



ORIGINAL ARTICLE

Nephroprotective effect and untargeted metabolomic analysis of celery extract against diethylene glycol induced nephrotoxicity in rats

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ABSTRACT

BACKGROUND

Diethylene glycol (DEG) is known for its nephrotoxic effects as a contaminant. Recent investigations suggest that celery extract potentially protects against DEG-induced renal toxicity, warranting an exploration of the non-targeted metabolomic profile of celery in renal tissue. This study aimed to determine celery nephroprotective effects and identify potential biomarkers using an untargeted metabolomic celery profile in DEG-induced kidney injury in male rats.

METHODS

An experimental study was conducted involving 24 male Wistar rats, randomized into control and three treatment groups receiving DEG at doses of 1, 3, and 5 g/BW for three days. After 14 days, all rats were sacrificed. For the celery nephroprotective and metabolomic study, 27 male rats were randomized into three groups: control, DEG group at 3g/BW, and DEG-celery group (receiving celery extract 200mg/kg BW single dose for 6 days). After 14 days, blood was tested for creatinine level and untargeted metabolomic analysis using liquid chromatography-high resolution mass spectrometry. One-way ANOVA was used to analyze creatinine data.

RESULTS

Diethylene glycol at doses of 1 and 3 g/kg BW caused mild renal damage, while 5 g/kg BW resulted in moderate damage. Creatinine levels in the normal, DEG-induced, and DEG-celery groups were 0.55 ± 0.14 , 0.71 ± 0.1 , and 0.86 ± 0.17 mg/dL, respectively, but not statistically significantly different between groups ($p > 0.05$). DEG lead tryptophan and phenylalanine levels increased 45-fold and 123-fold, respectively. Celery reduced levels of these metabolites 21-fold and 65-fold, respectively.

CONCLUSION

Diethylene glycol led to mild to moderate renal damage. Celery effectively diminished metabolite levels associated with renal damage, specifically tryptophan and phenylalanine.

Keywords: Diethylene glycol, celery, histopathology, metabolomics, rats

INTRODUCTION

Diethylene glycol (DEG) is a clear, colorless, odorless, sweet-tasting, viscous, and hygroscopic liquid miscible in water, alcohol, ether, acetone, and ethylene glycol. Diethylene glycol is widely employed in the chemical industry as a solvent, lubricant, and component in various consumer products, including antifreeze, brake fluids, and cosmetics.⁽¹⁾ Contamination of medicinal syrups with DEG can result in significant poisoning incidents. In substantial quantities (1–1.63 g/kg BW), DEG exhibits nephrotoxic properties and poses a potentially fatal risk to humans.⁽²⁾

Clinically, DEG poisoning evolves through three distinct phases. The initial phase is characterized by gastrointestinal symptoms, inebriation, and metabolic acidosis. The subsequent phase is marked by exacerbated metabolic acidosis, hepatotoxicity, and renal failure, which without appropriate supportive care often culminate in death.⁽³⁾ Diethylene glycol is metabolized in the bloodstream to 2-hydroxyethoxyacetaldehyde, which subsequently converts into 2-hydroxyethoxyacetic acid (2-HEAA) and ultimately into diglycolic acid (DGA).⁽⁴⁾ The accumulation of DGA to toxic levels results in proximal tubular necrosis, as it is the nephrotoxic agent implicated in DEG poisoning. The kidneys endure various forms of damage, including tubular toxicity, inflammation, glomerular injury, crystal nephropathy, and microangiopathy.⁽⁵⁾ Notable characteristics of glomerular damage include enlargement or narrowing of the glomerulus and the capsular space, as well as the presence of red blood cell casts. Tubular damage is characterized by degeneration, indistinct lumens, and necrotic cells.⁽⁶⁾

Diethylene glycol and other toxic compounds are among the many variables that can cause reduced functioning of the kidneys. According to Jamison et al.⁽⁷⁾ on the neurotoxicity and nephrotoxicity of DEG, male rats can develop nephrotoxicity beginning about 48 hours after receiving an oral dose of 4 g/kg to 6 g/kg administered every 12 hours.

