Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 12 No. 3 December 2025, 372-381
DOI: 10.20473/jfiki.v12i32025.372-381
Available online at https://e-journal.unair.ac.id/JFIKI/

Hypoxia-inducible Factor-2α Probably Mediated the Toxicities of Intravenous Beta-Cyclodextrin in Normal and Diabetic Rats' Kidneys

Anita Purnamayanti[1,2]*, Suharjono[3], Mahardian Rahmadi[3]
[1]Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Clinical and Community Pharmacy, Faculty of Pharmacy, Universitas Surabaya, Surabaya, Indonesia
[3]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: suharjono@ff.unair.ac.id
Orcid ID: 0009-0002-5862-6922

Submitted: 25 July 2025
Revised: 29 November 2025
Accepted: 9 December 2025

Abstract

Background*: Intravenous β-cyclodextrin administration poses an increased risk of toxicity.* ***Objective:*** *This study aimed to determine the nephrotoxicity of intravenous beta-cyclodextrin (BCD) and its underlying molecular mechanism.* ***Methods:*** *Sixteen Wistar rats were randomly assigned to the healthy or diabetic test group, which received a daily intravenous injection of water for injection or a 6% beta-cyclodextrin solution, respectively, for 28 days. Histopathology was analyzed semi-quantitatively on a 0-5 score basis, and RT-PCR was used to quantify the mRNA expression of HIF-1, HIF-2, IL-1, IL-6, IL-18, and eNOS.* ***Results:*** *There were no deaths in any of the groups, and renal function declined in the test groups. Histopathological findings revealed abnormalities in vascular integrity, cellular infiltration/damage, and necrosis in the kidney, while the relative expression of mRNA for hypoxia-inducible factors (HIFs), a marker of regional renal hypoxia, was upregulated.* ***Conclusion:*** *Intravenous administration of beta-cyclodextrin increases the risk of nephrotoxicity, and the molecular mechanisms underlying kidney injury are probably mediated by hypoxia inducible factors-2α (HIF-2α). We strongly suggest that future research should be conducted with an adequate sample size.*

Keywords: *beta-cyclodextrin, intravenous, toxicity, sub-chronic, death rate*

How to cite this article:
Purnamayanti, A., Suharjono & Rahmadi, M. (2025). Hypoxia-inducible Factor-2α Probably Mediated the Toxicities of Intravenous Beta-Cyclodextrin in Normal and Diabetic Rats' Kidneys. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 12(3), 372-381. http://doi.org/10.20473/jfiki.v12i32025.372-381

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

β-Cyclodextrin (BCD) is one of the most commonly used excipients in the food industry. The oral safety profile of beta-cyclodextrin is strongly correlated with its relatively low solubility and bioavailability, and is considered a "generally regarded as safe" (GRAS) excipient. In contrast to the established safety of oral BCD, intravenous injection administration in animal studies has been associated with nephrotoxicity. Most studies have analyzed the toxicity of medicinal products as a whole. Thus, toxicity studies of BCD without active substances are limited (Stella, 2008; EMA, 2017).

Diabetes is one of the leading causes of morbidity and mortality, causing approximately 3.4 million deaths, fatal complications, and a 338% increase in health spending. Hypertension, coronary artery disease (CAD), nephropathy, retinopathy, and cerebrovascular accidents (CVA) are examples of chronic diabetes complications (IDF, 2025). Recent developments in medical diagnostic technology have investigated the advantages of injecting nanoparticle formulations of BCD-based contrast agents for magnetic resonance imaging (MRI) and molecular probe imaging for computed tomography (CT) scanning. Diabetes is an underlying disease, and its chronic complications affecting the central nervous system (CNS) include CVA. Successful disease management comprises reliable diagnosis and safe and effective therapeutic management. One reliable method for diagnosing CVA. A more sophisticated supramolecular polymer of a per-methyl-BCD-based CT scanning contrast agent was developed by Sun et al. in 2021, which showed a high contrast level in the blood, kidney, and bladder (Sun et al., 2021).

High BCD levels in these organs may increase the risk of nephrotoxicity; therefore, further research is required. The ADME-Toxicity study focuses on two critical topics: efficacy and safety (or toxicity) factors, which are represented by BCD kinetics and dynamics in the body. Kubota *et al. (1996)* investigated the pharmacokinetics of BCD, including the absorption, distribution, and metabolism of intravenous and oral BCD, but did not examine the nephrotoxicity of IV and oral BCD. Intravenous delivery of BCD increases its concentration in the intact form, resulting in a 3–6-fold increase in nephrotoxicity. A dose-toxicity effect correlation was observed following a single intravenous BCD administration and subsequent intraperitoneal injection doses, resulting in increased vacuolation. It is frequently observed as a physiological or degenerative process in the kidney or liver (Frank et al., 1976; Kubota et al., 1996). However, the BCD toxicities and their molecular mechanisms were not further explained in these two studies.

Except for acute kidney injury, normal renal proximal epithelial cells did not express kidney injury molecule-1 (KIM-1). Therefore, in toxicology, increased vacuolation should be considered an early warning indicator of risk (Nishina et al., 2021; Lin & Liu, 2025). Neutrophil gelatinase-associated lipocalin (NGAL) is another marker for kidney damage. NGAL is released by renal tubule epithelial cells and increases in response to nephrotoxins or renal ischemia-reperfusion injury, rather than secondary to neutrophil activation (DeOliviera, 2019).

