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# Nephroprotective effects of ethanolic extract of *Ganoderma lucidum* against diethylene glycol-induced acute kidney injury in rats

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**ABSTRACT**

**Background**: Diethylene glycol (DEG) contamination in pharmaceutical preparations has caused numerous cases of acute kidney injury, particularly in pediatric populations. Current treatments are expensive and not readily accessible in resource-limited settings. *Ganoderma lucidum*, a medicinal mushroom rich in triterpenoids, flavonoids, and ergothioneine, may offer a natural therapeutic alternative.

**Objective**: This study evaluated the nephroprotective potential of ethanolic *G. lucidum* extract against DEG-induced acute kidney injury in rats.

**Methods**: Twenty-four male Wistar rats were divided into six groups: healthy control, DEG-induced control (5 g/kg BW), three *G. lucidum* treatment groups (250, 500, 750 mg/kg BW for 7 days plus DEG), and fomepizole control (5 mg/kg BW plus DEG). Renal function markers (β2-microglobulin, malondialdehyde) and electrolytes (sodium, potassium, chloride) were measured on day 11.

**Results**: DEG significantly elevated β2-microglobulin (0.73 vs. 0.29 ng/mL) and malondialdehyde levels (3.368 vs. 0.72 µmol/L) while reducing electrolytes. *G. lucidum* extract at 500 and 750 mg/kg BW significantly improved all parameters, achieving efficacy comparable to fomepizole ($p > 0.05$).

**Conclusion**: Ethanolic *G. lucidum* extract demonstrates significant nephroprotective effects against DEG-induced kidney injury, with 500 mg/kg BW as the optimal therapeutic dose.

**Keywords**: acute kidney injury, diethylene glycol, electrolyte homeostasis, *Ganoderma lucidum*, renal function

## Introduction

Diethylene glycol (DEG), a member of the ethylene glycol group of organic solvents, poses a significant public health threat due to its sweet taste and misuse as a solvent in pharmaceutical preparations, particularly in children's syrups. In 2022, the Ministry of Health of the Republic of Indonesia identified 102 children's prescribed medications containing ethylene glycol, diethylene glycol, and ethylene glycol butyl ether, compounds known to cause acute renal failure (ARF) [1]. This contamination has resulted in numerous pediatric fatalities, highlighting the urgent need for effective therapeutic interventions.

DEG-induced nephrotoxicity follows a well-characterized metabolic pathway. Upon ingestion, DEG is rapidly absorbed through the intestinal tract and undergoes hepatic metabolism by alcohol dehydrogenase, converting to glycol aldehyde, then to glycolic acid, and ultimately to oxalate [2]. The resulting oxalic acid has low solubility and forms calcium oxalate crystals that deposit in renal tubules, causing mechanical injury to kidney tissue. This process leads to acute kidney injury characterized by elevated blood urea nitrogen (BUN), creatinine, and β2-microglobulin (β2-M) levels, alongside electrolyte imbalances including sodium, potassium, and chloride dysregulation [3,4]. Additionally, DEG metabolism produces 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA), which induce oxidative stress and lipid peroxidation in renal proximal tubules, elevating

malondialdehyde (MDA) levels and promoting cellular apoptosis [5,6].

Current treatment protocols for DEG poisoning rely primarily on fomepizole and ethanol as antidotes, with hemodialysis reserved for severe cases involving metabolic acidosis [2,7]. However, these interventions are expensive, require specialized medical facilities, and may not be readily accessible in resource-limited settings. Furthermore, the invasive nature of hemodialysis makes it particularly challenging for pediatric patients. These limitations underscore the need for alternative therapeutic approaches using readily available, cost-effective natural compounds.

*Ganoderma lucidum* (Lingzhi mushroom), a saprophytic basidiomycete recognized by the American Herbal Pharmacopoeia and Therapeutic Compendium, contains numerous bioactive compounds with potential nephroprotective properties [8]. The mushroom's fruiting body is rich in triterpenoids, polysaccharides, flavonoids, and ergothioneine—an amino acid derivative of thiourea and histidine containing a sulfur atom in its imidazole ring [9,10]. These compounds have demonstrated immunomodulatory, antioxidant, and anti-inflammatory activities in various experimental models [11,12]. Notably, *G. lucidum* contains essential minerals including sodium, potassium, calcium, magnesium, and phosphorus, as well as B-complex vitamins (B1, B2, B6) and niacin [13,14]. The triterpenoid content has shown particular promise in treating various kidney diseases, while ergothioneine may serve as an amino acid source to support impaired renal function [15].

Despite the documented bioactive properties of *G. lucidum*, its specific efficacy against DEG-induced nephrotoxicity has not been systematically investigated. This study aimed to evaluate the nephroprotective potential of ethanolic *G. lucidum* extract in DEG-induced acute kidney injury in rats by measuring renal function markers (β2-M and MDA) and serum electrolyte levels (sodium, potassium, and chloride). Additionally, we sought to determine the optimal therapeutic dose of *G. lucidum* extract by comparing its effects at 250, 500, and 750 mg/kg body weight against the standard treatment fomepizole. We hypothesized that *G. lucidum* extract would mitigate DEG-induced nephrotoxicity through its antioxidant and mineral-replenishing properties, potentially offering a natural, accessible alternative for managing acute renal failure in resource-limited settings.