Celery (*Apium graveolens*) has been reported in a previous study to exhibit nephroprotective effects.⁽⁸⁾ This plant contains antioxidant compounds, particularly flavonoids such as apigenin and luteolin. The aforementioned study demonstrated that celery displays

nephroprotective properties against renal damage induced by nephrotoxic agents, as evidenced by significant reductions in biochemical kidney parameters, including creatinine.⁽⁸⁾ Creatinine, a waste product of muscle metabolism, is filtered by the kidneys and excreted in urine. In instances of DEG poisoning, impaired renal function results in the accumulation of creatinine in the bloodstream, leading to elevated serum creatinine levels. This elevation serves as a critical diagnostic criterion for assessing the severity of renal failure.^(8,9)

An *in vivo* test that was conducted on male Wistar rats showed that the ethyl acetate fraction of celery had an effective activity at a dose of 150 mg/kg BW which was significantly different from the negative control group ($p < 0.05$) as measured by a reduction in urea and creatinine levels in male Wistar rats induced by ethylene glycol.⁽¹⁰⁾ Previous studies did not detail histopathological kidney damage and its metabolite profile. Histopathological damage can be observed through several parameters, comprising endothelial, glomerular, tubular, and interstitial (EGTI) components. Metabolite profiles can provide information on which compounds cause kidney damage. The present study employed an untargeted metabolomic approach to analyze the metabolite profile of samples, encompassing amino acids, sugars, lipids, and nucleotides. Metabolomic analysis was utilized in earlier celery research to analyze the content of different types and enhance the storage method.^(11,12) Based on the above background, research is needed to explain histopathological kidney damage and its metabolite profile. The objective of this study was to determine nephroprotective effects and identification of potential biomarkers using untargeted metabolomics for celery in DEG-induced kidney injury.

METHODS

Research design

A post-test only laboratory experimental study with control, conducted from July – December 2023 in the Pharmacology Laboratory, Pharmacy Department, Faculty of Health Sciences, Integrated Research Laboratory, Jenderal Soedirman University, Purwokerto, the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada and the Police Forensic Laboratory Center, Bogor, Indonesia.

Plant material

Fresh celery was collected from Tawangmangu and the conversion of the simplicia into powder form was performed in the Tawangmangu Traditional Health Service - Pharmacy Service Unit Testing Laboratory.

Preparation of celery extract

Five hundred grams of celery powder was extracted using 96% ethanol at a 1:5 ratio for initial maceration, followed by extractions at a 1:4 ratio over three 24-hour periods. Filtration was conducted every 24 hours, and the resultant filtrate was concentrated using a rotary evaporator, yielding a solvent-free, viscous extract.

Experimental animals

Male Sprague Dawley rats, aged 2 months and weighing approximately 200 grams, were procured from the Faculty of Pharmacy, Muhammadiyah Purwokerto University, Indonesia. The rats were acclimatized for five days, provided with standard pelleted feed, allowed access to water ad libitum, and maintained under a 12:12-hour light-dark cycle. The sample size (number of study subjects) was determined based on the number of experimental repetitions, calculated using Federer's formula: $(k-1)(n-1) \geq 15$, where n = number of samples and k = number of treatments.

The nephrotoxicity study was conducted involving 24 male Wistar rats divided into 4 groups, while for the celery nephroprotective and metabolomic study, 27 rats were randomized into three groups.

Nephrotoxicity study on rats induced by DEG Experimental procedure

A post-test only control group design was employed. After acclimatization, the rats were randomized into four groups. Group I served as the control group, receiving only food and water. Groups II, III, and IV were administered diethylene glycol (DEG) orally at doses of 1, 3, and 5 g/kg body weight (BW) twice daily for three consecutive days. The weight of each rat was recorded every day. On day 14, all rats were sacrificed, and histopathological assessment of the kidneys was conducted using hematoxylin-eosin (H&E) staining.

Hematoxylin-eosin (H&E) staining

On day 14, all rats were euthanized via cervical dislocation. The kidneys and livers were

excised and fixed in 10% buffered formalin. Following fixation, tissue samples measuring 3–5 cm were embedded in paraffin, sectioned, and stained with H&E for histological examination. The staining procedure was performed by the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada.

Two trained researchers, under the supervision of a pathologist, independently evaluated the specimens using a binocular microscope (Optilab digital camera system) at 400x magnification.

Nephroprotective effect and metabolomic study of celery

Creatinine level and potential biomarker analysis was conducted using 27 rats divided into three groups, comprising the control group (receiving only food and water), DEG group (oral DEG at 3 g/kg BW, twice daily for three days), and celery treatment group (administered DEG orally at 3 g/kg BW twice daily for three days along with celery extract at a dose of 200 mg/kg BW, orally twice daily for six days). The celery extract was diluted, and rats weighing approximately 200 g were administered a dose of 40 mg/kg BW in 2 mL volume, dissolved in 0.1% Tween 80.