Other factors should also be considered, such as whether a particular disease results in an anatomically distinct pattern of kidney injury, which remains to be elucidated (DeOliviera, 2019). STZ is superior to Aloxan in inducing diabetes because of its higher specificity and lower toxicity (Peng *et al*., 2023). Wistar rats are generally considered more sensitive to STZ-induced diabetes, whereas Wistar-Kyoto rats are resistant. C57BL/6J mice are sensitive to diabetic injury but develop more pronounced progressive nephropathy, which could mask BCD nephrotoxicity, as well as a higher risk of mortality due to severe diabetes in the STZ-induced diabetes model (Furman, 2021; Liang & Liu, 2023). Therefore, this study aimed to investigate the nephrotoxicity of intravenous BCD and its possible molecular mechanism in normal and diabetic rats, based on prior research and the potential toxicity of BCD, despite its broad and vital applications in pharmaceuticals and diagnostics. The novelty of our study is that we analyzed the type of BCD nephrotoxicity in the glomerulus, tubular epithelial cells, and vascular integrity, as well as the possibility of BCD nephrotoxicity through its molecular mechanisms.

MATERIALS AND METHODS

Materials

BCD was produced by Sigma Aldrich and purchased from Laboratorium Solusi Indonesia, Surabaya, Indonesia. A 6% BCD solution was prepared by dissolving 6 g of BCD in 100 mL of water for injection. The volume injected into the tail vein was a maximum of 2 ml/kg, which is equivalent to a maximum of 120 mg BCD/kg rat body weight/day for 28 days. RNA Purification Kit (Jena Bioscience #PP-210S, Jena, Germany) was used to extract total RNA from the kidney tissues.

Tools

The GoScript Reverse Transcription System (Promega Corporation, #A2791, Madison, WI, USA)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

was used for cDNA synthesis, and the quantification of gene expression was performed using the MyGo Mini device for RT-qPCR.

Method

Animal handling

Sixteen male Wistar rats aged 8-9 weeks were obtained from PT. Riset AIRC, Surabaya, Indonesia. The rats were acclimated for 7 days in 12 12-hour light and 12-hour dark cycle housing prior to the study, provided with water, and fed *ad libitum*. Diabetes induction was performed using a single dose of streptozotocin (STZ) freshly prepared solution, adjusted to pH 3-4 with citrate buffer, administered intraperitoneally at 65 mg/kg rat body weight, as described previously (Furman et al., 2021). Diabetes was confirmed if random blood glucose was ≥ 250 mg/dL and remained in the diabetes range until all procedures were completed. All procedures were approved by the Research Ethics Committee, Faculty of Veterinary Universitas Airlangga, Surabaya, Indonesia (ethical approval no. 2).KEH.003.01.2024.

Preclinical experimental design

The sample size should be a minimum of six rats, according to the following calculation,

(4 group - 1) x (replication - 1) ⩾ 15

(3) x (5) ⩾ 15

replication = 6

However, according to the Research Ethics Committee's recommendation, based on the 3R principles, especially the "reduction principle," only four samples were approved for this study. We adhered to these recommendations.

The rats were randomly allocated to one of the following four groups: (i) control (C), (ii) diabetic control (D), (iii) healthy (BCD), or (iv) diabetic (D-BCD). All groups received daily intravenous injections for 28 days through the tail vein using either water for injection (C and D) or a 6% beta-cyclodextrin solution (BCD and D-BCD). All surviving animals were sacrificed on day 29. Intracardiac blood samples were collected, and serum was separated for the analysis of creatinine and BUN concentrations. The estimated glomerular filtration rate was calculated as follows: (Bessling et al., 2021).

When plasma creatinine is < 52 µmol/l, the equation is as follows:

$eGFR = 880 \times (W^{0.695}) \times (C^{-0.660}) \times (U^{-0.391})$

When plasma creatinine is ≥ 52 µmol/l, the equation is as follows:

$eGFR = 5862 \times (W^{0.695}) \times (C^{-1.150}) \times (U^{-0.391})$

In these equations, eGFR is in µl/min, W is weight in grams, C is creatinine in µmol/L, and U is urea in mmol/L.

Histopathology analysis

The kidneys were harvested and weighed, and the body weight ratio was calculated based on the absolute weight of the kidney and body weight in grams (DeOliviera et al., 2019). The Kidneys were perfusion-fixed with 3% paraformaldehyde via the abdominal aorta, as previously reported. The samples were then stored in ice-cold phosphate-buffered saline (PBS) and processed for paraffin embedding. For histological assessment, paraffin sections were cut to a thickness of 3 µm and stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and Mason's trichrome using standard procedures (Rosenberger, 2008). Histological evaluation was conducted independently by two pathologists across ten microscopic fields. Changes in renal morphology, including vascular integrity, necrosis, and cellular infiltration/damage, were assessed semi-quantitatively using a 0-5 score system, as previously described. Briefly, (a) there were three grades of abnormality with subsequent scores: grade 1 = 1, grade 2 = 4, and grade 3 = 10; (b) the percentage of the kidney slices affected were as follows: < 1% = 0, 1–5% = 1, 5 – 10% = 2, 10–< 20% = 3, 20–< 30% = 4, 30–40% = 5, and ≥ 40% = 6. The final semiquantitative score was calculated as grade score × percentage score, which was denoted as follows: 0 = no significant change (overall score < 1), +1 = mild damage (overall score 1-15), +2 = mild to moderate damage (overall score 15 to < 30), +3 = moderate damage (overall score 30 to < 45), +4 = moderate to severe damage (overall score 45 to < 60), and +5 = severe damage (overall score 60) (Yousef, et al., 2011). To confirm the potential toxicity induced by BCD, mRNA expression analysis was performed using reverse-transcription polymerase chain reaction (RT-qPCR). The mRNA expression of the molecular markers assessed included hypoxia-inducible factor-1α (HIF-1α), HIF-2α, endothelial nitric oxide synthase (eNOS), and the inflammation markers interleukins IL-1β, IL-6, and IL-18.

