

GREEN TEA EXTRACT ATTENUATES CIGARETTE SMOKE-INDUCED RENAL STRESS IN MICE

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Abstract

Cigarette smoke induces oxidative stress that impairs kidney function, as reflected by increased blood urea nitrogen (BUN) and creatinine levels. Green tea extract (GTE), rich in epigallocatechin gallate (EGCG), exhibits antioxidant properties with potential nephroprotective effects; however, its role in attenuating cigarette smoke-induced renal injury remains unclear. This study employed a true experimental design with a post-test-only control group approach using 25 male mice (aged 12 weeks, 20–25 g), randomly assigned into five groups (n = 5): negative control (C-), positive control (C+), and three treatment groups receiving GTE at 20 (T1), 40 (T2), and 60 mg/kg BW (T3). On day 37, serum BUN and creatinine levels were measured. The C+ group exhibited significantly elevated BUN (30.42 ± 4.15 mg/dL) and creatinine (0.50 ± 0.06 mg/dL) compared to C- (BUN: 17.14 ± 2.38 mg/dL; creatinine: 0.22 ± 0.04 mg/dL) ($p < 0.05$). GTE administration reduced both markers in a dose-dependent manner; the greatest attenuation was observed in T3 (BUN: 18.95 ± 3.09 mg/dL; creatinine: 0.27 ± 0.04 mg/dL), approaching C- values ($p < 0.05$). These findings suggest that GTE may attenuate cigarette smoke-induced renal dysfunction in mice; however, further studies are warranted to confirm its nephroprotective efficacy.

Keywords: BUN, Creatinine, Green Tea Extract, Nephroprotective, Tobacco Use

INTRODUCTION

Cigarette smoking constitutes the leading preventable cause of morbidity and mortality worldwide, with its pathophysiological consequences extending across virtually all organ systems including, though less prominently recognized, the renal system (Kopp, 2022). Tobacco combustion generates an estimated seven thousand distinct chemical compounds, among them nicotine, polycyclic aromatic hydrocarbons (PAHs), and reactive oxygen species (ROS), which collectively induce systemic oxidative stress and chronic low-grade inflammation that exceed endogenous antioxidant capacity (Umutoni et

al., 2024). The kidneys, functioning as the primary site of blood filtration and toxin elimination, are particularly susceptible to such cumulative chemical insult (Rush & Hook, 2022). Sustained exposure to tobacco smoke has been associated with progressive glomerulosclerosis, tubular dysfunction, and accelerated renal deterioration, ultimately manifesting as elevated serum blood urea nitrogen (BUN) and creatinine concentrations established biochemical markers of impaired renal function (Al-Dagestani, 2021). The escalating global burden of smoking-induced nephropathy underscores an urgent need to identify effective therapeutic or preventive strategies capable of attenuating disease progression prior to end-stage renal failure.

Camellia sinensis (green tea) represents a botanically derived candidate of growing pharmacological relevance. Historically regarded primarily as a dietary beverage, green tea has attracted considerable scientific interest owing to the bioactive properties of its principal polyphenolic constituents epigallocatechin gallate (EGCG), catechins, and flavonoids which exert potent antioxidant and anti-inflammatory effects through modulation of cellular redox signaling pathways (Capasso et al., 2025). Accumulating evidence suggests that these phytochemicals confer cytoprotective effects in hepatic, cardiac, and pulmonary tissues subjected to chronic oxidative damage (Zhou et al., 2025; Eng et al., 2018; Mokra et al., 2022). In preclinical models, green tea extract has demonstrated the capacity to scavenge free radicals, attenuate pro-inflammatory signaling cascades, and upregulate endogenous antioxidant enzyme activity, thereby substantially mitigating oxidative tissue injury (Mao et al., 2017). Its nephroprotective potential has been evaluated in experimental models of diabetic nephropathy and chemically induced renal toxicity (Gao et al., 2021; El-Desouky et al., 2019); however, its efficacy in the context of smoking-induced renal injury remains insufficiently characterized. The present study aimed to investigate whether green tea extract attenuates renal dysfunction in cigarette smoke-exposed *Mus musculus*, thereby contributing to the expanding body of evidence supporting its therapeutic utility. The findings may inform the development of evidence-based dietary interventions for the prevention or amelioration of smoking-related nephropathy, with direct implications for public health strategies targeting high-risk populations.

MATERIALS AND METHODS

This study used a true experimental design with a posttest-only control group design. On July 2, 2020, the Animal Care and Use Committee of Universitas Airlangga issued ethical approval (No. 2.KE.047.07.2020) for the study protocol described herein.