Blood plasma collection

Blood was collected via the orbital sinus region of the eye using a microhematocrit, placed in an EDTA vacutainer tube, and rotated until the EDTA was uniformly distributed. The specimen was then centrifuged at 4000 rpm for 15 minutes at 4°C to obtain clear plasma while minimizing damage to the cells and analytes. After plasma separation, specimens from the same treatment group were pooled.⁽¹³⁾

Among the three animal test groups, serum creatinine levels were measured for each rat. Normal serum creatinine levels in rats, as determined by the Jaffe method, range from 0.4 to 0.8 mg/dL.⁽¹⁴⁾

Identification of untargeted metabolomics using MS-DIAL

The spectral data from liquid chromatography-high-resolution mass spectrometry (LC-HRMS) analysis were converted into centroid format with .mzML extension using MSConvert (ProteoWizard Software Foundation, Palo Alto, US). The conversion continued from .mzML to .abf format using the ABF Converter (Reifycs Inc., Tokyo,

Japan). The .abf file was analyzed using MS-DIAL version 4.9.2 (RIKEN Center for Sustainable Resource Science, Kanagawa, Japan). All Ion Fragmentation (AIF) was employed in the non-targeted metabolomic analysis. Parameters for MS-DIAL included a mass tolerance of 0.01 Da to 0.05 Da, a mass range of 50 to 1000, a maximum nominal charge number of 2, four nominal threads, a minimum peak height of 1000 amplitude, and a mass slice width of 0.05 Da. A linear-weighted moving average with a smoothing level of three scans and a minimum peak width of five scans was configured for peak detection. The sigma window value was set to 0.5, and the mass spectra abundance cut-off was established at 100 amplitude for MS spectrum deconvolution. Ion species for adduct settings included [M+H]⁺, [M+NH₄]⁺, [M+Na]⁺, [M+K]⁺, [M+ACN+H]⁺, [2M+H]⁺, and [M]⁺ in positive mode. For metabolite identification, the was used, with a retention time tolerance of 100 minutes, accurate mass tolerance of 0.01 Da to 0.015 Da, and an identification score cut-off of 65%, reporting only the top hit. The gradient elution table is presented in Table 1.

Analysis using LC-HRMS

The collected plasma was mixed with acetonitrile in a 1:1 ratio and vortexed for 10 seconds, then centrifuged at 1000 rpm for 10 minutes at 4°C. The supernatant was filtered using a 0.22-micron syringe filter and transferred into a 2 mL HPLC autosampler vial.^(13,15) Plasma analysis was conducted using the ACQUITY UPLC® H-Class System LC and the Xevo G2-S Q-ToF mass spectrometer. Electrospray ionization was implemented in positive mode. The analytical column was a C18 ACQUITY UPLC® 1.8µm 2.1x100 mm, with a mobile phase comprising water containing 5 mM ammonium formate and acetonitrile with 0.05% formic acid. The total duration for gradient elution was 30 minutes, at a

flow rate of 0.2 mL/min, with a column temperature of 50°C and room temperature of 25°C. HRMS data were recorded over a mass range of m/z 50-1500, utilizing a low collision energy of 0 V and a high collision energy ramp of 40-70 V.⁽¹⁶⁾

Statistical analysis

The differences in rat body weight across experimental groups were analyzed to evaluate the relationship between treatments and changes in weight. Kidney damage scores were analyzed using the Kruskal-Wallis test, followed by Dunn's test, utilizing JASP. Kidney damage was assessed using the Endothelial Glomerular Tubular Interstitial (EGTI) scoring system, which evaluates endothelial, glomerular, tubular, and interstitial tissues.⁽¹⁷⁾

Creatinine data were analyzed using JASP. The Shapiro-Wilk test assessed normality, and one-way ANOVA evaluated statistical differences, with a p-value <0.05 being considered significant. If significant differences were found, Dunn's post hoc analysis was conducted to determine the degree of difference between group means.

For untargeted metabolomics analysis, data processing was conducted using MS-DIAL version 4.9.2, followed by filtering with MS-CleanR. This generated .csv files accessible via MS Excel for further interpretation. Descriptive visualizations, including tables and diagrams, were employed to present the detected metabolites based on a total identification score ≥70% and their ontology.

Ethical clearance

The animal experiments received approval from the Ethics Committee, Faculty of Health Sciences, Jenderal Soedirman University, under ethical approval number 1108/EC/KEPK/V2023.