RT-qPCR procedures

Freshly collected kidney specimens were snap-frozen in liquid nitrogen and stored at –80˚C until use. RNA Purification Kit (Jena Bioscience #PP-210S, Jena, Germany) was used to extract total RNA from kidney tissues, and cDNA synthesis was carried out using the GoScript Reverse Transcription System (Promega Corporation #A2791, Madison, WI, USA). The quantification of gene expression using the MyGo Mini device for RT-qPCR was conducted at 95 °C for 3 min,

P-ISSN: 2406-9388
E-ISSN: 2580-8303

followed by 40 cycles of 95 °C to 63 °C for 30 s, and 60 °C to 97 °C for 1 min. mRNA amplifications were performed for HIF-1α, HIF-2α, eNOS, IL-1β, IL-6, IL-18, and β-actin housekeeping genes. The following primers were used (all Thermo Fischer Scientific, Waltham, MA, USA): HIF-1 alpha 5'-CAACTGCCACCACTGATGAA-3' (forward), 5'-TGGGTAGAAGGTGGAGATGC-3' (reverse); HIF-2 alpha 5'-GCGACAATGACAGCTGACAA-3' (forward), 5'-CGGCATCTCGGGATTTCT-3' (reverse); eNOS 5'-CGAGATATCTTCAGTCCCAAGC-3' (forward), 5'-GTGGATTTGCTGCTCTCTAGG-3' (reverse); IL-1b 5'-CTCAATGGACAGAACATAAGCC-3' (forward), 5'-GGTGTGCCGTCTTTCATCA-3' (reverse); IL-6 5′-ATTGTATGAACAGCGATGATGCAC-3′ (forward), 5′-CCAGGTAGAAACGGAACTCCAGA-3′; IL-18 5′-GCAGTAATACGGAGCATAAA-3′ (forward), and 5′-ATCCTTCACAGATAGGGTCA-3′ (reverse).

Analysis of the BCD nephrotoxicity molecular mechanism

The expression of each mRNA renal hypoxia and inflammation parameter was normalized to the housekeeping gene β-actin in each sample. The results of the relative expression to β-actin are presented as the mean ± SEM, and one-way analysis of variance (ANOVA) or Kruskal-Wallis test was performed to identify the statistical significance with a p-value limit of ≤ 0.05, followed by Tukey's post hoc test using GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The deterioration in renal function was not statistically significant; however, all groups had zero death rates. The final renal function is excretion, which includes glomerular filtration, tubular secretion, and reabsorption. Since glomerular filtration eliminates nearly all chemicals, the glomerular filtration rate (GFR) plays a crucial role in assessing the severity of kidney disorders and the efficacy of interventions. Because GFR cannot be evaluated directly, it must be inferred from the serum level of an endogenous filtration marker (eGFR) or quantified based on the clearance of an exogenous substance that is only excreted by glomerular filtration (mGFR). In this study, body weight, serum creatinine, and urea levels were used to create an equation that yields estimated GFR (eGFR). Since creatinine is a byproduct of the metabolism of muscle tissues, an increase in its serum level is likely to occur in the following days. In this study, the mean eGFR of the diseased and control rats were 0.831 ml/min and 3.341 ml/min, respectively (Bessling et al., 2021; Gama et al., 2023; Lamb et al., 2024). The results showed that beta-cyclodextrin injected sub-chronically for 28 days could alter renal function, despite a half-decrease in eGFRs in the BCD and D-BCD groups, which was not statistically significant (p>0.05) (Table 1). The sample size in this study was very limited to 16 rats (n = 4/group), which led to a high rate of Type II errors (false negatives). This might explain the lack of significant differences in eGFR data between the groups, despite the precise observation of anuria symptoms. We further discuss this anuric state at the end of the Discussion.

Table 1. Estimated glomerular filtration rate (eGFR) and kidney to body weight ratio

Group	eGFR – pre-intervention%-(mL/ minute)	eGFR – post intervention (mL/ minute)	Sign	Kidney to Body Weight Ratio (g*100/g)	Sign
Control (C)	1.460±103.1	1.376 ±114.2	p > 0.05	0.655 ±0.25	p > 0.05
Diabetes (D)	0.920 ±22.0	0.822 ±80.3		0.535 ±0.05	
Healthy – BCD (BCD)	1.181 ±217.6	0.546 ±102.3		0.770 ±0.0	
Diabetes – BCD (D-BCD)	0.994±66.1	0.503 ±19.9		0.930 ±0.5	

eGFR = estimated glomerular filtration rate

Table 2. Renal tissue damage scores

Control (C)			Beta-cyclodextrin (BCD)		
Cellular Infiltration/ damage	Vascular integrity	Necrosis	Cellular Infiltration/ damage	Vascular integrity	Necrosis
0	0	0	+3 (30 to <45)	+2 (15 to <30)	+3 (30 to <45)
Diabetes (D)			Diabetes-Beta-cyclodextrin (D-BCD)		
Cellular Infiltration/ damage	Vascular integrity	Necrosis	Cellular Infiltration/ damage	Vascular integrity	Necrosis
+2 (15 to <30)	+2 (15 to <30)	+2 (15 to <30)	+2 (15 to <30)	+2 (15 to <305)	+3 (30 to <45)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

