Trop J Nat Prod Res, December 2024; 8(12): 9648 - 9653

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Subacute Administration of MCH-2 Syrup on the Renal Morphology of Wistar Rats

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ARTICLE INFO

Article history:
Received 23 July 2024
Revised 24 September 2024
Accepted 18 November 2024
Published online 01 January 2025

ABSTRACT

Biomedical research highlights the therapeutic potential of natural products, with plant-based formulations showing promise for immunomodulatory effects. However, concerns regarding their renal safety still need to be addressed. This study assessed the subacute toxicity of MCH-2 syrup in male Wistar rats, focusing on its effects on renal morphology through histopathological evaluation. The MCH-2 syrup was formulated using extracts of *Phyllanthus niruri* and *Morinda citrifolia*. Adult male Wistar rats were assigned to five groups (n=5). Three groups received MCH-2 syrup at doses of 100, 300, and 500 mg/kg body weight for 28 days. The positive control group received only the vehicle, while the negative control group was given syrup without the plant extract. Behavioural parameters, body weight, and renal histology were assessed, emphasizing indicators such as inflammation, haemorrhage, and necrosis in kidney tissues. The study reported no mortality or adverse clinical signs across the groups. Body weight increased consistently, suggesting an absence of stress or toxicity. Histopathological analysis revealed no significant differences in kidney weight among groups. The least renal tubule and endothelial damage were observed in the negative control and 500 mg/kg dose groups, whereas the highest damage occurred in the positive control group. Notably, higher doses of MCH-2 syrup correlated with reduced glomerular and endothelial damage, indicating a potential dose-dependent protective effect. These findings demonstrate that MCH-2 syrup does not induce significant renal toxicity in a subacute setting, supporting its safety profile for renal health and its potential as a therapeutic formulation.

Keywords: Phyllantus niruri, Morinda citrifolia, Subacute toxicity, Kidney, Histopathology.

Introduction

Exploring natural products for their therapeutic potential remains at the forefront of biomedical research, offering a rich source of bioactive compounds with diverse pharmacological activities.[1] Natural remedies have been utilized for centuries in traditional medicine, and modern research continues to uncover their molecular mechanisms and therapeutic efficacy. Among the vast array of medicinal plants, *Phyllanthus niruri* L. and *Morinda citrifolia* L. have gained widespread attention for their numerous health benefits.[2] These plants are lauded for their ability to combat several ailments, owing to their rich phytochemical content, which includes alkaloids, flavonoids, tannins, and polyphenols.[3] *Phyllanthus niruri*, commonly known as "chanca piedra" or "stonebreaker," has a long history of use in treating kidney stones and liver disorders. Modern pharmacological studies have confirmed its hepatoprotective, antioxidant, and immunomodulatory properties, making it a valuable candidate for further research in immune system modulation.[4] Polynesian traditional medicine celebrates *Morinda citrifolia*, widely known as noni, for its ability to address inflammation, infections, and pain. Recent scientific investigations have revealed its capacity to enhance immune function, reduce inflammation, and provide anti-cancer effects, positioning it as a versatile plant in natural therapeutics.[5]

With increasing interest in utilizing these plants for pharmaceutical applications, MCH-2 syrup, a formulation combining *P. niruri* and *M. citrifolia* in equal proportions, emerges as a novel herbal product with potential immunomodulatory properties. Immunomodulation, which involves regulating immune responses, plays an important role in managing several diseases, including autoimmune disorders, infections, and inflammatory conditions.[6] The combination of these two botanicals in MCH-2 syrup offers a potential synergistic effect that could enhance their therapeutic benefits.[7] However, while the therapeutic potential is promising, ensuring the safety of such formulations is paramount, particularly concerning the long-term effects on vital organs.

The kidneys play a vital role in detoxification and excretion, rendering them particularly susceptible to the toxic effects of various substances, including herbal medicines. Due to their critical functions in blood filtration, electrolyte balance, and blood pressure regulation, disruptions in renal function can result in severe systemic consequences. Although herbal formulations are widely regarded as safe, they often contain bioactive compounds that may exert nephrotoxic effects, particularly with prolonged use.[9] Consequently, it is imperative to assess the renal toxicity of novel herbal preparations, especially those intended for long-term administration or use by individuals with preexisting renal conditions.