Table 1. Gradient elution

Time (minutes)	Flow rate (mL/min)	Eluent A (%) water + ammonium formate (5mM)	Eluent B (%) acetonitrile + 0.05% formic acid
Initial	0.200	97	3
5	0.200	97	3
20	0.200	5	95
22	0.200	5	95
25	0.200	97	3
30	0.200	97	3

RESULTS

Nephrotoxicity study on rats induced by DEG

Body weight measurements were utilized to determine appropriate doses for feed and DEG administration. The average body weight of rats across all experimental groups is illustrated in Figure 1. Histological analysis of rat kidney tissue is presented in Figure 2 and Table 2.

Table 2 illustrates that average tubular tissue damage in normal control rats ranged from 0.07 to 0.32, indicative of minimal damage. In contrast, rats administered diethyleneglycol (DEG) at a dosage of 1 g/kg body weight (BW) displayed damage scores ranging from 2.14 to 2.65, signifying moderate damage. Rats treated with DEG at 3 g/kg BW recorded scores between 2.89 and 2.97, classified as moderate to severe damage, whereas rats receiving DEG at 5 g/kg BW exhibited scores ranging from 3.39 to 3.74, indicating severe damage.

The EGTI scoring system revealed that the variations in interstitial tissue damage across the treatment groups were significantly different ($p < 0.001$). Normal control rats scored between 0.33 and 0.47, while the 1 g/kg BW DEG group exhibited scores ranging from 0.78 to 1.02. Rats receiving DEG at 3 g/kg BW recorded scores between 0.85 and 1.15, and the 5 g/kg BW group had scores ranging from 1.46 to 1.54. These findings suggest an absence of damage in normal control rats, mild interstitial damage in those treated with DEG at 1 g/kg BW and 3 g/kg BW,

and mild to moderate interstitial damage in the 5 g/kg BW group.

Based on statistical analysis, the endothelium did not differ significantly between all groups ($p = 0.093$). In regard to the glomerulus, the normal group and the DEG 1 g/kg BW group did not differ significantly from each other, but differed significantly from the other two groups. The DEG 1 g/kg BW group did not differ significantly from the DEG 3 g/kg BW group, but differed significantly from the DEG 5 g/kg BW group. The DEG 3 g/kg BW group did not differ significantly from the DEG 5 g/kg BW group.

In the results of the statistical analysis of the tubules, the data from the normal group was not different from the DEG 1 g/kg BW group, but was significantly different from the DEG 3 g/kg BW and DEG 5 g/kg BW groups. The 1 g/kg BW group did not differ significantly from the DEG 3 g/kg BW group but was significantly different from the DEG 5 g/kg BW group. The DEG 3 g/kg BW group did not differ significantly from the DEG 5 g/kg BW group. Regarding the interstitium, the results of statistical analysis showed that the normal group was not significantly different from the DEG 1 g/kg BW group, but was significantly different from the DEG 3 g/kg BW and DEG 5 g/kg BW groups. The DEG 1 g/kg BW group was not significantly different from the DEG 3 g/kg BW group but was significantly different from the DEG 5 g/kg BW group. The DEG 3 g/kg BW group was significantly different from the 5 g/kg BW group (Table 2).