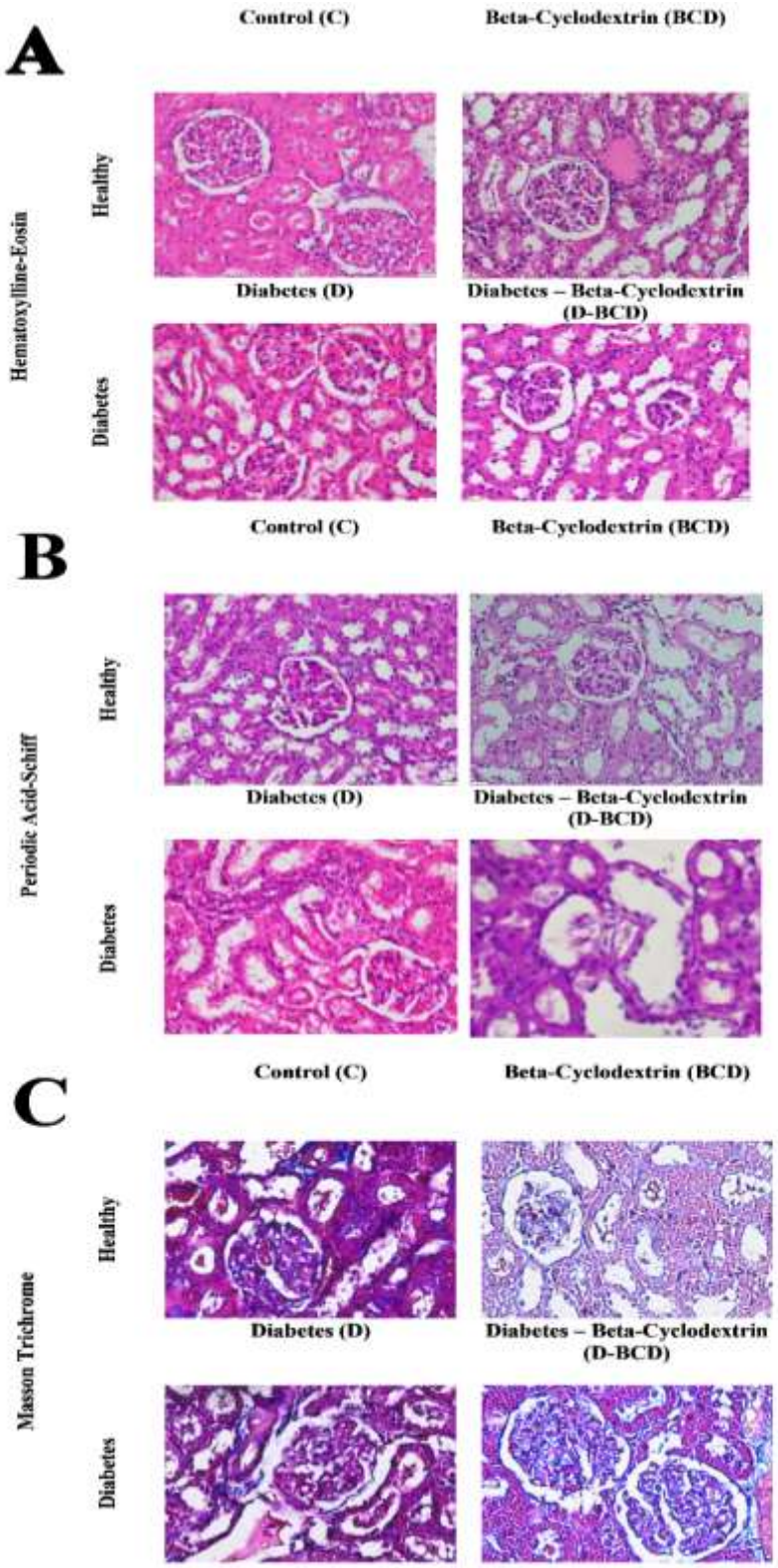

Figure 1. Photomicrograph of beta-cyclodextrin (BCD)-induced renal injury in healthy and diabetic rats. *A-C*: kidney histological injury represented by hematoxylin-eosin (HE) staining, with a relatively intact basal membrane without Kimmelstiel-Wilson nodule in the glomerulus, as shown by periodic acid-Schiff (PAS) staining, and no sign of fibrosis as reflected by Masson's trichrome staining (magnification: x400). *Bar-graph legends*: C = control, BCD = beta-cyclodextrin, D = diabetes, D-BCD = diabetes – beta-cyclodextrin group

