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Rabbits as an Experimental Model for Methanol-Induced Acute Kidney Injury

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ABSTRACT

Background: Methanol intoxication is associated with a high fatality rate and is further exacerbated by acute kidney injury (AKI). Recent studies have demonstrated that rabbits share similarities with humans in methanol metabolism via the alcohol dehydrogenase (ADH) pathway, unlike rats, where methanol is metabolized through the catalase pathway, leading to faster formic acid oxidation. However, the use of rabbits as a model for AKI due to methanol intoxication has not been explored.
Methods: This experimental study employed a randomized posttest-only control group design. Three groups were compared: a control group receiving aquabidest, a 2 g/kg BW methanol group, and a 4 g/kg BW methanol group. After 72 hours of treatment, the right kidney of each rabbit was biopsied and fixed in buffered formalin for histopathological examination using Periodic Acid-Schiff (PAS) staining. Tubular injury scores were calculated using a semi-quantitative scoring system and expressed as percentages. Statistical analyses were performed using one-way ANOVA followed by the LSD test.
Results: Significant differences were observed in the percentage of tubular injury between the control group and the 2 g/kg BW methanol group [7.41 ± 0.74% vs. 77.15 ± 1.66%; MD: -69.75 ± 1.61%; 95% CI: -71.19 to -66.3%; $P < 0.001$], as well as between the control group and the 4 g/kg BW methanol group [7.41 ± 0.74% vs. 95.96 ± 0.77%; MD: -88.56 ± 1.62%; 95% CI: -92 to -85.11%; $P < 0.001$].
Conclusion: Rabbits can serve as an effective model for studying acute kidney injury induced by methanol intoxication.
Keywords: methanol, acute kidney injury, experimental animal models, rabbits

INTRODUCTION

Methanol intoxication is associated with a high case fatality rate, which becomes significantly worse when acute kidney injury (AKI) occurs. Formic acid, the toxic end product of methanol metabolism, plays a central role in its toxicity. Tubular injury in the kidneys results from the osmotic

effects of elevated methanol levels and the cytotoxicity of formic acid on proximal tubular cells.(1) Additionally, metabolic acidosis, a hallmark of methanol toxicity, can indicate tubulointerstitial damage, reflecting worse kidney function and a higher risk of AKI.(2)

Species-specific differences in methanol metabolism contribute to its varied toxicity. In humans, methanol is metabolized by the alcohol dehydrogenase (ADH) pathway, leading to significant formic acid accumulation and metabolic acidosis. In contrast, rodents primarily metabolize methanol through the catalase pathway, which limits formic acid buildup. Rabbits, however, share similarities with humans, metabolizing methanol via the ADH pathway.(3) Studies have demonstrated that methanol exposure in rabbits causes kidney injury, including tubular epithelial swelling, degeneration, and hypoxic changes.(4)

Compared to rodents, rabbits more closely mimic the in vivo metabolism of methanol in primates, particularly regarding formic acid accumulation.(5) Methanol metabolism studies in rabbits have shown formic acid accumulation rates several times higher than in rodents, making rabbits a more suitable model for human methanol toxicity.(6) Moreover, the teratogenic potential of methanol in humans remains unknown, and its teratogenicity in rodents may not accurately reflect human developmental risks due to species-specific metabolic differences, as seen with other teratogens. Rabbits may better represent methanol metabolism in humans than rodents.(7)

Recent studies suggest that rabbits share significant similarities with humans in methanol metabolism through the alcohol dehydrogenase (ADH) pathway. However, no studies have used rabbits as a model for AKI due to methanol intoxication. Developing such a model is crucial for advancing our understanding of methanol-induced renal injury. This study aims to provide insights into the pathomechanisms and potential therapeutic interventions for methanol-induced AKI using rabbits model, serving as a foundation for future research.

MATERIALS & METHODS

Study Design

This study utilized a pure experimental design with a randomized posttest-only control group. Three groups were established for comparison: a control group receiving aquabidest, a 2 g/kgBW methanol group, and a 4 g/kgBW methanol group.

