GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

The Effect of Green Tea Extract (*Camellia sinensis*) on Renal Histopathology in Cigarette Smoke-Exposed Mice (*Mus musculus*)

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GSC Biological and Pharmaceutical Sciences, 2026, 34(02), 279-286

Publication history: Received on 03 January 2026; revised on 12 February 2026; accepted on 14 February 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.34.2.0066

Abstract

Cigarette smoke exposure induces oxidative stress that can cause renal histopathological damage. Green tea (Camellia sinensis) contains polyphenols, particularly epigallocatechin-3-gallate (EGCG), which possess potent antioxidant activity. This study aimed to analyze the effect of green tea extract administration on renal histopathology in cigarette smoke-exposed mice. This experimental study utilized 25 male mice (Mus musculus) aged 12 weeks, divided into five groups: C- (negative control), C+ (cigarette smoke exposure), T1 (cigarette smoke exposure + GTE 20 mg/kg BW), T2 (cigarette smoke exposure + GTE 40 mg/kg BW), and T3 (cigarette smoke exposure + GTE 60 mg/kg BW). Treatment was administered for 36 days. Parameters observed included glomerular necrosis, tubular cell necrosis, and tubular cell degeneration using Hematoxylin-Eosin staining. Data was analyzed using Kruskal-Walli's test followed by Mann-Whitney test. The C+ group demonstrated significant histopathological damage with scores of glomerular necrosis (7.12±1.22), tubular cell necrosis (5.09±0.75), and tubular cell degeneration (3.31±0.55) compared to C- ($p<0.05$). GTE administration reduced damage scores in a dose-dependent manner. The 60 mg/kg BW dose (T3) provided the most optimal protective effect with scores of glomerular necrosis (3.13±0.87), tubular cell necrosis (3.04±0.47), and tubular cell degeneration (1.52±0.56), showing no significant difference from C- for the degeneration parameter ($p>0.05$). Green tea extract exhibits protective effects against renal histopathological damage induced by cigarette smoke exposure, with an optimal dose of 60 mg/kg BW.

Keywords: Green Tea Extract; Cigarette Smoke; Renal Histopathology; Necrosis; Degeneration

1. Introduction

Cigarette smoke exposure has become a critical public health issue both globally and nationally. Cigarette smoke contains more than 7,000 chemical compounds, hundreds of which are toxic and carcinogenic. Harmful components such as nicotine, tar, carbon monoxide, and free radicals contained in cigarettes can trigger oxidative stress in the body [1]. Oxidative stress occurs when the amount of free radicals exceeds endogenous antioxidant capacity, subsequently triggering lipid peroxidation and cellular structural damage in various vital organs) [2].

The kidney is a major excretory organ that is highly susceptible to systemic toxicity from cigarette smoke exposure. As an organ responsible for blood filtration, the kidney is directly exposed to harmful metabolites circulating in the

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bloodstream. Chronic cigarette smoke exposure is known to induce microscopic damage to renal tissue, which histopathologic ally manifests as tubular epithelial degeneration, tubular cell necrosis, and damage to glomerular structures. If this condition persists, renal filtration function will decline and risks progressing to chronic kidney failure [3].

To minimize tissue damage due to oxidative stress, exogenous antioxidant intake is required. Green tea (Camellia sinensis) is one of the plants rich in polyphenolic compounds, particularly catechins such as epigallocatechin-3-gallate (EGCG). This compound possesses strong antioxidant activity by donating electrons to stabilize free radicals and enhancing antioxidant enzyme activity in the body [4,5] The potential of green tea in protecting internal organs as cytoprotective has been extensively studied [6–10], however its specific effects on repairing renal histopathological structures exposed to cigarette smoke still require further investigation.

2. Materials And Methods

2.1. Study Design and Ethical Approval

This study was a true experimental design with a posttest-only control group design. The research protocol received ethical approval from the Animal Care and Use Committee, Universitas Airlangga, Surabaya, Indonesia, with ethical certificate number 2.KE.047.07.2020.