This study aims to evaluate the potential adverse effects of MCH-2 syrup on renal tissue through histopathological examination and biochemical marker analysis. Investigating the toxicological profile of this novel formulation is essential for determining its safety and therapeutic viability. Moreover, these findings will serve as a

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Citation: Monica E, Rollando R, Susanto H, Hendra GA, Putra ES, Nugraha DP, Lazuardy A. Effects of Subacute Administration of MCH-2 Syrup on the Renal Morphology of Wistar Rats. Trop J Nat Prod Res. 2024; 8(12): 9648 – 9653 https://doi.org/10.26538/tjnpr/v8i12.44

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

foundation for future research into its efficacy in treating immune-related disorders, thereby contributing to the broader understanding of its clinical applicability.

Materials and Methods

Materials used

The study utilized the following materials: purified water, methylparaben, sucrose, sorbitol, sodium benzoate, tutty fruity flavour, diethyl ether, and neutral buffered formalin (10%). The laboratory equipment employed in the study included Ohaus analytical balance, Taffware I-2000 scales, a binder brand oven, an infusion pan, a Memmert WNB 10 water bath, an IKA T25 digital ULTRA-TURRAX, micros (MCX50LED) light microscope, an Opti Lab Plus digital camera, network scissors, tweezers, an oral probe, and a 1 cc syringe.

Source of plant materials

Phyllanthus niruri and *Morinda citrifolia* were collected in December 2023 from the Herbal Materia Medica Laboratory in Batu, East Java, Indonesia. The collection sites are located within the geographic coordinates of S 11° 09' 41" to S 11° 18' 52" latitude and E 182° 72' 32" to E 182° 62' 11" longitude. Batu experiences an annual rainfall of approximately 3,290 mm, with a mean relative humidity of 77% and an average temperature of 23.5°C, providing an optimal environment for the growth of medicinal plants. The plant specimens were identified by Dr. Budi Sumarta, a botanist affiliated with the Pharmacognosy Laboratory, Department of Pharmacy, Universitas Ma Chung. Specimen voucher numbers FA:054-MACHUNG-2023 and FA:055-MACHUNG-2023 were assigned to *Phyllanthus niruri* and *Morinda citrifolia* for reference and documentation purposes.

Ethical approval

The study received approval from the Research Ethics Commission of Brawijaya University (reference number 830-KEP-UB).

Source of animals

Adult male Wistar rats, weighing 150–250 g and aged 4–5 months, were obtained from the Veterinary and Animal Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Brawijaya University. The rats underwent a one-week acclimation period in a controlled environment. During this time, they had unlimited access to food, while the housing conditions were kept clean, with a temperature of 22±3°C, relative humidity of 30 to 70%, and a 12-hour light/dark cycle.

Preparation of plant extracts

Separate batches of 500 grams of powdered *Phyllanthus niruri* and *Morinda citrifolia* were macerated individually in 2 L of solvent for one hour. The solvent volume and extraction duration were selected based on established practices for herbal extractions. After maceration, the mixtures were filtered to separate the liquid filtrate containing the extracted bioactive compounds from the solid residue (pomace). The filtrate was then concentrated under reduced pressure to produce a dense extract. This concentrated extract was subsequently processed for use in the formulation of MCH-2 syrup, designed to evaluate its immunomodulatory effects.

Preparation of the herbal syrup

The preparation of MCH-2 syrup followed a series of precise steps to ensure proper formulation and homogenization. First, 87.5 mL of purified water was heated to 90°C, and 0.3 g of methylparaben was dissolved in it. This solution was mixed with 150 g of sucrose and stirred for 10 minutes at 40°C. In a separate step, 214 mL of purified water was combined with 50 g of sorbitol and stirred until fully dissolved. The two solutions were homogenized at 40°C for 5 minutes. Next, 10 g of *Morinda citrifolia* extract was added and homogenized for 5 minutes, followed by 10 g of *Phyllanthus niruri* extract, which was homogenized for 15 minutes. Subsequently, 0.5 g of sodium benzoate and 1.25 g of tutti-frutti flavour were incorporated and homogenized for 5 minutes. Purified water was added to bring the total volume to 500 mL, and the mixture was homogenized for another 5 minutes to ensure uniformity.[2]