Animal and Housing

The male rabbits used in this study served as the animal model, as they were injected intraperitoneally with methanol in doses of either 2 g/kgBW or 4 g/kgBW. The rabbits' ages, expressed in weeks and calculated from birth, ranged from 5 to 12 months, while their body weights, measured in grams, ranged from 2,000 to 3,000 g. The sex of each rabbit (male) was confirmed by a veterinarian. Eligible rabbits were acclimatized for one week in individual cages to minimize stress. Housing conditions included a well-ventilated room with indirect sunlight exposure. Rabbits were fed standard pellets constituting 10%–15% of their body weight daily and provided with 100–200 ml of mineral water per day. To avoid interference from external antioxidants, vegetable supplementation was discontinued three days prior to treatment.

Baseline Assessment

Prior to methanol or aquabidest administration, blood samples were collected to measure baseline serum creatinine levels. Only rabbits with serum creatinine levels <2.5 mg/dL were included in the study.

Treatment Administration

Methanol was administered intraperitoneally as a 40% solution. The control group received 10 ml of aquabidest, while the treatment groups were injected with methanol doses of 2 g/kgBW and 4 g/kgBW, respectively. A single injection

was performed for each rabbit using a 23G needle.

Tissue Collection and Histopathological Examination

After 72 hours, nephrectomy of the right kidney was performed under sevoflurane inhalation anesthesia. The kidney samples were fixed in buffered formalin for histopathological analysis. Periodic Acid-Schiff's (PAS) staining was used to assess tubular injury, which was scored using a semi-quantitative system. Tubular injury was evaluated blindly across 10 high-power fields (400x magnification) and expressed as the percentage of damaged tubules relative to the total tubules. Tubular injury was defined as dilation, atrophy, cast formation, vacuolation, epithelial degeneration, cell detachment, brush border loss, and basement membrane thickening.

Ethical Considerations

Ethical approval for the study was obtained from Institutional Review Board of Prof. Dr. I.G.N.G. Ngoerah General Hospital (Number: 3034/UN14.2.2.VII.14/LT/2024) and conducted in accordance with the ARRIVE guidelines and the principles outlined in the Guide for the Care and Use of Laboratory Animals.(8)

STATISTICAL ANALYSIS

Data analysis was performed in three stages. First, descriptive statistics were used to calculate the mean, standard deviation (SD), minimum, and maximum values for each group. The tubular injury scores were analyzed based on the normality of data distribution. Normality testing was conducted using the Shapiro-Wilk test, which was appropriate for sample sizes <30 per group. A p-value >0.05 indicated normal distribution. Homogeneity of variance was assessed using Levene's test, with a p-value >0.05 indicating homogeneity.

For normally distributed and homogeneous data, mean tubular injury scores were compared among groups using One-Way ANOVA, with a p-value <0.05 indicating significant differences. For non-normal or non-homogeneous data, transformation was attempted to achieve normality and homogeneity. If this was unsuccessful, the Kruskal-Wallis test was used as a non-parametric alternative.

RESULT

The Tubular Injury Score, assessed semi-quantitatively from PAS-stained kidney biopsies, showed no intra-observer variability, as confirmed by Bland-Altman analysis, with all data within the 95% limits of agreement. The histopathological examination of kidney tissues stained with PAS revealed distinct differences between the control and methanol-treated groups. Figure 1 shows representative histological images from each group. In the control group (Figure 1A), the corticomedullary region of the kidney displayed normal tubular structures without significant pathological findings. In contrast, the group treated with 2 g/kgBW methanol (Figure 1B) exhibited marked tubular injury, including dilation, epithelial degeneration, and vacuolation. The group treated with 4 g/kgBW methanol (Figure 1C) showed severe tubular injury, characterized by pronounced epithelial detachment, brush border loss, and cast formation. These findings align with the quantitative assessment of Tubular Injury Scores, which demonstrated a dose-dependent increase in tubular damage with methanol administration.