2.2. Green Tea Extract (GTE) Preparation

Green tea leaves (Camellia sinensis) were obtained from the Wonosari tea plantation, Malang Regency, East Java. The extraction process was performed using the maceration method: 1000 g of finely powdered green tea leaves were macerated in 8 L of 96% ethanol at room temperature for 72 hours. The resulting macerate was then concentrated using a rotary evaporator (50°C, 45 rpm) for 5 hours and continued with lyophilization following the procedure of Khairun's et al. (2020). The dry extract was then suspended in 1% Na-CMC solution to achieve the desired dose concentration [11].

2.3. Experimental Animals and Treatment

Twenty-five healthy male mice (Mus musculus), 12 weeks old and weighing 20–25 g, were housed in standard cages with rice husk bedding, provided commercial pellet feed and water ad libitum, and maintained under controlled laboratory conditions. After a 7-day acclimatization period, the mice were randomly assigned to five groups (n=5/group): a negative control group (C-) receiving 0.5 mL 1% Na-CMC without smoke exposure; a positive control group (C+) receiving cigarette smoke exposure and 0.5 mL 1% Na-CMC; and three treatment groups (T1, T2, and T3) receiving smoke exposure along with green tea extract (GTE) at dosages of 20 mg/kg BW, 40 mg/kg BW, and 60 mg/kg BW, respectively.

The study lasted for 36 days. Green tea extract was administered once daily via oral gavage (0.5 mL/mouse). Cigarette smoke exposure for groups C+, T1, T2, and T3 was conducted in a special chamber (31 × 19 × 22 cm). Exposure sessions were performed for 20 minutes using one kretek cigarette (nicotine content 2.2 mg) per session [12].

2.4. Sample Collection and Histopathological Examination

On day 37, mice were euthanized by cervical dislocation under anesthesia. Kidney organs were harvested and fixed in 10% Neutral Buffered Formalin (NBF) solution for histopathological preparation with Hematoxylin-Eosin (HE) staining. Microscopic observation was conducted to evaluate tissue structural changes, including glomerular necrosis characterized by the presence of damage or cell death in glomerular structures, tubular cell necrosis identified by the death of tubular epithelial cells such as pyknotic nuclei, karyorrhexis, or karyolysis, and tubular cell degeneration involving cellular changes in the form of cytoplasmic swelling (albuminoid or hydropic degeneration). Semi-quantitative assessment was performed in five fields of view using a light microscope (Optilab IRIS 4) with the assistance of Opti lab SIGMA 4K connected to a computer [5].

2.5. Statistical Analysis

Observation data were processed using IBM SPSS Statistics version 25.0 software. Normality test was performed using Shapiro-Wilk and variance homogeneity test using Levene's test. Data were presented as mean ± standard deviation (SD). Comparison between treatment groups for histopathological damage scores was analyzed using the non-parametric Kruskal-Walli's test, followed by Mann-Whitney test to determine significant differences between groups.

3. Results

Based on microscopic observation of renal histopathological preparations in mice (Mus musculus), cigarette smoke exposure was proven to induce significant structural damage to renal tissue, including glomerular necrosis, as well as tubular cell necrosis and degeneration. Administration of green tea extract (GTE) demonstrated an effect on improving these histopathological features at various dose levels. A summary of semi-quantitative assessment results for renal damage parameters is presented in Table 1.

Table 1 Renal Histopathological Damage Scores in Mice across Various Treatment Groups

Treatment Group	Glomerular Necrosis (Means)	Tubular Cell Necrosis (Means)	Tubular Cell Degeneration (Means)
C-	1.44a±0.48	2.49a±0.72	1.15a±0.21
C+	7.12d±1.22	5.09c±0.75	3.31c±0.55
T1	5.05c±0.63	4.76c±0.89	2.80bc±0.49
T2	4.90c±0.48	3.26b±0.28	2.67b±0.36
T3	3.13b±0.87	3.04ab±0.47	1.52a±0.56

Note: Different superscripts (A, B, C, D) in the same column indicate significant differences based on Mann-Whitney test ($p<0.05$).