Subacute toxicity test on the herbal syrup

Before the experimental procedures, the rats were fasted for 14 hours but allowed to drink water. They were then individually weighed and administered the test chemicals through oral gavage. The animals were divided into five groups of 5 male rats. The sub-acute toxicity study of the test formulation was conducted following OECD-407 guidelines over 28 days. Laboratory procedures followed CPCSEA guidelines. The animals were housed under standard laboratory conditions. The control group received the vehicle orally, while the study groups were administered the test formulation at 100, 300, and 500 mg/kg body weight per day. Toxicity symptoms were monitored individually at 30 minutes, 4, and 24 hours after the first dose, and continuously for 28 days. Observations included signs of toxicity, changes in body weight, and mortality. At the end of the experiment, histopathological examinations were performed on the surviving rats.[5]

Histological examination

Preparation of the histopathological slides of the rats' kidneys involved fixation, dehydration, clearing, infiltration, embedding, sectioning, affixing, and mounting. In this study, histopathological parameters of rat kidneys were meticulously observed to evaluate the potential effects of MCH-2 syrup administration over a subacute period. The evaluation included an assessment of kidney tubules, where changes, such as loss of brush border in tubular cells, basement membrane damage, inflammation, hyaline cast formation, and necrosis, were identified and scored from 0 to 4 based on the extent of affected areas. Additionally, kidney endothelium was evaluated for swelling and damage, while the glomerulus was assessed for changes, such as Bowman's capsule thickening, glomerular plate retraction, and glomerular fibrosis. Furthermore, tubular interstitial conditions were evaluated to identify signs of inflammation, haemorrhage, and necrosis, with scores ranging from 0 to 4 depending on the extent of tissue damage.[6]

Statistical analysis

Several statistical tests were performed to analyze the data effectively. A one-way analysis of variance (ANOVA) was used to assess differences in means across multiple samples in a single direction. This method is suitable for analyzing continuous data in a normal distribution. To confirm the normality of the data, the Shapiro-Wilk test was employed, examining the raw data directly without any prior transformations or preprocessing, such as creating frequency distribution tables. Additionally, the Kruskal-Wallis test, a non-parametric test based on ranks, was utilized to determine if there were significant differences among two or more groups of independent variables concerning a dependent variable. This test is particularly well-suited for evaluating differences in numerical data (interval/ratio) and ordinal scales, providing robust statistical comparisons even when assumptions of normality and equal variance are not met. Data from the histopathological observations were analyzed using the non-parametric Kruskal-Wallis test to evaluate the significance of findings obtained from different treatment groups. Collectively, these statistical methods provided a comprehensive analysis of the findings across different experimental groups in the study.[7]

Results and Discussion

Effect of the MCH-2 formulation on the body weight of rats

During the subacute toxicity observation period, several critical factors must be monitored, including the behaviour of the test animals and any changes in their autonomic nervous system, sensory system, eyes, skin, and neuromuscular system.[8] Among these, monitoring body weight changes is crucial, especially for detecting significant fluctuations. Significant weight loss, characterized by a reduction exceeding 20% relative to the control group or a decrease of more than 25% sustained over seven days, is a critical indicator of potential illness or distress in experimental animals. Such weight loss is often accompanied by

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

reduced food intake, providing additional evidence of compromised health status. Figure 1 presents the body weight observations of the experimental rats, demonstrating an overall increase in body weight across all treatment groups. This weight gain can be attributed to several factors. Firstly, the absence of stress indicators throughout the study suggests that the animals maintained a stable physiological state. Secondly, the rats effectively adapted to the experimental environment, likely enhancing their overall well-being. This adaptation may have positively influenced their appetite, increasing food consumption and weight gain.

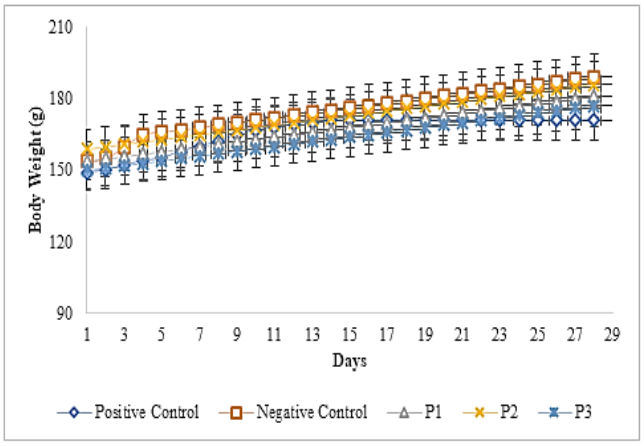

Figure 1: Effect of MCH-2 syrup on body weight changes in male rats.
Data are expressed as mean ± SEM (n = 5).