## Method

### Ethical approval

This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital, Surakarta, Indonesia (Ethical Clearance No: 1.129/V/HREC/2024). All experimental procedures followed institutional guidelines for the care and use of laboratory animals.

### Experimental design

This study employed a completely randomized design (CRD) with six treatment groups and four replications, totaling 24 experimental animals. The treatment groups were as follows:

- Group 1 (C1): Healthy control (no treatment)
- Group 2 (C2): Positive control (DEG-induced, 5 g/kg BW for 5 days)
- Group 3 (C3): *G. lucidum* extract 250 mg/kg BW for 7 days + DEG 5 g/kg BW for 5 days
- Group 4 (C4): *G. lucidum* extract 500 mg/kg BW for 7 days + DEG 5 g/kg BW for 5 days
- Group 5 (C5): *G. lucidum* extract 750 mg/kg BW for 7 days + DEG 5 g/kg BW for 5 days
- Group 6 (C6): Fomepizole 5 mg/kg BW for 7 days + DEG 5 g/kg BW for 5 days (standard treatment control)

*G. lucidum* extract and fomepizole were administered daily via oral gavage for 7 days. DEG was administered via oral gavage once daily for 5 consecutive days, beginning on day 3 of the treatment period. All parameters were measured on day 11.

### Experimental animals

Twenty-four male Wistar rats (*Rattus norvegicus*) aged 2 months with body weights of approximately

200 ± 20 g were obtained from the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, Indonesia. Rats were housed in individual cages under controlled conditions (temperature 22-25°C, 12-hour light/dark cycle, relative humidity 50-60%) at the Animal House, Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia.

Prior to experimental treatment, all animals underwent a 7-day acclimatization period. During acclimatization and throughout the experimental period, rats were provided with standard commercial pellet feed (AD II) twice daily (morning and evening) and drinking water *ad libitum*. Animal health status was monitored daily throughout the study period.

## Preparation of *Ganoderma lucidum* ethanolic extract

Fresh fruiting bodies of *G. lucidum* were obtained from local cultivators in Purwokerto, Indonesia, and authenticated by the Mycology Laboratory, Faculty of Biology, Jenderal Soedirman University. The fruiting bodies were cleaned, sliced thinly, and dried in an oven at 50°C for 48 hours. The dried material was then ground into powder to obtain simplicia.

Five hundred grams of *G. lucidum* simplicia powder was weighed using an analytical balance and placed in a glass beaker. Maceration was performed using absolute ethanol and distilled water at a ratio of 1:5 (w/v). The mixture was homogenized using a magnetic stirrer for 10 minutes, covered with aluminum foil, and incubated at room temperature for 24 hours. The macerate was filtered through Whatman No. 1 filter paper to obtain the first filtrate. The remaining residue was re-macerated with fresh ethanol solvent under the same conditions. Both filtrates were combined and concentrated using a rotary evaporator (Heidolph, Germany) at 45°C and reduced pressure until a thick extract was obtained [16]. The extract was stored at 4°C until use.

For treatment administration, the ethanolic extract of *G. lucidum* was dissolved in 5% dimethyl sulfoxide (DMSO) and distilled water to achieve final concentrations of 250 mg/kg BW, 500 mg/kg BW, and 750 mg/kg BW. Fresh solutions were prepared daily before administration.

## Preparation of test compounds

Diethylene glycol (DEG) powder (purity ≥99%, Sigma-Aldrich) was dissolved in 0.9% NaCl solution to achieve a final concentration of 5 g/kg BW. The solution was prepared fresh daily and administered via oral gavage at a volume of 1 mL per 200 g body weight.

Fomepizole (4-methylpyrazole, Sigma-Aldrich) was dissolved in sterile distilled water to achieve a concentration of 5 mg/kg BW. The solution was prepared under aseptic conditions and stored at 4°C for no longer than 7 days.

## Blood sample collection and serum preparation

On day 11 of the experiment, rats were anesthetized using ketamine-xylazine (80:10 mg/kg BW, intramuscular injection) after 12 hours of fasting. Blood samples (approximately 4 mL) were collected from the retroorbital plexus using heparinized hematocrit capillary tubes. Blood was immediately transferred to sterile Eppendorf tubes and allowed to clot at room temperature for 30 minutes.

Serum was separated by centrifugation at 3,000 rpm for 15 minutes at 4°C using a refrigerated centrifuge (Eppendorf 5424R, Germany). The supernatant (serum) was carefully transferred to new sterile tubes using micropipettes and stored at -20°C until biochemical analysis.

## Biochemical analyses

All biochemical analyses were performed at the Toxicology Laboratory, Faculty of Biology, Jenderal Soedirman University, and Medico Labora Laboratory, Purwokerto, Indonesia.