Renal histopathological observations revealed that cigarette smoke exposure in the positive control group (C+) triggered significant tissue damage compared to the negative control group (C-), with the highest scores for glomerular necrosis (7.12±1.22), tubular cell necrosis (5.09±0.75), and tubular cell degeneration (3.31±0.55). Microscopically, this damage was characterized by glomerular necrosis (Figure 1, Red Arrow) and various tubular changes including albuminoid degeneration (Figure 2, Yellow Arrow), karyopyknotic necrosis (Figure 2, Red Arrow), karyorrhexis (Figure 2, Orange Arrow), and karyolysis (Figure 2, White Arrow). Green tea extract (GTE) administration gradually reduced the level of damage, with the 60 mg/kg BW dose (T3) providing the most optimal protective effect. Statistically, particularly for the tubular cell degeneration parameter, results showed no significant difference ($p > 0.05$) from the negative control group, thus maintaining glomerular and renal tubular cell structures close to normal conditions (Figures 1 and 2, Green Arrow).

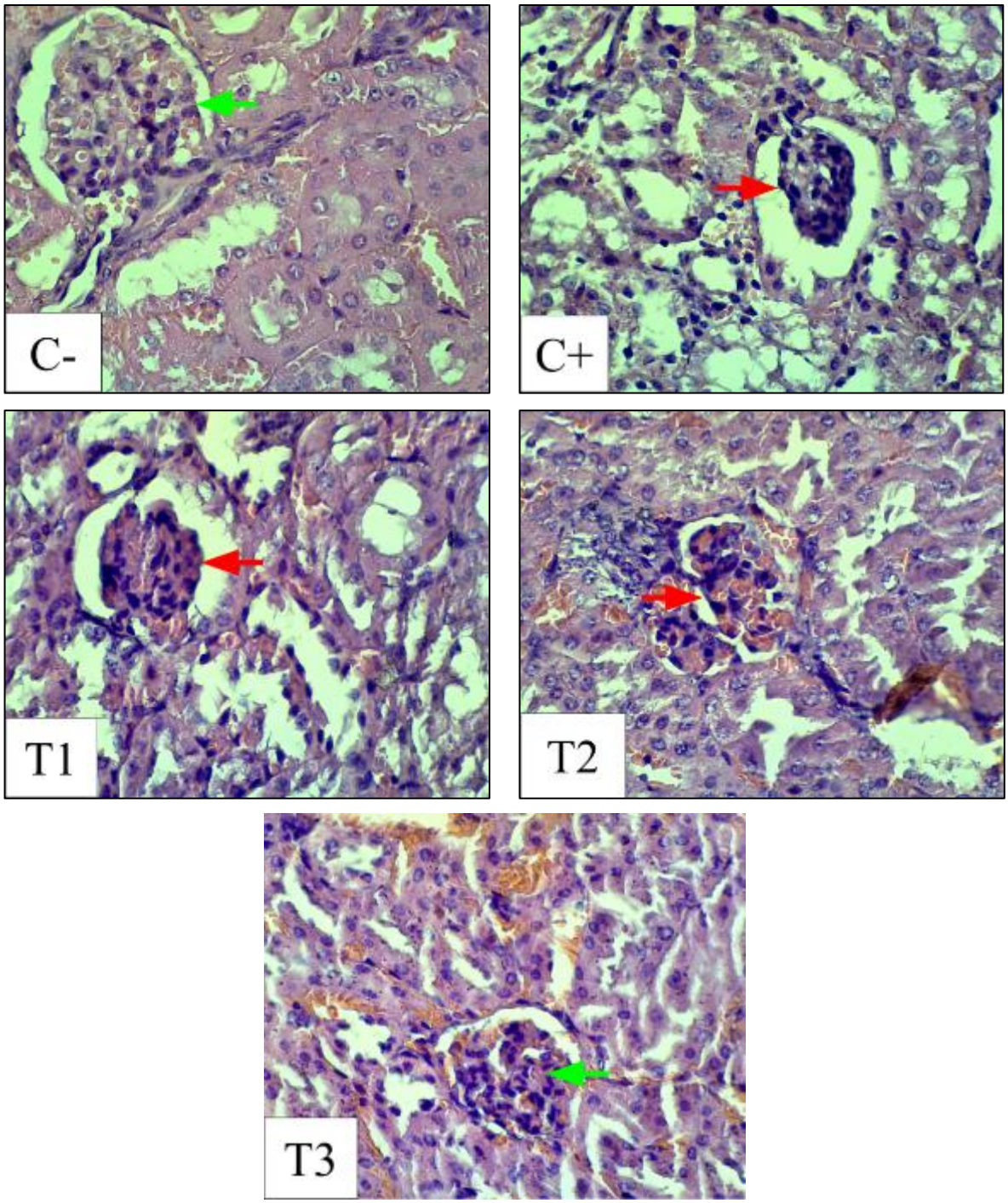

Figure 1 Histology of Glomerular Structure in Cigarette Smoke-Exposed Mice Treated with Green Tea Extract. Green arrow indicates normal glomerulus; Red arrow indicates necrotic glomerulus