Toxicity effects of the MCH-2 formulation on experimental rats

Pathological evaluations, including macroscopic and microscopic histopathological examinations of the kidneys, are fundamental for detecting cellular-level toxicity associated with administering test formulations. Subacute oral toxicity testing involves administering the test formulation at varying dose levels to multiple groups over an extended period,[9] facilitating the identification of potential toxic effects from prolonged exposure rather than the immediate effects observed in acute toxicity studies.[10] This study recorded no mortality in any test groups throughout the 28-day experimental period. The absence of fatalities suggests that any kidney damage that may have occurred did not progress to a life-threatening stage. The subacute toxicity protocol also closely monitored individual animals for signs of toxicity, encompassing autonomic responses, behavioural changes, sensory and neuromuscular responses, and eye and skin alterations.[11] The results indicated that the MCH-2 formulation did not induce toxic symptoms in the experimental groups. The lack of adverse effects observed throughout the 28-day testing period supports the conclusion that the MCH-2 formulation is safe and does not elicit toxicity under subacute testing conditions.[12]

Effect of the MCH-2 formulation on the kidney weight and histology

As displayed in Figure 2, there were no statistically significant differences in kidney weights across the various groups. Renal tubule damage can result from several mechanisms, including the loss of the brush border, thickening of the basal membrane, inflammation, cast formation, and necrosis.[13] The brush border, consisting of numerous long microvilli, is crucial for increasing the surface area available for absorption. The loss of this brush border is marked by cellular lesions that disrupt cell integrity and polarity, particularly in the proximal tubules. The basal membrane serves as a critical boundary between tubules and interstitial cells.

In the present study, the observed damage in the renal tubules of rats primarily involved the loss of the brush border. The proximal tubules, which are essential for re-absorption, demonstrate this loss. Reabsorption in these tubules occurs through pinocytosis, where proteins adhere to the brush border of the luminal membrane, allowing for complete absorption as the membrane segment is internalized. The loss of the brush border in the proximal tubules indicates membrane integrity and polarity disruptions due to exposure to toxic substances. Histopathological assessments revealed that the negative control and the 500 mg/kg body weight/day treatment groups had lower damage levels than the positive control and other treatment groups. The positive control group displayed the highest average damage score, indicating the most severe impairment. Statistical analysis revealed a significant ($p < 0.05$) difference in the treatment of tubular damage. Subsequent analysis showed significant differences between the positive and negative control groups, with the positive control group exhibiting a higher damage score compared to the negative control. The three treatment groups demonstrated similar levels of tubular damage, with scores lower than the positive control but higher than the negative control (Figure 3).

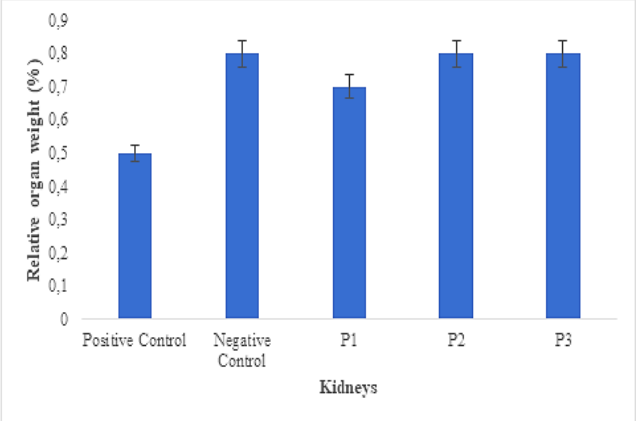

Figure 2: The relative weight of kidney in subacute toxicity in rats.
Data are expressed as mean ± SEM (n = 5); One-way analysis of variance (ANOVA) was performed followed by Tukey's multiple comparison test.