The Shapiro-Wilk test confirmed that the data were normally distributed, with homogeneous variances ($p>0.05$). Statistical analysis using One-Way ANOVA revealed significant differences in Tubular Injury Scores among the study groups ($p<0.001$). Further analysis with the LSD test demonstrated significant differences between the control group and the 2 g/kgBW methanol group ($7.41 \pm 0.74\%$ vs. $77.15 \pm 1.66\%$; MD: -69.75; 95% CI: -71.19 to -66.30; $p < 0.001$), as well as between the control group and the 4 g/kgBW methanol group ($7.41 \pm 0.74\%$ vs. $95.96 \pm 0.77\%$;

MD: -88.56; 95% CI: -92.00 to -85.11; $p < 0.001$). Additionally, a significant difference was observed between the 2 g/kgBW and 4 g/kgBW methanol groups (77.15 ± 1.66% vs. 95.96 ± 0.77%; MD: -18.81; 95% CI: -22.25 to -15.37; $p < 0.001$). The comparison of Tubular Injury Scores among the study groups is presented in Figure 2.

These findings indicate that methanol administration at both 2 g/kgBW and 4 g/kgBW doses resulted in significantly higher Tubular Injury Scores compared to the control group. Furthermore, the 4 g/kgBW dose caused significantly greater tubular injury than the 2 g/kgBW dose, suggesting a dose-dependent effect of methanol on tubular damage. These findings further confirm the histopathological observations and highlight the dose-response relationship of methanol-induced renal injury.

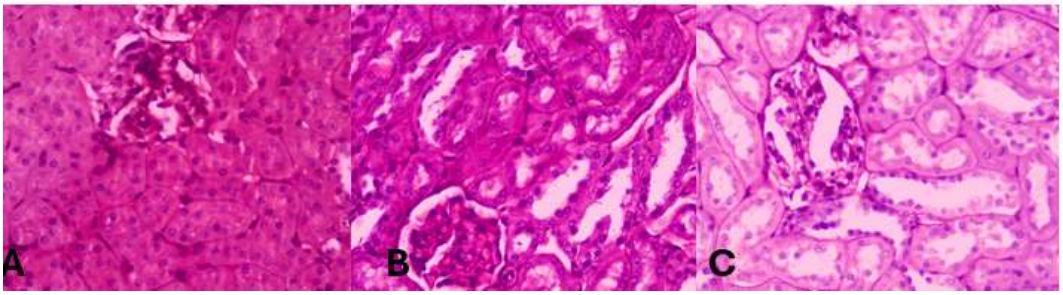

Figure 1. Histopathological images of rabbit kidneys. (A) Histopathology of the corticomedullary region in the control group. (B) Histopathology of the corticomedullary region in the 2 g/kgBW methanol group. (C) Histopathology of the corticomedullary region in the 4 g/kgBW methanol group.