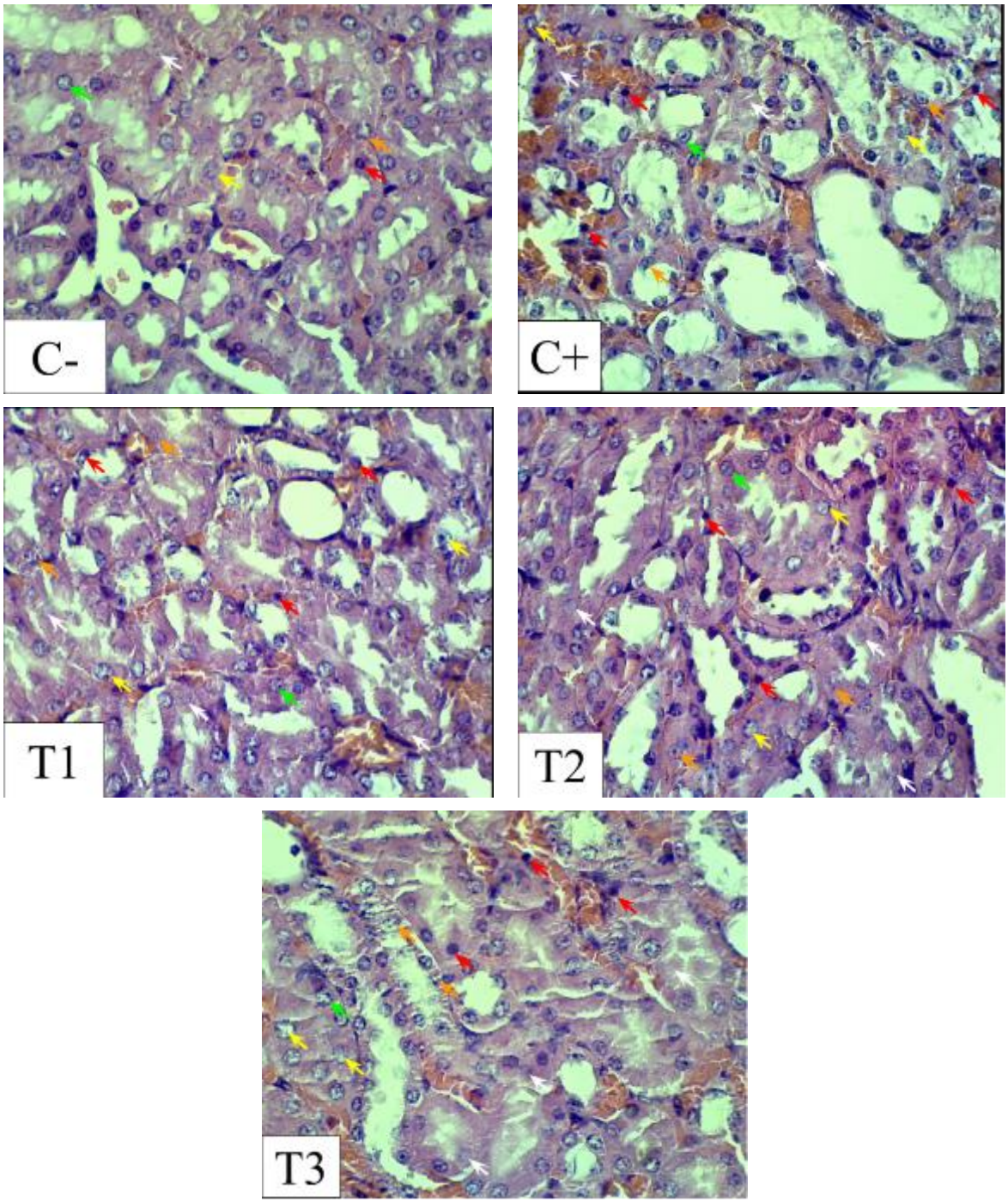

Figure 2 Histology of Tubular Structure in Cigarette Smoke-Exposed Mice Treated with Green Tea Extract. Green arrow indicates normal tubular cells; Yellow arrow indicates degenerated tubular cells; Red arrow indicates tubular cells with karyopyknotic necrosis; Orange arrow indicates tubular cells with karyorrhectic necrosis; and White arrow indicates tubular cells with karyolitic necrosis

4. Discussion

Cigarette smoke contains more than 7,000 harmful chemical compounds, including nicotine, tar, carbon monoxide, and various free radicals such as reactive oxygen species (ROS) [1]. When ROS enter the body through cigarette smoke inhalation, an imbalance occurs between free radical production and endogenous antioxidant defense system capacity. This condition triggers oxidative stress that can cause lipid peroxidation in cell membranes, DNA damage, and denaturation of structural and functional proteins in renal tissue [2].

The kidney is an organ highly vulnerable to the toxic effects of cigarette smoke due to its function as a filtration organ continuously exposed to harmful metabolites circulating in the blood. Prolonged oxidative stress can cause endothelial dysfunction in renal blood vessels, hemodynamic disturbances, and activation of inflammatory pathways that contribute to progressive renal tissue damage [3].

4.1. Glomerular Necrosis

The positive control group exposed to cigarette smoke showed the highest glomerular necrosis score (7.12±1.22), which was significantly higher than the negative control group (1.44±0.48). The glomerulus serves as the main filtration unit in the kidney, and damage to this structure can impair kidney function [13].