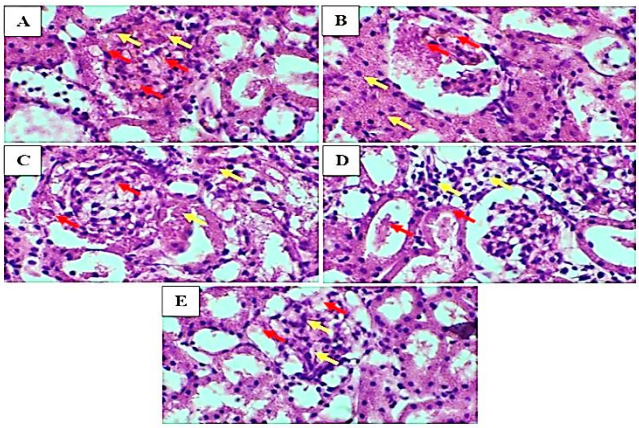

Figure 3: Histopathology of the kidney tubules.
A: positive control; B: negative control; C: 100 mg/kg body weight/day; D: 300 mg/kg body weight/day; E: 500 mg/kg body weight/day; Red arrow: necrosis; Yellow arrow: inflammation.

The renal tubules are categorized into distal and proximal types. Distal tubules, located in the cortex after the loop of Henle, are responsible for excreting excess or waste substances, forming secondary urine. These tubules reabsorb bicarbonate and water and transport or secrete ions, such as sodium, hydrogen, chloride, calcium, ammonia, and magnesium.[14] Proximal tubules reabsorb primary urine, including glucose, salts, water, and amino acids. They are convoluted and end in the loop of Henle, characterized by a brush border that absorbs approximately 60-65% of the filtered water, along with nutrients, ions,

vitamins, and small plasma proteins. Substances are transported through the tubular wall and absorbed by the tubular capillaries.[15]
Damage to the proximal tubules is frequently associated with xenobiotic substances.[16] When a chemical compound enters the proximal tubules, it is absorbed into the urine through the epithelial cells at high concentrations. This damage, often linked to the renal excretion of drugs, adversely affects kidney function. Factors contributing to this include the high blood flow to the kidneys, which delivers significant amounts of drugs and chemicals. Tubular damage can impair the body's ability to excrete excess fluids, leading to fluid accumulation and increased blood pressure. Moreover, the inability to eliminate unnecessary substances can result in their accumulation and subsequent toxic effects.[17]

Effect of the MCH-2 formulation on the endothelium
Endothelial cell damage is assessed by swelling, structural damage, and cell loss.[13] Swelling of endothelial cells often results from partial or complete occlusion of capillary lumens, leading to the constriction of fenestrae. Such endothelial damage is typically attributed to early ischaemia and hypoxia, which induce cellular dysfunction and swelling, compromising endothelial integrity. Loss of endothelial cells results in endothelial leakage, allowing fluid and proteins to move from the intravascular space to the interstitial space.[18] In the present study, damage to the endothelium in rat kidney tissues manifested as edge damage and endothelial swelling. Endothelial swelling is observed when congestion in the renal capillaries causes cellular expansion. The nuclei of these swollen cells are frequently adjacent to hemosiderin, a pigment formed from the breakdown of red blood cells or an accumulation of iron-derived granular pigment due to systemic conditions.[19] Histopathological examination revealed that the positive control group exhibited the highest level of endothelial damage, including edge damage. In contrast, the negative control group and the three treatment groups primarily exhibited endothelial swelling. A trend was observed indicating that higher doses of the test solutions were associated with reduced damage, as evidenced by lower damage scores in the 500 mg/kg body weight/day group. The analysis of endothelial parameters indicated significant effects of the treatment groups. The results demonstrated that the positive control group had the highest damage scores, while the 300 and 500 mg/kg body weight/day treatment groups exhibited lower damage scores compared to the other groups. This suggested that higher doses of the MCH-2 formulation are associated with minimal endothelial damage (Figure 4).
Damage to endothelial cells can contribute to the widening and hardening of glomerular walls, leading to decreased blood flow and potential entry of toxic substances, which may cause congestion or blockage within the capillary lumen. This results in the swelling of podocytes and mesangial cells and blockage in the glomerular capillaries, disrupting the endothelial function necessary for effective glomerular filtration.[20] The endothelium of the glomerular capillary walls contains numerous pores, making the glomerular capillary membrane more permeable to water and solutes than other capillaries. These endothelial cells are negatively charged, which helps prevent the passage of plasma proteins. Also, the glomerular capillary walls produce nitric oxide (a vasodilator) and endothelin-1 (a vasoconstrictor) to regulate blood flow.