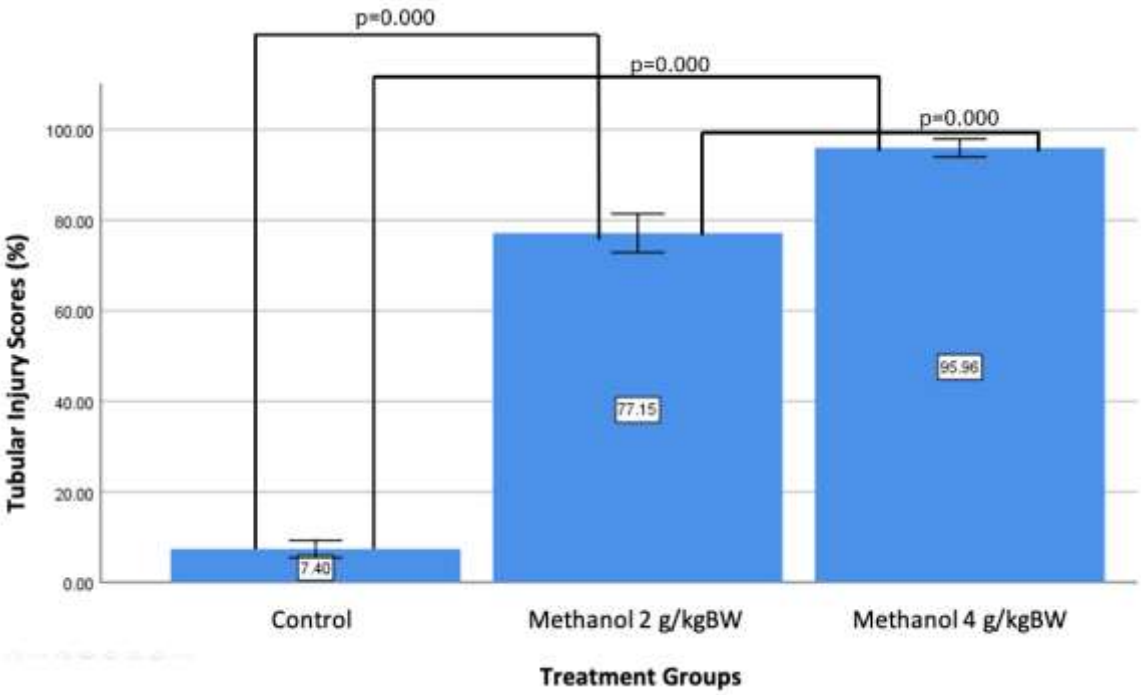

Figure 2. Comparison of Tubular Injury Scores among treatment groups. The bar chart shows the mean Tubular Injury Scores for the control group, 2 g/kgBW methanol group, and 4 g/kgBW methanol group, with error bars representing the standard deviation. Statistically significant differences ($p < 0.001$) are indicated between all groups.

DISCUSSION

Most laboratory animals, such as rodents, do not exhibit signs of methanol intoxication. In contrast, humans and primates experience metabolic acidosis and ocular toxicity following methanol ingestion due to the

accumulation of formic acid in the blood.(9) Rodents efficiently oxidize formic acid almost completely, whereas dogs exhibit only minor formic acid accumulation. Although all species metabolize formic acid via folate-dependent pathways, there are interspecies differences in the ability to metabolize formic acid.(10) Rodents metabolize formic acid approximately twice as rapidly as primates.(5)

Methanol metabolism occurs predominantly through alcohol dehydrogenase (ADH) in humans and catalase in rodents, resulting in differences in the pharmacokinetics of formic acid metabolites.(6) Comparative studies of methanol and formic acid pharmacokinetics in male CD-1 mice, New Zealand white rabbits, and cynomolgus monkeys revealed distinct elimination kinetics. Following a single intraperitoneal injection of 0.5 or 2 g/kg methanol, the plasma clearance of methanol in mice was 2.5 times higher than in rabbits, with peak plasma concentrations similar across species. Formic acid accumulation was significantly higher in rabbits, approximately 3.8 times greater than in mice. These findings suggest that rabbits may more accurately reflect in vivo methanol metabolism in primates, particularly in terms of formic acid accumulation, compared to rodents.(6)

Recent studies have further demonstrated species-specific differences in enzymatic pathways for methanol metabolism. Unlike rodents, which primarily utilize catalase, rabbits appear to metabolize methanol via ADH, similar to primates and humans. This similarity results in slower methanol clearance in vivo and substantially increased formic acid accumulation in rabbits compared to rodents.(6) For example, male New Zealand white rabbits exhibit pharmacokinetics that align more closely with primates, as evidenced by slower methanol clearance and significantly greater formic acid accumulation than observed in rodents.(7) Consequently, rabbits may represent a more appropriate animal model for evaluating methanol-induced toxicity and associated risks in humans.(11)

Currently, no studies have explored methanol's toxic effects on acute kidney injury (AKI) from a histopathological perspective using animal models. This study establishes methanol doses capable of inducing pathological features of AKI in rabbits, providing a foundation for future research. These findings suggest that rabbits are a suitable model for studying methanol-induced AKI and could support further investigations into pathophysiological mechanisms and therapeutic interventions.

CONCLUSION

This study successfully established a rabbit model for methanol-induced acute kidney injury (AKI), demonstrating that methanol administration at doses of 2 g/kgBW and 4 g/kgBW induces significant tubular injury in a dose-dependent manner. The findings highlight the suitability of rabbits as a experimental model for methanol toxicity research due to their metabolic similarity to humans, particularly in the accumulation of formic acid. This model provides a valuable foundation for future investigations into the pathophysiological mechanisms of methanol-induced AKI and the evaluation of potential therapeutic interventions. Further studies are needed to expand on these findings, including exploring long-term outcomes and refining treatment strategies for methanol toxicity.

Declaration by Authors

Ethical Approval: Approved

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

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