Cigarette smoke causes glomerular damage through three main mechanisms. First, nicotine causes blood vessel constriction, reducing blood flow to the glomerulus and causing tissue damage [14]. Second, free radicals from cigarette smoke trigger inflammation by increasing inflammatory proteins (TNF-α, IL-1β, IL-6), which attract inflammatory cells and worsen tissue damage [15]. Third, oxidative stress causes cell death in the glomerulus [16].

Green tea extract showed dose-dependent protection against glomerular necrosis. The P1 group (20 mg/kg BW) had a score of 5.05±0.63, P2 group (40 mg/kg BW) scored 4.90±0.48, and P3 group (60 mg/kg BW) showed the best results with 3.13±0.87. The 60 mg/kg BW dose provided the most effective protection, although it was still different from the negative control group.

4.2. Tubular Cell Necrosis

The positive control group showed a tubular cell necrosis score of 5.09±0.75, much higher than the negative control (2.49±0.72). Necrosis was identified by changes in cell nuclei: karyopyknotic (shrunken, dark nuclei), karyorrhexis (fragmented nuclei), and karyolysis (dissolved nuclei).

Tubular cells are particularly vulnerable to cigarette smoke damage because they have high energy demands and directly contact toxic substances. Excessive free radicals cause mitochondrial damage, energy depletion, and calcium imbalance, leading to cell death [3].

Green tea extract provided protection against tubular necrosis. Group P1 (4.76±0.89) showed no significant improvement from the positive control, indicating that 20 mg/kg BW was insufficient. However, groups P2 (3.26±0.28) and P3 (3.04±0.47) showed significant improvements. Importantly, the P3 group was not significantly different from the negative control, indicating optimal protection at 60 mg/kg BW.

