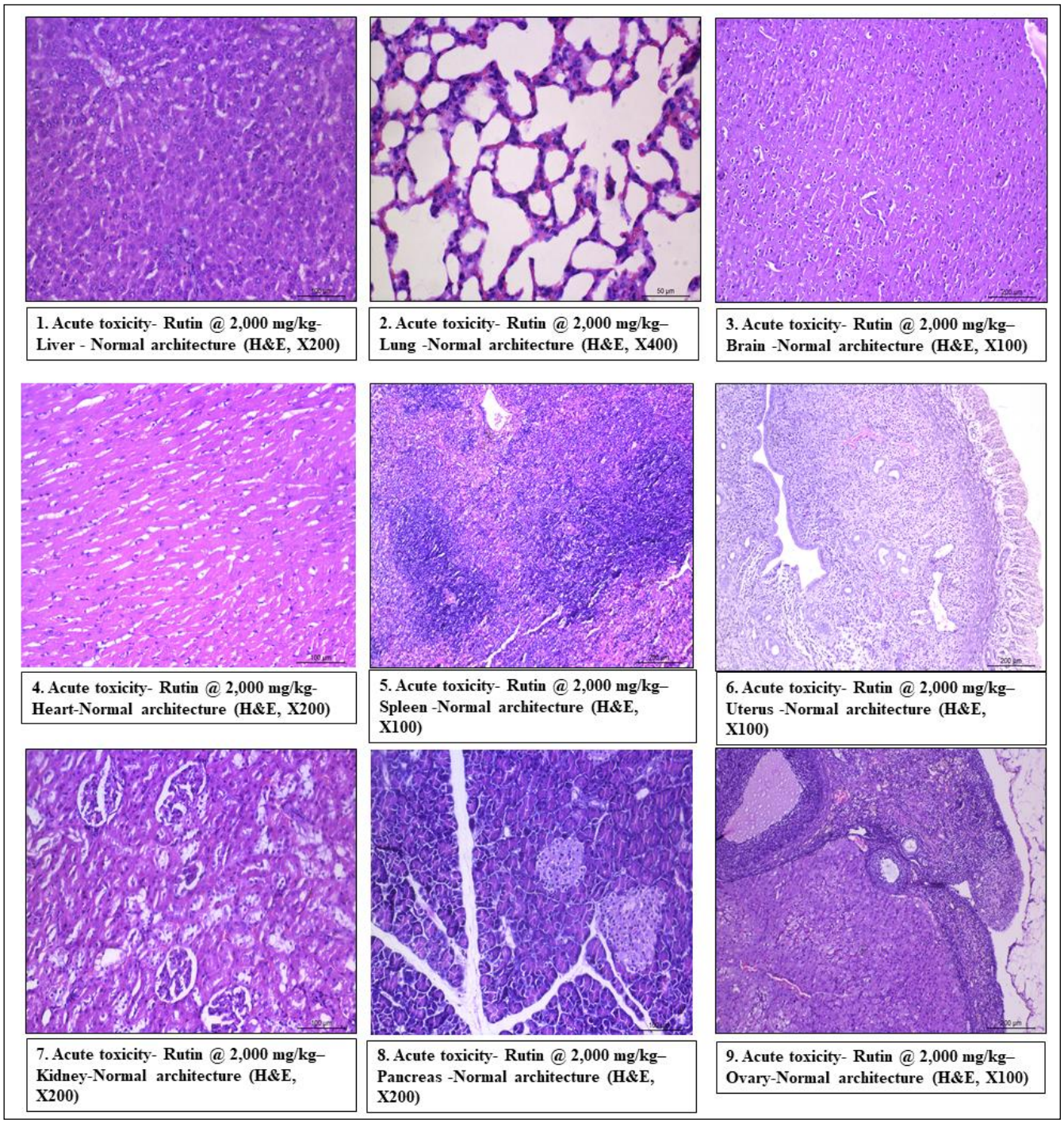

Figure 4: Histopathology of major organs of the rats treated with oral rutin

experimental conditions. Histopathological micrographs of representative organs were shown in Figure 4

4. CONCLUSION

Oral administration of rutin at 2,000 mg kg^{-1} produced no mortality and maintained stable body weight, normal feed and water intake, and largely unchanged relative organ weights with the exception of a modest increase in relative liver weight. Renal and hepatic biochemical indices remained within physiological limits, with preserved antioxidant status and lipid peroxidation profiles. Histopathology of major organs showed intact architecture without degenerative or inflammatory lesions. Minor biochemical and organ-specific variations were considered adaptive rather than toxicologically relevant. Overall, rutin demonstrated good tolerability and low acute oral toxicity, indicating a wide safety margin at high doses.

5. FUTURE RESEARCH

Future studies should focus on subacute and sub chronic toxicity to assess potential cumulative effects, particularly on hepatic function. Mechanistic investigations addressing hepatic metabolism and oxidative stress pathways, along with pharmacokinetic and metabolite profiling, would further clarify safety and support rational dose selection for long term use.

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