## β2-Microglobulin (β2-M) measurement

Serum β2-M levels were measured using a rat-specific enzyme-linked immunosorbent assay

(ELISA) kit (BT Laboratories, Shanghai, China) according to the manufacturer's protocol. Briefly, 50 µL of standard or sample was added to each well, followed by incubation at 37°C for 60 minutes. After washing, 100 µL of horseradish peroxidase (HRP)-conjugated detection antibody was added and incubated for 30 minutes. Following another wash step, 100 µL of tetramethylbenzidine (TMB) substrate was added and incubated for 15 minutes in the dark. The reaction was stopped with 50 µL of stop solution, and absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA). Results were expressed as ng/mL.

### Malondialdehyde (MDA) measurement

Serum MDA levels were determined using the thiobarbituric acid reactive substances (TBARS) method. Briefly, 200 µL of serum was mixed with 1 mL of 10% trichloroacetic acid (TCA) and centrifuged at 3,000 rpm for 10 minutes. The supernatant (500 µL) was mixed with 500 µL of 0.67% thiobarbituric acid (TBA) and heated in a boiling water bath for 15 minutes. After cooling, absorbance was measured at 532 nm using a spectrophotometer (Shimadzu UV-1800, Japan). MDA concentration was calculated using the molar extinction coefficient ($1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$) and expressed as µmol/L.

### Electrolyte measurement

Serum sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) concentrations were measured using the ion-selective electrode (ISE) method with an automated electrolyte analyzer (Medica EasyLyte Plus, USA) according to the manufacturer's instructions. Results were expressed as mEq/L. Quality control samples were run with each batch to ensure accuracy and precision.

### Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test.

Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was performed as a post-hoc test to identify specific group differences. Results were considered statistically significant at $p < 0.05$.

## Results

### β2-Microglobulin levels

The effect of *G. lucidum* extract on serum β2-M levels in DEG-induced rats is presented in Figure 1. The positive control group (C2) exposed to DEG showed significantly elevated β2-M levels (0.73 ng/mL) compared to the healthy control group (C1, 0.29 ng/mL) ($p < 0.05$), indicating severe renal tubular damage. Treatment with *G. lucidum* extract at all doses significantly reduced β2-M levels compared to the positive control. The β2-M levels in treatment groups were as follows: 250 mg/kg BW (C3, 0.65 ng/mL), 500 mg/kg BW (C4, 0.37 ng/mL), and 750 mg/kg BW (C5, 0.35 ng/mL). The fomepizole treatment group (C6) showed β2-M levels of 0.36 ng/mL. Statistical analysis revealed that *G. lucidum* extract at doses of 500 mg/kg BW and 750 mg/kg BW were not significantly different from fomepizole treatment ($p > 0.05$), demonstrating comparable efficacy to the standard therapeutic agent.

### Malondialdehyde levels

The effect of *G. lucidum* extract on serum MDA levels in DEG-induced rats is shown in Figure 2. DEG exposure in the positive control group (C2) resulted in a marked increase in MDA levels (3.368 µmol/L) compared to the healthy control (C1, 0.72 µmol/L) ($p < 0.05$), indicating significant oxidative stress and lipid peroxidation. Administration of *G. lucidum* extract effectively reduced MDA levels in all treatment groups: 250 mg/kg BW (C3, 1.95 µmol/L), 500 mg/kg BW

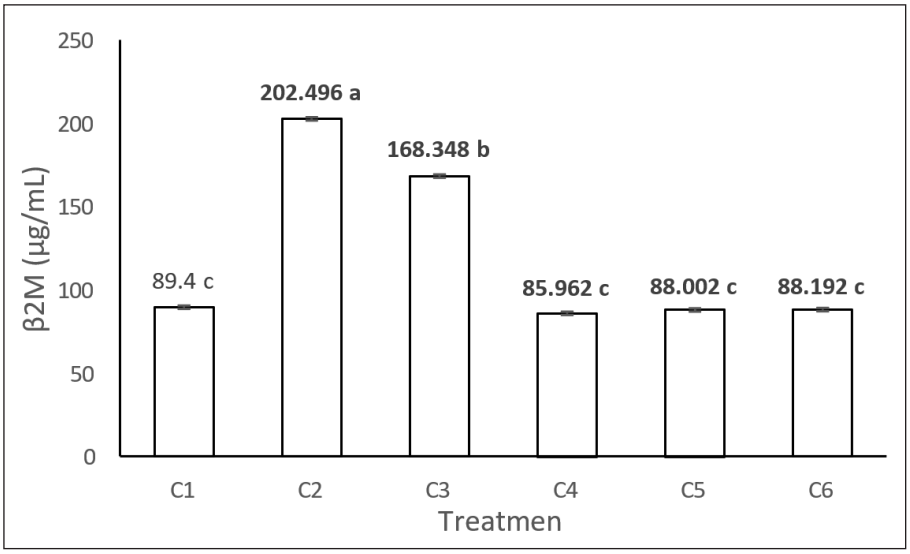


**Figure 1. Effect of *Ganoderma lucidum* ethanolic extract on serum β2-microglobulin levels in diethylene glycol-induced rats.** Data are presented as mean ± SD (n = 4 per group). Different letters above bars indicate significant differences among groups according to Duncan's Multiple Range Test ($p < 0.05$). C1: healthy control; C2: DEG-induced positive control (5 g/kg BW); C3: *G. lucidum* 250 mg/kg BW + DEG; C4: *G. lucidum* 500 mg/kg BW + DEG; C5: *G. lucidum* 750 mg/kg BW + DEG; C6: fomepizole 5 mg/kg BW + DEG.