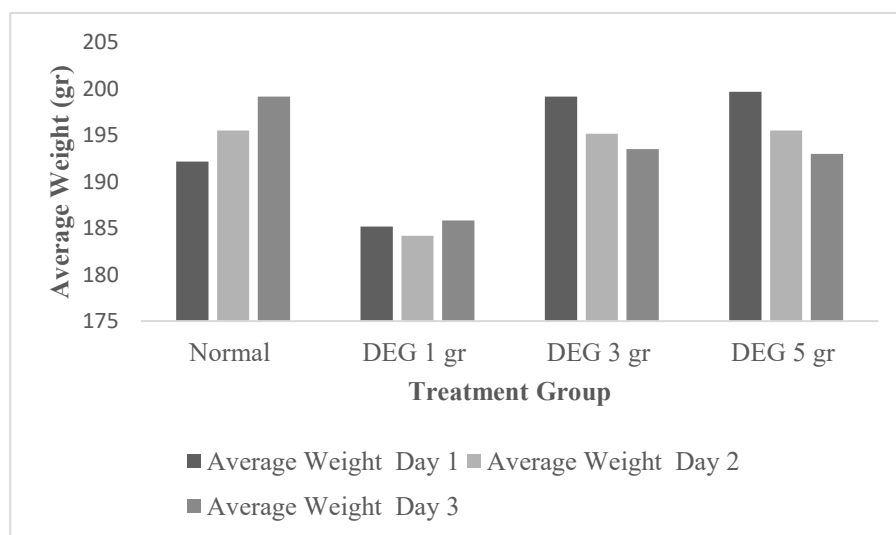


Figure 1. Comparison of mean rat body weight by treatment group on days 1-3

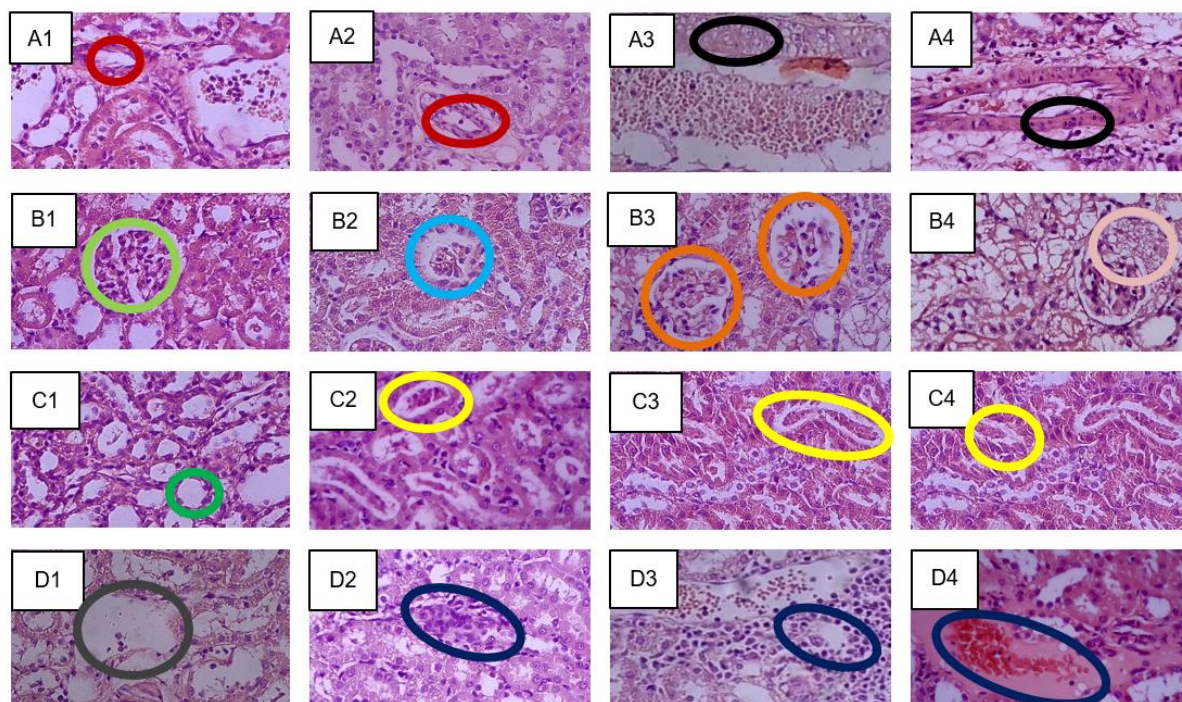


Figure 2. Histopathological profile of rat kidney
 Normal Group (A1-D1), DEG 1g/kg BW (A2-D2), DEG 3 g/kg BW (A3-D3), DEG 5 g/kg BW (A4-
 ● normal endothelium ● endothelial swelling ● normal glomerulus ● glomerular refraction
 ● Bowman's capsule thickening ● glomerular fibrosis ● normal tubules ● tubular necrosis
 ● normal interstitium ● interstitial inflammation

Table 2. Average histological damage based on EGTI scoring after 14 days intervention

	Treatment groups				p value
	Non-DEG induced (n=6)	DEG 1g/kg BW (n=6)	DEG 3 g/kg BW (n=6)	DEG 5 g/kg BW(n=6)	
Endothelium	0.33 ± 0.06	0.43 ± 0.08	0.60 ± 0.08	0.63 ± 0.10	0.093
Glomerulus	0.06 ± 0.06 ^a	1.23 ± 0.29 ^{ac}	2.23 ± 0.12 ^{bc}	2.50 ± 0.10 ^b	< 0.001
Tubule	0.20 ± 0.12 ^a	2.40 ± 0.25 ^{ac}	2.93 ± 0.04 ^{bc}	3.56 ± 0.17 ^b	< 0.001
Interstitialium	0.40 ± 0.07 ^a	0.90 ± 0.12 ^{ac}	1.00 ± 0.15 ^{bc}	1.50 ± 0.04 ^d	< 0.001

Note : Values presented as mean ± SD

The Kruskal-Wallis test for the endothelial parameter resulted in $p > 0.05$ → indicating no significant differences. However, the post hoc analysis Dunn's p indicating significant differences, denoted by the different superscripted letters a - d.