Effect of the MCH-2 formulation on the glomerulus
The glomerulus, a crucial component of the nephron, is responsible for plasma ultrafiltration and the filtration of water and dissolved substances into the bloodstream. It consists of a network of capillaries lined with endothelial cells, situated between the capillaries and Bowman's capsule, with an associated basement membrane. In kidney tissue sections, the glomerulus appears as an elongated or rounded structure comprising clusters of capillaries with a high density of red blood cells and surrounded by small spaces. The glomerulus is supplied by the afferent arteriole, which delivers blood to the glomerulus and is drained by the efferent arteriole, which carries blood away. Glomerular cell damage is assessed through several key indicators: thickening of Bowman's capsule, glomerular retraction, and glomerular fibrosis.[13] The thickening of Bowman's capsule is attributed to the proliferation of parietal epithelial cells, resulting in a multilayered structure encased in collagen-like material that extends into Bowman's space. This process is characterized by glomerular retraction, which involves wrinkling of the capillary wall and alterations in the tubular membrane. Glomerular retraction describes the contraction or expansion of the glomerular capsule in the space between the capsule and the glomerulus. Glomerular fibrosis is marked by the presence of fibrous tissue with numerous collagen fibres, which may lead to haemorrhage or damage to the kidney's epithelial cells.[22]
In the present study, glomerular damage was characterized by thickening of Bowman's capsule and glomerular retraction. The thickening of Bowman's capsule was attributed to exposure to toxic substances, which caused cell proliferation, hypertrophy, and alterations in tubular structure. The glomerular retraction was due to the pulling of the glomerular capsule, which increased the distance between Bowman's capsule and the glomerulus.[23] Histopathological assessment of kidney preparations indicated that the positive control group exhibited retraction of the glomerular laminae, while the negative control group and the treatment groups showed thickening of Bowman's capsule. The positive control group had the highest damage score, suggesting the most severe impact, whereas the 500 mg/kg body weight/day group recorded the lowest score, indicating minimal damage (Figure 5).

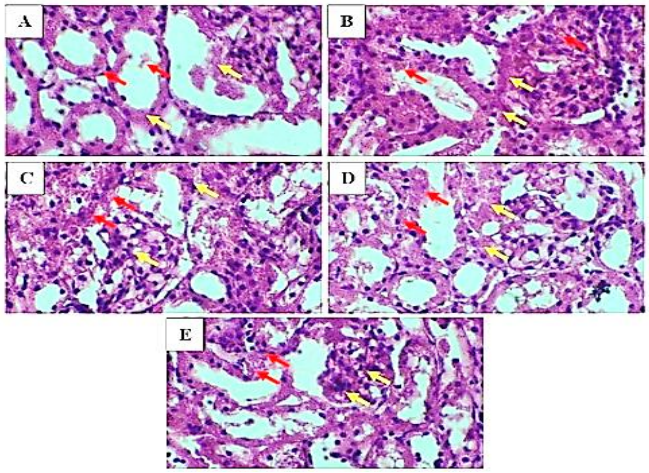

Figure 4: Histopathology of the kidney endothelium. A: positive control; B: negative control; C: 100 mg/kg body weight/day; D: 300 mg/kg body weight/day; E: 500 mg/kg body weight/day; Red arrow: necrosis; Yellow arrow: inflammation.

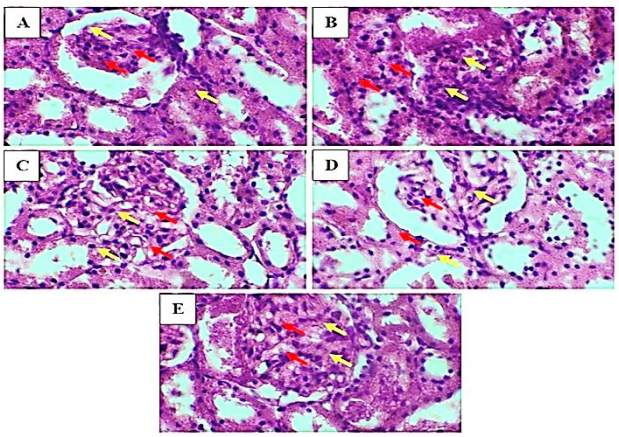

Figure 5: Histopathology of the kidney glomerulus. A: positive control; B: negative control; C: 100 mg/kg body weight/day; D: 300 mg/kg body weight/day; E: 500 mg/kg body weight/day; Red arrow: necrosis; Yellow arrow: inflammation.