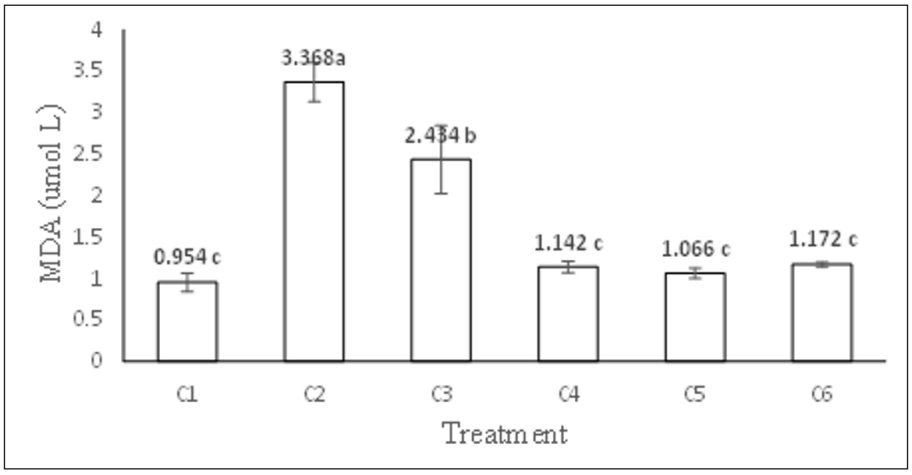


**Figure 2. Effect of *Ganoderma lucidum* ethanolic extract on serum malondialdehyde levels in diethylene glycol-induced rats.** Data are presented as mean ± SD (n = 4 per group). Different letters above bars indicate significant differences among groups according to Duncan's Multiple Range Test ($p < 0.05$). C1: healthy control; C2: DEG-induced positive control (5 g/kg BW); C3: *G. lucidum* 250 mg/kg BW + DEG; C4: *G. lucidum* 500 mg/kg BW + DEG; C5: *G. lucidum* 750 mg/kg BW + DEG; C6: fomepizole 5 mg/kg BW + DEG.

(C4, 0.94 µmol/L), and 750 mg/kg BW (C5, 0.79 µmol/L). The fomepizole group (C6) demonstrated MDA levels of 0.85 µmol/L. ANOVA followed by Duncan's test showed that *G. lucidum* extract at 500 mg/kg BW and 750 mg/kg BW produced MDA levels statistically similar to fomepizole ($p > 0.05$) and approaching normal values observed in healthy controls.

## Potassium levels

Serum potassium levels across treatment groups are presented in Figure 3. The positive control group (C2) exhibited severe hypokalemia with potassium levels of 2.276 mEq/L, significantly lower than the healthy control (C1, 5.12 mEq/L) and below the normal physiological range (3.5-5.0 mEq/L) ($p < 0.05$). Treatment with *G. lucidum*

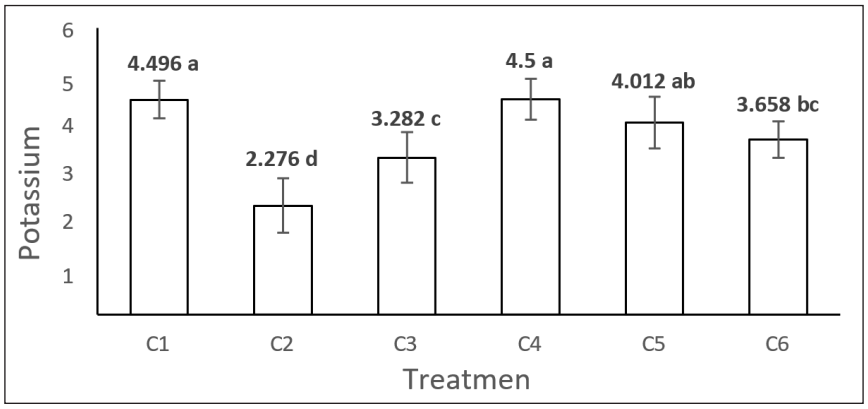


**Figure 3. Effect of *Ganoderma lucidum* ethanolic extract on serum potassium levels in diethylene glycol-induced rats.** Data are presented as mean ± SD (n = 4 per group). Different letters above bars indicate significant differences among groups according to Duncan's Multiple Range Test ($p < 0.05$). Normal physiological range for potassium is 3.5-5.0 mEq/L. C1: healthy control; C2: DEG-induced positive control (5 g/kg BW); C3: *G. lucidum* 250 mg/kg BW + DEG; C4: *G. lucidum* 500 mg/kg BW + DEG; C5: *G. lucidum* 750 mg/kg BW + DEG; C6: fomepizole 5 mg/kg BW + DEG.