P-ISSN: 2406-9388
E-ISSN: 2580-8303

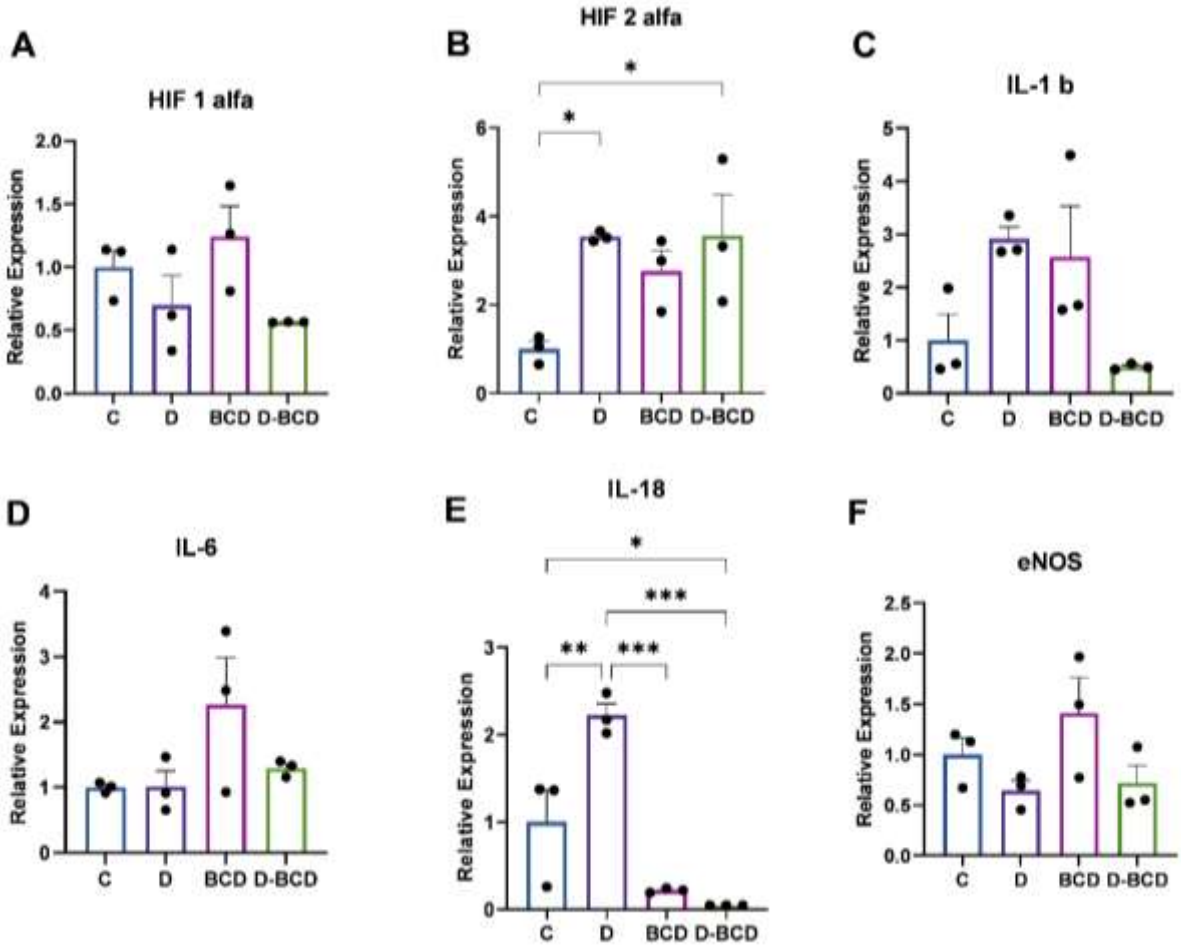

Figure 2. Photomicrograph of renal injury-related gene expression in healthy and diabetic rats. *A-F*: Gene expression of hypoxia-inducible factors (A = HIF-1α and B = HIF-2α), inflammations (C = IL-1β, D = IL-6, E = IL-18), and endothelial nitric oxide synthase (F = eNOS). *Bar-graph legends*: C = control, BCD = beta-cyclodextrin, D = diabetes, D-BCD = diabetes – beta-cyclodextrin group

The measured GFR or eGFR from serum creatinine determined by the enzymatic technique ± urine albumin-to-creatinine ratio can yield a more accurate result (Bessling, et al., 2021; Gama et al., 2023; Lamb, et al., 2024). Acute kidney injury (AKI) in humans can be identified by a decrease in urine volume < 0.5 ml/ kg body weight that lasts for at least 6 h or by an increase in serum creatinine ≥ 0.3 mg/ dl in the 48 h or > 50% in the previous 7 d, which further lowers eGFR (KDIGO, 2012). In both healthy and diabetic rats in the BCD and D-BCD groups, we observed an oliguric to anuric state, which made it impossible to accurately measure the urine levels of the tubular damage markers kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid-binding protein (LAFB). Sensitive and specific acute kidney injury parameters include urinary KIM-1, creatinine, and NGAL, which require a minimum urine sample volume of 3 mL for enzyme-linked immunosorbent assay (ELISA) analysis Jin et al., 2017). In our study, urine samples collected from rats were less than 1.5 mL. Thus, this is the reason why we were unable to perform urinary KIM, NGAL, protein, creatinine, and LAFB analysis. To examine renal tissue damage, we performed a histological evaluation.

Based on the histological features of HE, PAS, and MT staining, there was generally mild to moderate structural damage in diabetes, possibly due to the early stages of diabetes, and the diabetic complications had not yet manifested. The histological findings are presented in Table 2. Histopathology of typical early diabetic nephropathy typically reveals glomerular hypertrophy and dilated tubules, which can be observed by hematoxylin-eosin staining, as well as thickening of the glomerular basement membrane, as observed in periodic acid-Schiff (PAS)- stained tissue. In this study, dilated tubules and mild thickening of the glomerular basement membrane were observed in the D-BCD group (Figure 1.B) without renal fibrotic tissue findings, as demonstrated by Masson's trichrome staining, or nodular glomerulosclerosis (Kimmelstiel-Wilson nodules in the glomerulus), as demonstrated by PAS

P-ISSN: 2406-9388
E-ISSN: 2580-8303

staining (Figure 1. B and C). Kimmelstiel-Wilson nodules, albuminuria, and fibrosis are essential parameters for characterizing advanced diabetic nephropathy (Zimpelmann et al., 2000; US Department of Health and Human Services, 2023).