Figure 1 presents a discernible trend of weight gain in rats administered diethylene glycol (DEG) at a dosage of 1 g/kg BW between Day 2 and Day 3 of treatment. In contrast, rats receiving the alternative dosages exhibited weight loss. Body weight changes are deemed adverse when a significant weight loss of 10% from the initial weight is documented. The observed weight gain in rats treated with DEG at 1 g/kg BW may be attributable to the insufficient toxic effects of this dosage, which could have facilitated an improvement in their condition by Day 3.

Furthermore, rats in the normal control group demonstrated appropriate weight gain from Day 1 to Day 3.

Histopathological examination of the kidneys in rats treated with DEG at dosages of 3 g/kg BW and 5 g/kg BW indicated the presence of glomerular atrophy (tuft retraction), characterized by an expanded space between Bowman's capsule and the glomerulus, resulting from glomerular shrinkage. Conversely, rats administered DEG at 1 g/kg BW exhibited mild to moderate glomerular damage. Glomerular atrophy is attributed to the

infiltration of toxic compounds into the glomerular filter, leading to renal morphological shrinkage and damage to tubular cells, which function as barriers to the glomerular filter.

Nephroprotective effect and metabolomic study of celery

Creatinine levels were quantified using the Jaffe method, a colorimetric technique that involves the reaction of creatinine with picric acid in an alkaline solution. This method is based on the formation of a reddish-colored complex in an alkaline environment as creatinine reacts with picric acid. The intensity of the red color is proportional to the concentration of creatinine in the sample, measured using UV spectrophotometry at a wavelength of 510 nm. The average creatinine levels are depicted in Figure 3.

The mean serum creatinine levels (mg/dL) in the DEG group were higher than those in both the DEG-extract and control groups. The creatinine

results were analyzed using JASP software. Given that the data were normally distributed and homogeneous, ANOVA was conducted, resulting in significant differences between normal, DEG, and DEG-extract groups ($p=0.004$), then continued with multiple comparison test.

The raw data from LC-HRMS were converted for compatibility with MS-DIAL software and filtered using MS-CleanR. The filtered data were exported in .csv format for subsequent processing in MS Excel. The results of the non-targeted metabolomics profiling analyzed with MS-DIAL are summarized in Table 3. According to Table 3, all metabolites exhibited an increase following DEG induction in rats. Notably, tryptophan levels significantly increased 45-fold, from 15318 to 697312, while phenylalanine levels demonstrated a 123-fold increase, from 4132.2 to 509126.6. Following the administration of celery extract, both tryptophan and phenylalanine levels decreased substantially.