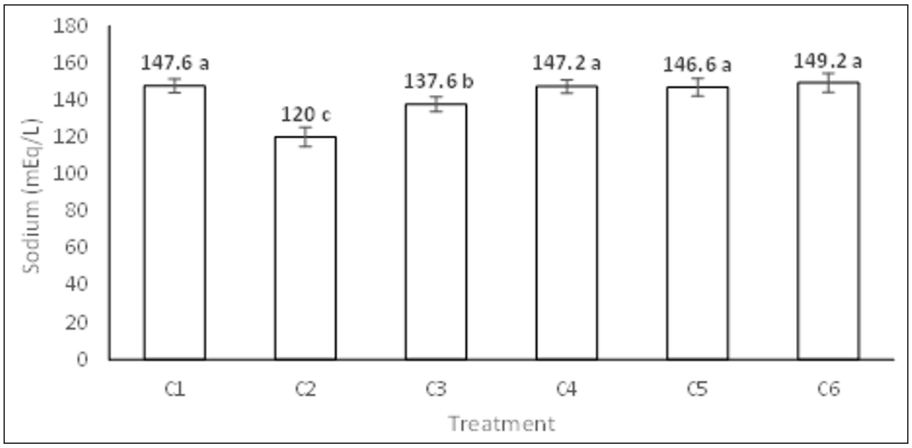


**Figure 4. Effect of *Ganoderma lucidum* ethanolic extract on serum sodium levels in diethylene glycol-induced rats.** Data are presented as mean ± SD (n = 4 per group). Different letters above bars indicate significant differences among groups according to Duncan's Multiple Range Test ($p < 0.05$). Normal physiological range for sodium is 135-145 mEq/L. C1: healthy control; C2: DEG-induced positive control (5 g/kg BW); C3: *G. lucidum* 250 mg/kg BW + DEG; C4: *G. lucidum* 500 mg/kg BW + DEG; C5: *G. lucidum* 750 mg/kg BW + DEG; C6: fomepizole 5 mg/kg BW + DEG.

extract progressively restored potassium levels: 250 mg/kg BW (C3, 2.94 mEq/L), 500 mg/kg BW (C4, 4.16 mEq/L), and 750 mg/kg BW (C5, 4.53 mEq/L). The fomepizole treatment (C6) resulted in potassium levels of 4.05 mEq/L. Statistical analysis indicated that *G. lucidum* extract at 500 mg/kg BW and 750 mg/kg BW achieved potassium restoration comparable to fomepizole treatment ($p > 0.05$), with values returning to the normal physiological range.

## Sodium levels

The effect of treatments on serum sodium levels is displayed in Figure 4. DEG-induced rats in the positive control group (C2) developed hyponatremia with sodium levels of 120 mEq/L, significantly lower than healthy controls (C1, 142 mEq/L) and below the normal range (135-145 mEq/L) ($p < 0.05$). Administration of *G. lucidum* extract dose-dependently increased sodium levels: 250 mg/kg BW (C3, 128 mEq/L), 500 mg/kg BW (C4, 139 mEq/L), and 750 mg/kg BW (C5, 141 mEq/L). The fomepizole group (C6) showed sodium levels of 138 mEq/L. Duncan's post-hoc test revealed that *G. lucidum* extract at doses of 500 mg/kg BW and 750 mg/kg BW were not significantly different from fomepizole treatment ($p > 0.05$), effectively restoring sodium homeostasis to near-normal levels.