Statistical analysis of glomerular observations revealed significant differences among the treatment groups. The highest significance value ($p = 0.023$) was obtained from the analysis, indicating that the treatment groups had a notable effect on the glomerular parameters. Furthermore, comparisons revealed significant ($p = 0.012$) differences between the positive control group and the negative control and treatment groups, with the latter showing less severe damage than the positive control. The negative control group did not exhibit significant differences in damage compared to the treatment groups, suggesting that the MCH-2 preparation did not cause severe glomerular damage. A trend was also observed, indicating that higher doses of the MCH-2 preparation were associated with reduced damage. This was evident from the scoring values among the negative control, 300, and 500 mg/kg body weight/day groups, which were lower and not significantly different from each other.[24]

Damage to the glomerulus arises from inflammation and proliferation of the Bowman's capsule epithelium, which narrows Bowman's space. Increased capillary permeability and filtration may lead to the leakage of plasma proteins and red blood cells from the glomerulus, compromising the glomerular filtration membrane and causing swelling in Bowman's space.[25] This swelling reduces the glomerular filtration rate and increases capillary permeability, allowing blood cells, proteins, and toxic substances to pass through due to impaired filtration. As a result, these substances accumulate in the glomerulus, leading to increased pressure and glomerular shrinkage.[26]

Effect of the MCH-2 formulation on the interstitial tubule

Damage to interstitial tubular cells in the kidneys is primarily identified by inflammation, haemorrhage, and necrosis. Inflammation is a protective response characterized by swelling, redness, heat, and the infiltration of inflammatory cells. Haemorrhage refers to the leakage of red blood cells from damaged vessels, often resulting from increased pressure within the vessel due to toxic exposure.[13] In the present study, histopathological assessments revealed that interstitial tubular damage, including inflammation, haemorrhage, and necrosis exceeding 25%, was observed only in the positive control group. The negative control and treatment groups exhibited similar levels of inflammation and haemorrhage, with higher doses of the MCH-2 preparation showing a trend toward reduced damage. Specifically, the 500 mg/kg body weight/day dose group showed the least damage compared to the other doses and the negative control group (Figure 6). Statistical analyses confirmed significant differences among the groups, with the positive control group showing the most severe damage. The 500 mg/kg body weight/day group demonstrated significant ($p < 0.05$) differences from the 300 mg/kg body weight/day group, suggesting that higher doses of the MCH-2 preparation may offer greater protection against tubular damage. Tubular damage, often resulting from blockages due to protein deposits and increased interstitial pressure, can lead to severe conditions, such as tubular necrosis and impaired kidney function. Interstitial tubules, or tubulointerstitial cells, respond to stimuli by facilitating fluid movement from the circulation to the tissue, mitigating damage from dead cells, pathogens, or irritants. Necrosis, characterized by cell swelling, organelle damage, and subsequent inflammatory responses, occurs when toxic substances directly affect epithelial tubular cells, leading to cell nucleus shrinkage and functional impairment.[27]

Phytochemicals from plants, such as *Phyllanthus niruri* and *Morinda citrifolia*, contribute to nephroprotection. Flavonoids in *P. niruri*, such as quercetin, help mitigate inflammation by inhibiting prostaglandin synthesis, reducing capillary permeability, and alleviating oxidative stress. Additionally, quercetin promotes kidney health by dissolving calcium deposits in kidney stones,[28] increasing sodium excretion, and boosting urine volume, thereby enhancing glomerular filtration rates and aiding in eliminating nephrotoxic substances.[29] Similarly, *M. citrifolia*, a plant rich in flavonoids and alkaloids, supports kidney health by protecting against functional disturbances and oxidative stress. Quercetin, found in *M. citrifolia* and *P. niruri*, has anti-inflammatory properties, aids in detoxification, strengthens kidney structure, and helps maintain biochemical balance.[30]

Conclusion

The study findings revealed no observable symptoms of toxicity in the test animals. None of the subjects exhibited adverse effects, such as body weight reduction, diminished strength, abnormal bowel movements, involuntary muscle contractions, or fluid retention. However, the administration of MCH-2 syrup demonstrated notable effects on kidney histology, highlighting the potential for subclinical changes that may not manifest as overt toxicity symptoms. These results emphasize the critical importance of comprehensive toxicological evaluations that integrate physiological observations with detailed histopathological analyses. Such rigorous assessments are essential to ensure the safety and efficacy of new pharmacological formulations, providing a robust foundation for their therapeutic application.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by me.

Acknowledgements

The authors would like to thank the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for support through the Fundamental Research-Regular grant (No. 051/SP2H/PT/LL7/2024).

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