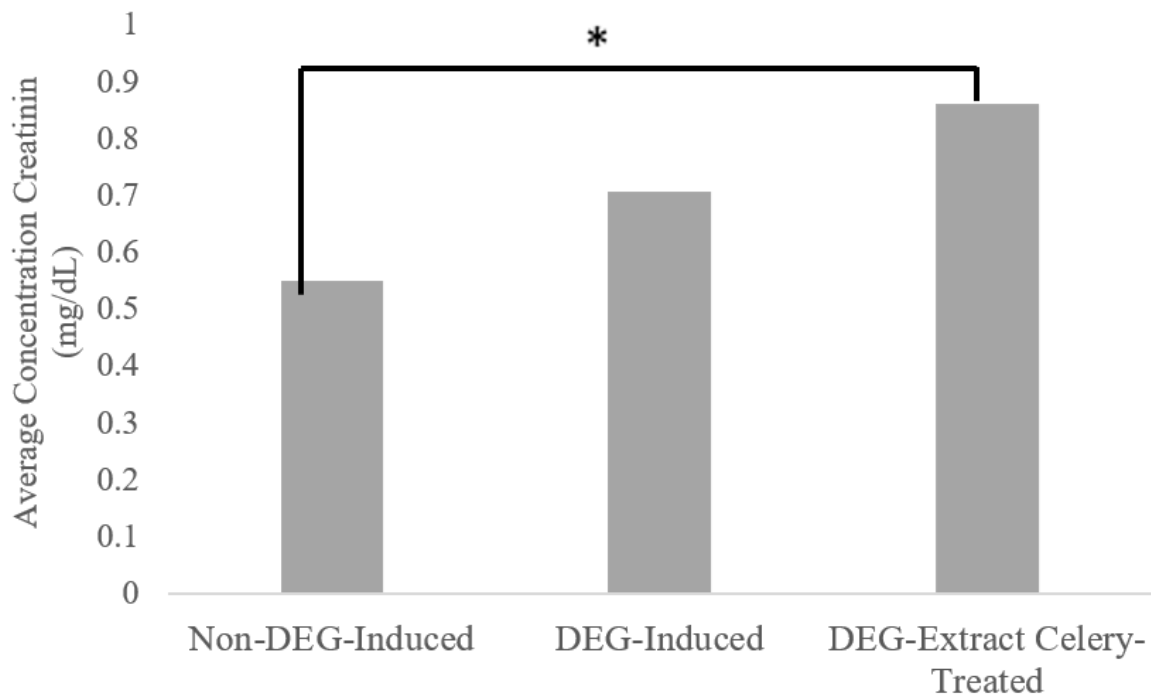


Figure 3. Comparison of mean ceatinine levels between groups after 14 days intervention. The DEG-celery extract-treated group showed significant differences compared to the normal group. * p -value < 0.05 (significant difference)

Note. “DEG-Extract Celery Treated” should be read as “DEG-Celery Extract Treated”

Table 3. Ten representative metabolites with the highest total identification scores in rat plasma in DEG-Celery extract treated, DEG-induced, and non-DEG-induced groups after 14 days intervention

RT	Metabolite name	Average peak area in rat plasma			Total identification score
		DEG-Celery-extract treated	DEG-induced	Non-DEG-induced	
7.29	L-Tryptophan	556264.4	697312.0	15318.0	95.4
6.97	L-Tryptophan	674226.2	1118479.0	43026.0	95.3
18.60	1-pentadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine	4157624.3	5273673.0	15819.0	95.1
3.46	L-Phenylalanine	268058.5	509126.6	4132.2	94.2
20.97	1-Stearoyl-sn-glycero-3-phosphocholine	96043736.0	118535712.0	Not detected	91.9
19.58	PC (16:0/0:0)	11927699.0	12057462.0	Not detected	89.2
4.13	L-Phenylalanine	41860.3	98764.8	37536.5	86.7
20.64	Lyso-PC 18:0	20358629.0	18138694.0	4545.9	85.8
19.68	Lyso-PC 18:1	8376492.8	11625487.0	3274.7	85.3
19.41	Choline	26534307.0	35266000.0	Not detected	84.7

Note : RT : Retention time

DISCUSSION

Over the course of the DEG dosage treatment period, the weight of each rat was recorded every day. The body weight of the rats in the normal group increased compared to the groups receiving DEG at 3 g/kg BW and 5 g/kg BW, respectively. Following exposure to a drug, changes in the test animals' body weight often indicate the toxicity status.⁽¹⁸⁾

While there was no significant damage to endothelial tissue, there was substantial damage to glomerular, tubular, and interstitial characteristics following exposure to DEG. Acute kidney injury (AKI) in the tubules and interstitial tissues represents a physiological response to the toxic effects of drugs or ethylene glycol (EG). The physicochemical characteristics of xenobiotics, their concentration, and the length of time they are in contact with renal tissue, all affect the causes of toxicity and kidney damage. As a result, damage occurs to the glomerulus and capillary microcirculation, which includes the glomerulus, endothelia, and renal tubular epithelial cells. The results indicate that DEG-induced kidney damage predominantly affects tubular and glomerular tissues, characterized by severe tubular necrosis

and glomerular fibrosis.⁽¹⁴⁾ These findings are consistent with previous studies on kidney histopathology following DEG administration, which reported severe acute tubular necrosis affecting both proximal and distal tubules. Tubular necrosis is characterized by microvacuolar degeneration of tubular epithelial cells, progressing to necrosis and detachment from the tubular basement membrane.⁽⁴⁾ Notably, while earlier research did not report interstitial inflammation, our study identified mild interstitial damage.⁽⁴⁾ Furthermore, this investigation observed glomerular fibrosis, a response to tissue injury associated with inflammation and tissue regeneration during the inflammatory process.^(18, 19) Endothelial tissue swelling was also noted, a phenomenon not addressed in Jamison's research. The DEG group's mean blood creatinine levels were higher than those of the celery extract and control groups. The association between creatinine levels has not yet been the subject of any study reports. There have been reports of a nephroprotective effect of celery when using the cancer medication cisplatin. Celery preserved healthy renal function and prevented kidney damage. Better cisplatin clearance from kidney tubules and a decrease in reactive oxygen species

(ROS) generated by the inflammatory response could be the cause.⁽⁸⁾ After DEG induction in rats, all metabolites showed an increase. Significantly, phenylalanine levels showed a 12-fold increase, whereas tryptophan levels rose 45-fold.