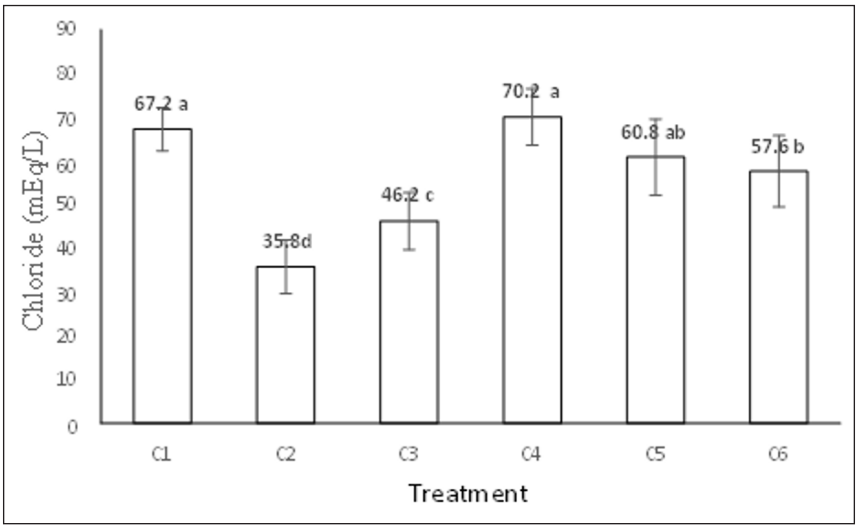


**Figure 5. Effect of *Ganoderma lucidum* ethanolic extract on serum chloride levels in diethylene glycol-induced rats.** Data are presented as mean ± SD (n = 4 per group). Different letters above bars indicate significant differences among groups according to Duncan's Multiple Range Test ($p < 0.05$). C1: healthy control; C2: DEG-induced positive control (5 g/kg BW); C3: *G. lucidum* 250 mg/kg BW + DEG; C4: *G. lucidum* 500 mg/kg BW + DEG; C5: *G. lucidum* 750 mg/kg BW + DEG; C6: fomepizole 5 mg/kg BW + DEG.

## Chloride levels

Serum chloride concentrations across all treatment groups are shown in Figure 5. The positive control group (C2) exhibited hypochloremia with chloride levels significantly reduced compared to healthy controls (C1) ($p < 0.05$). Treatment with *G. lucidum* extract resulted in dose-dependent restoration of chloride levels: 250 mg/kg BW (C3), 500 mg/kg BW (C4), and 750 mg/kg BW (C5). The fomepizole treatment group (C6) also demonstrated improved chloride levels. Statistical analysis showed that all doses of *G. lucidum* extract significantly increased chloride levels compared to the positive control ($p < 0.05$). Furthermore, extract doses of 500 mg/kg BW and 750 mg/kg BW were not significantly different from fomepizole treatment ($p > 0.05$), indicating comparable therapeutic efficacy in restoring electrolyte balance.

## Discussion

The present study demonstrates that ethanolic extract of *G. lucidum* provides significant nephroprotective effects against DEG-induced acute kidney injury in rats, as evidenced by improvements in renal function markers (β2-M and MDA) and restoration of electrolyte homeostasis (sodium, potassium, and chloride). Notably, *G. lucidum* extract at doses of 500 mg/kg BW and 750 mg/kg BW showed therapeutic efficacy comparable to fomepizole, the standard pharmaceutical treatment for glycol poisoning.

The elevation of β2-M levels in DEG-exposed rats reflects impaired glomerular filtration and tubular dysfunction, consistent with acute kidney injury pathophysiology. β2-M, a low-molecular-weight protein (11.8 kDa), is normally filtered by glomeruli and efficiently reabsorbed by proximal tubular cells [17]. When renal tubules are damaged, reabsorption capacity decreases, resulting in elevated serum β2-M concentrations. The reduction of β2-M levels following *G. lucidum* treatment suggests preservation of tubular function and restoration of protein reabsorption capacity. This protective effect may be attributed to ergothioneine, a sulfur-containing amino acid abundant in *G. lucidum* fruiting bodies ([15,18]. Ergothioneine, derived from thiourea and histidine, contains a sulfur atom in its imidazole ring structure, which may facilitate cellular uptake and provide substrate for protein synthesis, thereby supporting tubular cell regeneration and function.

The marked increase in MDA levels observed in DEG-exposed rats indicates severe oxidative

stress and lipid peroxidation in renal tissue. DEG undergoes hepatic metabolism to produce toxic metabolites, including 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA), both of which generate reactive oxygen species (ROS) and induce lipid peroxidation in proximal tubular cells [5,6]. The ability of *G. lucidum* extract to significantly reduce MDA levels can be attributed to its rich content of triterpenoids and flavonoids, which function as potent antioxidants [19,20]. These compounds donate hydrogen atoms to neutralize free radicals, thereby interrupting lipid peroxidation cascades and protecting cellular membranes from oxidative damage. The near-normalization of MDA levels at higher extract doses (500 and 750 mg/kg BW) suggests robust antioxidant capacity comparable to fomepizole's protective mechanism.

The development of hypokalemia, hyponatremia, and hypochloremia in DEG-exposed rats reflects severe disruption of renal electrolyte regulation, a hallmark of acute kidney injury. Normal kidney function requires intact tubular reabsorption and secretion mechanisms to maintain electrolyte balance [21]. DEG-induced tubular damage compromises these mechanisms, leading to excessive electrolyte loss and metabolic acidosis [5,22]. The observed hypokalemia (2.276 mEq/L vs. normal 3.5-5.0 mEq/L) represents a particularly dangerous condition that can lead to cardiac arrhythmias and muscle weakness.

*G. lucidum* extract effectively restored electrolyte homeostasis through multiple mechanisms. First, the mushroom contains significant amounts of essential minerals including sodium, potassium, calcium, magnesium, and phosphorus [14], which can directly supplement depleted electrolyte stores. Second, by reducing oxidative damage and supporting tubular cell function (as evidenced by reduced β2-M and MDA levels), *G. lucidum* may enhance tubular reabsorption capacity for electrolytes. The dose-dependent restoration of sodium, potassium, and chloride levels, with optimal effects at 500-750 mg/kg BW, suggests both direct mineral supplementation and functional improvement of renal tubular mechanisms.