4.3. Tubular Cell Degeneration

Tubular cell degeneration is a reversible condition that occurs before cell death. The positive control group had a degeneration score of 3.31±0.55, significantly higher than the negative control (1.15±0.21). Degeneration appeared as cell swelling due to fluid and protein buildup inside the cells.

Cigarette smoke causes degeneration by damaging the cell's sodium-potassium pump due to energy depletion. This allows sodium and water to accumulate inside cells, causing them to swell. If untreated, this can progress to cell death [17].

Green tea extract showed excellent protection against degeneration. Groups P1 (2.80±0.49) and P2 (2.67±0.36) showed significant improvements compared to the positive control. Most notably, group P3 (1.52±0.56) was not significantly different from the negative control ($p>0.05$), indicating that 60 mg/kg BW effectively maintained normal cell structure even with cigarette smoke exposure.

4.4. Protective Mechanisms of Green Tea Extract

Green tea extract, primarily through its major bioactive compound epigallocatechin-3-gallate (EGCG), exerts nephroprotective effects via multiple interconnected mechanisms. EGCG acts as a potent direct antioxidant by scavenging reactive oxygen species (ROS) and thereby preventing oxidative damage to cellular components such as membranes, proteins, and DNA [18]. In addition, it enhances endogenous antioxidant defenses by activating the nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway, which promotes the upregulation of key antioxidant enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Talebi et al., 2021). EGCG also attenuates inflammation by inhibiting the nuclear factor-kappa B (NF-κB) signaling pathway, leading to reduced production of proinflammatory cytokines and decreased inflammatory cell infiltration into

renal tissue [19]. Furthermore, it modulates apoptosis by upregulating anti-apoptotic proteins such as Bcl-2, downregulating pro-apoptotic proteins such as Bax, and inhibiting caspase-3 activation, collectively preserving renal cell viability under oxidative stress conditions [20] hese multifaceted actions highlight the therapeutic potential of green tea polyphenols in protecting against oxidative stress-related kidney injury.

4.5. Dose-Response Effects

This study showed a clear relationship between green tea extract dose and protection level. The 60 mg/kg BW dose (P3) consistently provided the best protection across all parameters, followed by 40 mg/kg BW (P2) and 20 mg/kg BW (P1). Higher doses deliver more EGCG and other protective compounds to kidney tissue, providing stronger antioxidant defense against cigarette smoke damage. Notably, the 60 mg/kg BW dose achieved near-normal protection for tubular degeneration, suggesting this may be close to the optimal dose.

4.6. Clinical Implications and Study Limitations

This study provides evidence that green tea extract can protect kidneys from cigarette smoke damage. These findings are particularly important for smokers and those exposed to secondhand smoke. Regular green tea consumption could be a simple and accessible strategy to reduce kidney damage risk. However, this study has limitations. First, it used mice, so results cannot be directly applied to humans without clinical trials. Second, the 36-day duration was relatively short and may not reflect long-term cigarette exposure effects. Third, the study only measured tissue damage without biochemical markers like urea, creatinine, or oxidative stress indicators, which would provide a more complete picture of kidney function and protection mechanisms.

5. Conclusion

This study demonstrates that cigarette smoke exposure induces significant renal histopathological damage, including glomerular necrosis, tubular cell necrosis, and tubular cell degeneration. Green tea extract administration shows significant protective effects against this damage with a clear dose-response pattern. The 60 mg/kg BW dose provides the most optimal protective effect, particularly for the tubular cell degeneration parameter, which shows results not significantly different from the negative control group. This protective effect is presumably mediated through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms of polyphenolic compounds, particularly EGCG, contained in green tea.

Compliance with ethical standards

Acknowledgments

We thank the Faculty of Medicine, Universitas Airlangga, for granting ethical clearance for this study (approval number: 2.KE.047.07.2020). We also acknowledge all laboratory staff and technicians for their valuable assistance and support during the research process.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This study was approved by the Animal Care and Use Committee, Universitas Airlangga, Surabaya, Indonesia (Ethical Certificate No. 2.KE.047.07.2020).

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