The complexity of tryptophan metabolic pathways and the diverse properties of tryptophan-derived metabolites have been associated with various pathophysiological states. Tryptophan metabolism proceeds along three major pathways in the gastrointestinal tract: (1) the kynurenine pathway in both epithelial and immune cells; (2) the serotonin pathway in enterochromaffin cells; and (3) the indole pathway influenced by gut microbiota. Endogenous tryptophan metabolites (e.g., serotonin and melatonin) and microbial tryptophan catabolites (e.g., indole and indole-3-aldehyde) are implicated in hypertension and kidney disease. Tryptophan-derived uremic toxins, primarily from the indole and kynurenine pathways, exhibit prooxidant, proinflammatory, procoagulant, and pro-apoptotic effects. In patients with chronic kidney disease (CKD), serum tryptophan levels are reduced, whereas metabolites of the kynurenine pathway are increased.⁽²⁰⁻²²⁾

In the context of renal failure, various metabolites accumulate in the body due to impaired excretion. Among these are metabolites of tryptophan (TRP), such as indoxyl sulfate and kynurenine, which induce vascular damage during chronic kidney impairment. Experimental evidence suggests that TRP metabolites are implicated in the progression of chronic kidney disease, with oxidative stress identified as a key mechanism exacerbating these conditions. Given that blood levels of these metabolites significantly increase in renal failure and generate reactive oxygen species (ROS) that can lead to endothelial injury, it is plausible that products of TRP metabolism represent a critical link in the frequent occurrence of CKD.⁽²³⁾ In the context of cisplatin treatment and septic conditions, kidney injury represents a significant adverse effect. Metabolomics profiling may facilitate the identification of nephrotoxic mechanisms and biomarkers associated with cisplatin-induced injury. Seven key metabolic pathways were disrupted, including the tricarboxylic acid cycle (TCA cycle); biosynthesis of phenylalanine, tyrosine, and tryptophan; phenylalanine metabolism; glycerophospholipid metabolism; taurine and hypotaurine metabolism; D-glutamine and D-glutamate metabolism; as well as nicotinate

and nicotinamide metabolism. These pathways are integral to energy generation, amino acid metabolism, and lipid metabolism; their disruption may contribute to oxidative stress injury, inflammation, and cell membrane damage.^(24,25)

The limitation of our study was that the preparation of kidney organ specimens in rats necessitated an extended duration of over 1.5 months, thereby increasing the risk of organ damage during formalin storage. The clinical implication of this investigation was that it elucidated specific doses of diethylene glycol (DEG) (3 g/kg and 5 g/kg body weight) that precipitate histopathological damage to rat kidneys. These findings may provide a foundational basis for the development of a nephrotoxic animal model aimed at investigating DEG toxicity, thus enhancing the assessment of DEG's toxic effects in humans.

Comprehensive evaluations of renal damage—including the assessment of blood urea nitrogen (BUN) levels and glomerular filtration rate (GFR)—should be conducted to complement the findings of this study.

CONCLUSION

Kidney damage resulting from DEG exposure occurs at doses of 3 g/kg and 5 g/kg body weight, as evidenced by histopathological changes in endothelial tissues, glomeruli, tubules, and interstitial areas. These doses may provide a basis for the development of a nephrotoxic animal model for studying DEG toxicity. However, analysis of serum creatinine levels in rats administered DEG at 3 g/kg BW did not reveal a significant difference compared to the normal and celery extract groups, although levels were found to be elevated in relation to the other groups. Furthermore, celery extract significantly reduced metabolites, such as tryptophan and phenylalanine, which are implicated in kidney damage.

Conflict of Interest

The authors declare no conflicts of interest concerning this study.

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Author Contributions

THW and HE wrote the manuscript, designed the experiment and revised the manuscript, HW analyzed the data, GM, TM and FO collected the research data in the laboratory. All authors read and approved the final manuscript.

Data Availability Statement

Data is available from the corresponding author upon request.

Declaration the Use of AI in Scientific Writing

We declare that we do not use AI in our scientific writing

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