The comparable efficacy of *G. lucidum* extract (500 and 750 mg/kg BW) with fomepizole represents a significant finding with potential clinical implications. Fomepizole, a competitive inhibitor of alcohol dehydrogenase, prevents the conversion of glycols to toxic metabolites and is considered the gold standard for treating ethylene glycol and DEG poisoning [7]. However, fomepizole is expensive and requires intravenous administration, limiting its accessibility in resource-poor settings where DEG contamination of medications is most problematic. The demonstration that *G. lucidum* extract achieves similar therapeutic outcomes suggests it could serve as a complementary or alternative therapy, particularly in regions with limited access to advanced medical care.

The dose-response relationship observed in this study indicates that 500 mg/kg BW represents the optimal therapeutic dose, offering maximal benefit without requiring higher doses. While the 250 mg/kg BW dose showed beneficial effects compared to untreated controls, it did not achieve the same level of efficacy as higher doses or fomepizole treatment. The similarity in outcomes between 500 and 750 mg/kg BW doses suggests a plateau effect, where additional increases in dosage do not confer proportional benefits, likely due to saturation of relevant biochemical pathways or absorption limitations.

These findings support the potential development of *G. lucidum*-based interventions for DEG-induced nephrotoxicity, particularly in pediatric populations where such poisoning events have caused significant morbidity and mortality. The mushroom's well-established safety profile, widespread cultivation, and traditional use in Asian medicine facilitate its potential translation to clinical applications. However, several considerations warrant attention. First, the bioactive compound composition of *G. lucidum* can vary based on cultivation conditions, extraction methods, and fruiting body maturity, necessitating standardization protocols for therapeutic preparations. Second, while this animal model demonstrates proof-of-concept, human studies would need to establish appropriate

dosing, pharmacokinetics, and safety profiles in poisoning contexts.

Future research should investigate the molecular mechanisms underlying *G. lucidum*'s nephroprotective effects, including specific signaling pathways involved in antioxidant defense, cellular repair, and electrolyte regulation. Additionally, studies examining the temporal aspects of treatment—such as optimal timing of administration relative to toxin exposure and duration of treatment required for complete recovery—would enhance clinical utility. Comparative studies with other natural compounds possessing nephroprotective properties could identify synergistic combinations for enhanced efficacy. Finally, histopathological examination of kidney tissue could provide direct evidence of structural protection and regeneration following *G. lucidum* treatment.

This study has several limitations that should be acknowledged. First, the relatively short treatment duration (7 days) and observation period (11 days) may not fully capture long-term effects or potential delayed toxicity. Second, while we measured key biomarkers of kidney function and oxidative stress, direct histopathological examination of kidney tissue would provide more comprehensive assessment of structural damage and repair. Third, this study did not investigate the specific bioactive compounds responsible for the observed effects or their individual contributions to nephroprotection. Fourth, the study was conducted in healthy young male rats, which may not fully represent the human pediatric population most at risk for DEG poisoning or account for sex-related differences in drug metabolism and toxicity responses.

## Conclusion

This study demonstrates that ethanolic extract of *G. lucidum* effectively protects against DEG-induced nephrotoxicity in rats by reducing oxidative stress, preserving renal tubular function, and restoring electrolyte homeostasis. The optimal therapeutic dose was determined to be 500 mg/kg BW, which achieved efficacy comparable to fomepizole across all measured parameters. These findings suggest that *G. lucidum* may represent a promising natural therapeutic option for managing acute kidney injury induced by glycol poisoning, particularly in settings where conventional treatments are unavailable or inaccessible. Further research is warranted to translate these findings to clinical applications and elucidate the specific molecular mechanisms underlying the nephroprotective effects of *G. lucidum*.

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## Declaration of interest

The authors declare no conflicts of interest with any private, public, or academic entities related to the information contained in this manuscript. No financial or personal relationships influenced the work reported in this paper.

## Author contributions

H: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing, Investigation. HDKT: Data curation, Formal analysis, Investigation,

Methodology, Resources, Validation, Writing – original draft.