Histopathological analysis revealed renal abnormalities that correlated with gene expression patterns, particularly the upregulation of hypoxia-inducible factor 2α (HIF-2α), as shown in Figure 1. A. Hematoxylin-eosin (HE) staining revealed vacuolation within the renal tubular epithelial cells and interstitial inflammation in the BCD, diabetes, and D-BCD groups. In contrast, the glomerular and tubular basement membranes were largely preserved compared to those in the control group. These observations are consistent with previous studies investigating the toxicological effects of BCD, including a 52-week oral administration study, a single-dose intravenous administration study, and a 7-day repeated subcutaneous injection study. While no signs of nephrotoxicity were reported following oral administration, minor vacuolation was observed after intravenous injection. In contrast, high-dose subcutaneous administration (0.98 g/kg body weight) induced the presence of elongated or needle-like cytoplasmic crystals and giant lysosomes, initially localized in the proximal convoluted tubules (Frank et al., 1976; Bellringer et al., 1995; Kubota et al., 1996). The nephrotoxic doses reported in these prior studies were approximately 1.6 times higher than the dosage used in the present study, which may account for the absence of mineralization and crystalline structures in our results.

An increase in vacuolation may result in significant damage to renal epithelial cells, subsequently triggering the activation of innate immune responses. This includes the recruitment of systemic macrophages and activation of resident macrophages within the tubular epithelium. Histopathological examination revealed inflamed tubular epithelial cells characterized by heightened eosinophilia on hematoxylin-eosin (HE) staining, along with increased interstitial cellularity within the nephron. Despite these alterations, periodic acid–Schiff (PAS) staining demonstrated the preservation of tubular basement membranes, and Masson's trichrome (MT) staining did not indicate any fibrotic changes. Furthermore, the presence of sloughed epithelial cells or casts within the tubular lumens observed in this study is consistent with the findings reported by Frank *et al*. (1976). Mechanistically, kidney injury molecule-1 (KIM-1) is upregulated in the proximal tubular epithelium during injury, where it functions as a non-specific resident of the macrophages. KIM-1 facilitates the phagocytosis of cellular debris, with the resultant bodies being shed into the tubular lumen, where they are visible as sloughs or casts. Similarly, neutrophil gelatinase-associated lipocalin (NGAL) is induced during acute kidney injury, particularly in distal nephron segments, including the distal tubules, connecting tubules, and collecting ducts (DeOliveira et al., 2019).

In renal tissue, hypoxia-inducible factor 1α (HIF-1α) is predominantly localized in the collecting ducts and the thick ascending limb (TAL) of the nephron, whereas hypoxia-inducible factor 2α (HIF-2α) is mainly expressed in endothelial and interstitial cells (Rosenberger et al., 2008; Smith *et al*., 2011; Kletkiewicz *et al*., 2018). As depicted in Figure 1, these distribution patterns are consistent with our data, which demonstrate a statistically significant upregulation ($p < 0.05$) in the relative mRNA expression of HIF-2α in the experimental group compared to that in the control group (E). HIF-2α expression is known to increase under prolonged hypoxic conditions, typically persisting beyond 72 h post-injury, and is considered to play a protective role in the context of acute kidney injury by initiating adaptive cellular survival pathways (Rosenberger et al., 2008; Smith *et al*., 2011; Kletkiewicz et al., 2018).

Our findings showed a significant upregulation ($p<0.05$) in the relative mRNA expression of IL-18 following 28 days of intravenous BCD administration in healthy rats compared to that in the D-BCD group (H). Marked tubular dilations were observed in the D-BCD group compared to the normal control (C) and diabetes (D) groups. Interestingly, IL-18 expression was downregulated in the D and D-BCD groups. Notably, all pro-inflammatory cytokines evaluated in this study—IL-1β, IL-6, and IL-18—were consistently downregulated, which contrasts with previous findings suggesting that IL-18 plays a key role in mediating certain human autoimmune conditions, including type 1 diabetes mellitus (Zimpelmann et al., 2000; Zhao et al., 2024). This discrepancy may be attributed to the early stage diabetes model employed in this study, characterized by six weeks of sustained hyperglycemia without the onset of diabetic nephropathy.

STZ-induced type 1 diabetes in a rat model confirmed the infiltration of the innate immune system, specifically macrophages, in the glomerulus and renal interstitial cells from the early phase of type 1 diabetes, resulting in a proinflammatory condition. IL-18 was previously known as an interferon-γ (IFN-γ) producing factor, and may be released by both macrophages and renal tubular epithelial cells. In the presence of abundant IFN-γ and tumor necrosis factor-α (TNF-α),