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## References

1. Kaur J, Kyle PB. Ethylene glycol toxicity. Toxicology Cases for the Clinical and Forensic Laboratory. Elsevier; 2022. pp. 51-54. https://doi.org/10.1016/B978-0-12-815846-3.00031-4
2. Madathodi ARK, Andrews MA, Madhavan I. Ethylene glycol poisoning: An unusual cause of hyperglycemia-A case report. Asia Pac J Med Toxicol. 2015;4: 55-57.
3. Petejova N, Martinek A, Zadrazil J, Teplan V. Acute toxic kidney injury. Ren Fail. 2019;41: 576-594. https://doi.org/10.1080/0886022X.2019.1628780
4. Tanasescu A, Macovei RA, Tudosie MS. Outcome of patients in acute poisoning with ethylene glycol-Factors which may have influence on evolution. J Med Life. 2014;7: 81-86.
5. Landry GM, Martin S, Mcmartin KE. Diglycolic acid is the nephrotoxic metabolite in diethylene glycol poisoning inducing necrosis in human proximal tubule cells in vitro. Toxicological Sciences. 2011;124: 35-44. https://doi.org/10.1093/toxsci/kfr204
6. Robinson CN, Latimer B, Abreo F, Broussard K, McMartin KE. In-vivo evidence of nephrotoxicity and altered hepatic function in rats following administration of diglycolic acid, a metabolite of diethylene glycol. Clin Toxicol (Phila). 2017;55: 196-205. https://doi.org/10.1080/15563650.2016.1271128
7. Kruse JA. Methanol and ethylene glycol intoxication. Crit Care Clin. 2012;28: 661-671. https://doi.org/10.1016/j.ccc.2012.07.002
8. Salvatore MM, Elvetico A, Gallo M, Salvatore F, Dellagreca M, Naviglio D, et al. Fatty acids from Ganoderma lucidum spores: Extraction, identification and quantification. Applied Sciences. 2020;10: 1-12. https://doi.org/10.3390/app10113907
9. Ahmad MF. Ganoderma lucidum: Persuasive biologically active constituents and their health endorsement. Biomedicine & Pharmacotherapy. 2018;107: 507-519. https://doi.org/10.1016/j.biopha.2018.08.036
10. Baby S, Johnson AJ, Govindan B. Secondary metabolites from Ganoderma. Phytochemistry. 2015;114: 66-101. https://doi.org/10.1016/j.phytochem.2015.03.010
11. Zhong L, Yan P, Lam WC, Yao L, Bian Z. Coriolus versicolor and Ganoderma lucidum related natural products as an adjunct therapy for cancers: A systematic review and meta-analysis of randomized controlled trials. Front Pharmacol. 2019;10. https://doi.org/10.3389/fphar.2019.00703
12. Adeyi AO, Awosanya SA, Adeyi OE, James AS, Adenipekun CO. Ganoderma lucidum ethanol extract abrogates metabolic syndrome in rats: In vivo evaluation of hypoglycemic, hypolipidemic, hypotensive and antioxidant properties. Obes Med. 2021;22: 1-12. https://doi.org/10.1016/j.obmed.2021.100320
13. Bishop KS, Kao CH, Xu Y, Glucina MP, Paterson RR, Ferguson LR. From 2000 years of Ganoderma lucidum to recent developments in nutraceuticals. Phytochemistry. 2015;114: 56-65. https://doi.org/10.1016/j.phytochem.2015.02.015
14. Lin MS, Yu ZR, Wang BJ, Wang CC, Weng YM, Koo M. Bioactive constituent characterization and antioxidant activity of Ganoderma lucidum extract fractionated by supercritical carbon dioxide. Sains Malays. 2015;44: 1685-1691.
15. Chen S. The pharmacological effects of triterpenoids from Ganoderma lucidum and the regulation of its biosynthesis. Adv Biol Chem. 2020;10: 55-65. https://doi.org/10.4236/abc.2020.102005
16. Ratnaningtyas NI, Andarwati S, Ekowati N, Purwati ES, Sukmawati D. Effects of Ganoderma lucidum extract on diabetic rats. Biosaintifika: Journal of Biology & Biology Education. 2018;10: 642-647. https://doi.org/10.15294/biosaintifika.v10i3.15356
17. Liabeuf S, Lenglet A, Desjardins Lucie and Neirynck N, Glorieux G, Lemke H-D, Vanholder R, et al. Plasma beta-2 microglobulin is associated with cardiovascular disease in uremic patients. Kidney Int. 2012;82: 1297-1303. https://doi.org/10.1038/ki.2012.301
18. Kalaras MD, Richie JP, Calcagnotto A, Beelman RB. Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. Food Chem. 2017;233: 429-433. https://doi.org/10.1016/j.foodchem.2017.04.109
19. Rohmah M, Sayuti K, Wahyuni D. Antioxidant activity of red dragon fruit peel extract (Hylocereus polyrhizus). Jurnal Teknologi Pertanian Andalas. 2014;18: 35-39.
20. Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: A review of recent research. Carbohydr Polym. 2019;90: 1395-1410. https://doi.org/10.1016/j.carbpol.2012.07.026
21. Ashman N, Yaqoob M. Metabolic acidosis, hypokalaemia and acute renal failure with a normal urine output. Nephrology Dialysis Transplantation. 2000;15: 1083-1085. https://doi.org/10.1093/ndt/15.7.1083
22. Seo JW, Lee JH, Son IS, Kim YJ, Kim DY, Hwang Y, et al. Acute oxalate nephropathy caused by ethylene glycol poisoning. Kidney Res Clin Pract. 2012;31: 249-252. https://doi.org/10.1016/j.krcp.2012.09.007