P-ISSN: 2406-9388
E-ISSN: 2580-8303

macrophage differentiation leads to the activation of M1 macrophages, which initiates the pro-inflammatory phase, potentially causing renal damage. IL-18 may accelerate glomerular injury, whereas podocyte hypertrophy occurs as a result of IL-6 activation under hyperglycemic conditions. IFN-γ and IL-6 may also activate HIF-1α in the renal tubules under hypoxic conditions; however, high glucose levels inhibit the stabilization of HIF-1α (Youssef et al., 2024). Wang et al. (2021) demonstrate that the expression of HIF-1α mRNA was significantly reduced in various AKI models following the depletion of systemic monocytes/macrophages. A key factor in AKI, regardless of causality, is regional renal hypoxia, which leads to an increase in nitric oxide (NO) production, an endogenous vasodilator, through eNOS (Rosenberger et al., 2008). As part of the diabetes disease continuum, a shift from M1 to M2 macrophages may occur, which facilitates tissue repair and inhibits further inflammation, possibly due to a compensatory mechanism. In contrast, this macrophage transition could lead to maladaptive repair and fibrosis as the disease progresses to overt diabetic kidney disease (Youssef et al., 2023; Zhao et al., 2024; Ji et al., 2025). These findings are consistent with our results, which revealed the upregulation of HIF-2α. HIF-2α is predominantly expressed in capillary endothelial cells as an adaptive mechanism under hypoxic conditions, potentially compensating for renal damage by downregulating proinflammatory cytokines. Thus, we hypothesized that HIF-2α plays a crucial role in iv BCD-induced renal hypoxia in both healthy and diabetic rat groups; however, this requires further elucidation.

Consequently, intravenous BCD administration did not appear to provoke acute kidney injury under these conditions, although further investigation is required. A notable limitation of this study was the occurrence of anuria during the final 8 h preceding necropsy in both the BCD and D-BCD groups, which precluded the measurement of acute kidney injury biomarkers such as the urinary albumin-to-creatinine ratio, KIM-1, NGAL, and other urinary markers. A limitation of this study was its underpowered nature, which led to a type II error, resulting in false negatives owing to the limited sample size. This led to a lack of statistical significance for eGFR as an important renal function parameter, despite the precise observation of an anuric state. STZ-induced diabetic rats exhibit glomerular and tubular hypertrophy, a thickened glomerular basement membrane, enhanced renal blood flow, and glomerular hyperfiltration in the early phase of diabetes as a compensatory mechanism to eliminate high glucose levels in the kidney. However, as the disease progresses, microalbuminuria, accompanied by the accumulation of matrix metalloproteinase (MMP) in the mesangium, leads to progressive glomerulosclerosis due to the activation of the renal renin-angiotensin system, specifically the angiotensin II (Ang II) pathway. Ang II is a growth factor that promotes the production of pro-sclerotic transforming growth factor-β (TGF-β), afferent arterial vasoconstriction, and subsequent reduction in urine production (Zimpelmann et al., 2000). Zimpelmann et al. (2000) showed that renal Ang II levels significantly increased ($p < 0.04$) within two weeks of STZ induction, whereas there were no statistically significant differences in plasma renin activity and creatinine levels in STZ-induced rats.

CONCLUSION

Intravenous administration of beta-cyclodextrin increases the risk of nephrotoxicity in diabetic rats, and HIF-2α may mediate the molecular mechanisms underlying kidney injury. We strongly suggest conducting future research with an adequate sample size.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Dean of the Faculty of Pharmacy at Universitas Airlangga for facilitating the internal research funding for this project.

AUTHOR CONTRIBUTIONS

Conceptualization, S.; Methodology, M.R., Validaton, M.R.; Formal Analysis, A.P.; Investigation, A.P.; Resources, M.R.; Data Curation, M.R.; Writing - Original Draft, A.P.; Writing - Review & Editing, A.P., S., M.R..; Visualization, A.P.; Supervision, M.R.; Project Administration, M.R.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Bellringer ME, Smith TG, Read R, Gopinath C, Olivier Ph. (1995). β-Cyclodextrin: 52-Week Toxicity Studies in the Rat and Dog. *Fd Chem. Toxic.* 1995;33(5):367-37

Bessling PJ, Pieters TT, Nguyen ITN, deBree PM, Willekes N, Dijk AH, Bovee DM, Hoorn EJ, Rookmaker MB, Gerristen KG, Verhaar MC, Gremmels H, Joles JA. (2021). A plasma creatinine- and urea-based equation to estimate

glomerular filtration rate in rats. *Am J Physiol Renal Physiol* 320: F518–F524, 2021.

DeOliviera BD, Xu K, Shen TH, Callahan M, Kiryluk K, D'Agati VD, Tatonetti NP, Barasch J, Devarajan P. (2019). Molecular nephrology: types of acute tubular injury. *Nat Rev Nephrol*, 15(10): 599–612. doi:10.1038/s41581-019-0184-x.

EMA Committee of Human Medicinal Products Report. *Question and Answer on Cyclodextrin Used as an Excipient in Human Medicinal Products*. London: European Medicines Agency - Science Medicines Health. (2017). pp 1-9.

Frank DW, Gray JE, Weaver RN. (1976). Cyclodextrin Nephrosis in The Rats. *Am. J. Pathol.*, 83(2): 367-382.

Furman BL. (2021). Streptozotocin-induced diabetic models in mice and rats. *Curr Protocols*, *1*, e78. doi: 10.1002/cpz1.78

Gama RM, Griffiths K, Vincent RP, Peters AM, Bramham K. (2023). Performance and pitfalls of the tools for measuring glomerular filtration rate to guide chronic kidney disease diagnosis and assessment. *J Clin Pathol*, 2023;76(7):442-49. [online abstract]

International Diabetes Federation. (2025). IDF Diabetes Atlas

Ishiguro T, Takeda J, Fang X, Bronson H, Olson DM. (2016) Interleukin (IL)-1 in rat parturition: IL-1 receptors 1 and 2 and accessory proteins abundance in pregnant rat uterus at term – regulation by progesterone. *Physiol Rep*, 4 (14):1-12.

Jin Y, Shao X, Sun B, Miao C, Li Z, Shi Y. Urinary kidney injury molecule-1 as an early diagnostic biomarker of obstructive acute kidney injury and development of a rapid detection method. Molecular Medicine Report 15: 1229-1235, 2017.

Kidney Disease Improving Global Outcomes. (2012). Clinical Practice Guideline for Acute Kidney Injury. *Kidney International*, Suppl. Vol 2, Issue 1, March 2012. https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf

Kletkiewicz, H, Hyjek, M., Jaworski, K., Nowakowska, A. & Rogalska, J. (2018). Activation of hypoxia-inducible factor-1α in rat brain after perinatal anoxia: role of body temperature, *Intl J of Hyperthermia*, 34:6, 824-833

Kubota Y, Fukuda M, Muroguchi M, Koizumi K. (1996). Absorption, distribution, and excretion of beta-cyclodextrin and glucosyl-beta-cyclodextrin in rats. *Biol Pharm Bull*, 19(8):1068-72.

Lamb EJ, Barratt J, Brettell EA, Cockwell P, Dalton RN, Deeks JJ, Eaglestone G, Pellatt-Higgins T, Kalra PA, Khunti K, Loud FC, Ottridge RS, Potter A, Rowe C, Scandrett K, Sitch AJ, Stevens PE, Sharpe CC, Shinkins B, Smith A, Sutton AJ, Taal MW. (2024). Accuracy of glomerular filtration rate estimation using creatinine and cystatin C for identifying and monitoring moderate chronic kidney disease: the eGFR-C study. *Health Technol Assess*, 28(35):1-169.

Liang Y & Liu J. 2023. Animal Models of Kidney Disease: Challenges and Perspectives. *KIDNEY360* 4: 1479–1493, 2023.

Lin S, Hao C, Liu B-C, Eds. 2025. *Comprehensive Frontier of Kidney Disease*. New Jersey: World Scientific Publishing.Nishina H, Sakairi T, Sato H, Mizukawa M, Ozawa Y, Nishikawa S. (2021). Karnovsky's fixative prevents artifacts appearing as vacuolation derived from tissue processing in kidneys treated with antisense oligonucleotide.

Reynolds PS. A guide to sample size for animal-based study. (2024). Florida: Wiley6- Blackwell.

Rosenberger C, Khamaisi M, Abassi Z, Shilo V, Weksler-Zangen S, Goldfarb M, Shina A, Zibertrest F, Eckard K-U, Rosen S, Heyman SN. (2008). Adaptation to hypoxia in the diabetic rat kidney. *Kidney International*, 73, 34–42.

Smith BJ, Clarke SL, McKale RD, Shawron KM, Rendina E, Peterson SK, Lucas EA (2011). Hypoxia Inducible Factor-2a Is Translationally Repressed in Response to Dietary Iron Deficiency in Sprague-Dawley Rats. *J. Nutr*. 141: 1590–96.

Stella VJ, He Q. (2008). Cyclodextrins. *Toxicol Pathol.* 2008; 36:30-42

Sun M, Zhang H-Y, Liu B-W, Liu Y. (2013). Construction of a Supramolecular Polymer by Bridged Bis(permethyl-β-cyclodextrin)s with Porphyrins and Its Highly Efficient Magnetic Resonance Imaging. *Macromol* 46, 4268−4275.

Thomas JM, Ling YH, Huuskes B, Jelinic M, Sharma P, Saini N, Ferens DM, Diep H, Krishnan SM, Kemp-Harper BK, O'Connor PM, Latz E, Arumugam TV, Guzik TJ, Hickey MJ, Mansell A, Sobey CG, Vinh A, Drummond GR. (2021).

IL-18 (interleukin-18), produced by renal tubular epithelial cells, promotes renal inflammation and injury during Deoxycorticosterone/Salt-induced hypertension in mice. *Hypertension*, 78:1296-1309.

United States Department of Health and Human Services. National Toxicology Program. (2023). Urinary System - Kidney. In: Non-neoplastic Lesion Atlas - a Guide for standardizing terminology in toxicologic pathology for rodents. https://ntp.niehs.nih.gov/atlas/nnl/urinary-system/kidney. The website was last updated on July 18, 2023. Assessed: 5 July 2024.

Wu Z, Yao H, Xu H, Wang Y, Hu W, Lou G, Zhang L, Huang C, Jiang C, Zhou S, Shi Y, Chen X, Yang L, Xu Y, Wang Y. (2020). Inhibition of eNOS by L-NAME resulting in rat hind limb developmental defects through PFKFB3 mediated angiogenetic pathway. *Sci Rep*, 10:16754

Yousef JM, Chen JG, Hill PA, Nation RL, Li J. (2011). Melatonin Attenuates Colistin-Induced Nephrotoxicity in Rats. Antimicrobial Agents and Chemotherapy, 2011;55(9):4044–49.

Youssef N, Noureldein MH, Riachi ME, Haddad A, Assaad AEid. Macrophage polarization and signaling in diabetic kidney disease: a catalyst for disease progression. *Am J Physio Ren Physiol* 326: F301–F312, 2024.

Zimpelmann J, Kumar D, Levine DZ, Wehbi G, Imig JD, Navar LG, Burns. (2000). Early diabetes mellitus stimulates proximal tubule renin mRNA expression in the rat. *Kidney Int*, 2000;58:2320–30.

Zhao L, Hu H, Zhang L, Liu Z, Huang Y, Liu Q, Jin L, Zhu M, Zhang L. (2024). Inflammation in diabetes complications: molecular mechanisms and therapeutic interventions. *MedComm*, 5:e516.

P-ISSN: 2406-9388
E-ISSN: 2580-8303