Preparation of Green Tea Extract (GTE)

A total of 1000 g of dried green tea leaves were finely powdered and extracted by maceration in 96% ethanol (8 L) at room temperature for 72 hours. The obtained macerate was concentrated using a rotary evaporator at 50°C with a rotation speed of 45 rpm for approximately 5 hours. The concentrated extract was then freeze-dried to obtain a dry extract following the method described by Khoirunnisa et al. (2019). The resulting dry extract was subsequently suspended in 1% Na-CMC to achieve the required dosage for administration//.

Experimental Design and Treatment

This study employed a true experimental design with a post-test-only control group design. The design involved five groups of subjects, with three groups treated with green tea formulation at sequential doses of 20, 40, and 60 mg/kg body weight (T1, T2, and T3), one group without cigarette smoke exposure receiving only 0.5 mL of 1% Na-CMC solution (negative control group, C-), and one group exposed to cigarette smoke and given 0.5 mL of 1% Na-CMC (positive control group, C+). The sample in this study consisted of 25 male mice (*Mus musculus*) weighing 20-25 g and aged 12 weeks. A sample size of five animals per group is consistent with the minimum recommended for preliminary in vivo pharmacological studies conducted under the 3Rs principles of animal research (replacement, reduction, and refinement), and is comparable to group sizes reported in analogous experimental models. It is acknowledged, however, that the absence of a formal a priori power calculation represents a methodological limitation; future studies should incorporate power analysis to ensure adequate statistical power and to enhance reproducibility of findings.

A special room measuring 31×19×22 centimeters was used to expose the mice to cigarette smoke for 20 minutes per session. Each exposure session utilized one clove cigarette with a nicotine content of 2.2 mg. Green tea extract was administered orally by gavage 30 minutes prior to each smoke exposure session. The smoke concentration within the chamber was not instrumentally measured; however, a standardized exposure protocol was maintained by using one

cigarette per session of fixed duration. The chamber was ventilated and cleaned between sessions to prevent residual smoke accumulation. Each exposure session involved all mice within the respective group simultaneously (five mice per session per group (Fadhilah et al., 2023).

Sample Collection and Biochemical Analysis

On day 37, the mice were euthanized by cervical dislocation under anesthesia. Blood was drawn via cardiac puncture from the left ventricle into EDTA-coated tubes (plasma) and plain tubes (serum). Samples incubated for 15 minutes at 37°C and centrifuged (3000 rpm, 15 minutes, 4°C) to split serum, which was kept at -20°C. Plasma creatinine and BUN levels were analyzed using an automated clinical analyzer (RA 1000; Technicon Instruments, USA) (Rahmatullah, 2024).

Statistical Analysis

The data analysis process in this study utilized the SPSS 30th edition. Each measurement result is displayed in the format of the mean value accompanied by a standard deviation (mean ± SD). Data normality was tested using the Shapiro-Wilk method, while the Levene test was used to test for homogeneity of variance. Comparisons between experimental groups for serum BUN and creatinine levels were performed using one-way ANOVA followed by Tukey's post hoc test.

RESULT

Green tea extract (*Camellia sinensis*) impacts on serum blood urea nitrogen (BUN) and creatinine levels in mice (*Mus musculus*) exposed to cigarette smoke were determined in five treatment groups: negative control (C-), positive control (C+), and three treatment groups that received various doses of green tea extract (T1, T2, T3). Results, expressed as mean ± standard deviation (SD), are presented in **Table 1**, **Figure 1** and **Figure 2**.

Table 1. Serum BUN and Creatinine Levels Across Treatment Groups

Treatment Group	BUN Serum Levels (mg/dl) (Mean ± SD)	Creatinine Serum Levels (mg/dl) (Mean ± SD)
C-	17.14 ^a ± 2.38	0.22 ^a ± 0.04
C+	30.42 ^c ± 4.15	0.50 ^d ± 0.06
T1	23.62 ^c ± 3.71	0.39 ^{cd} ± 0.03
T2	23.69 ^{bc} ± 3.68	0.34 ^{bc} ± 0.04
T3	18.95 ^{ab} ± 3.09	0.27 ^{ab} ± 0.04

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T3	18.95 ^{ab} ± 3.09	0.27 ^{ab} ± 0.04

Note: Differences in superscripts a, b, c, d in the same column indicate significant differences ($p < 0.05$).

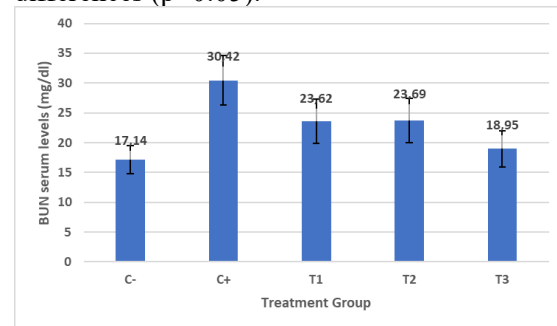


Figure 1. BUN serum levels in mice (*Mus musculus*) exposed to cigarette smoke and treated with different doses of green tea extract.

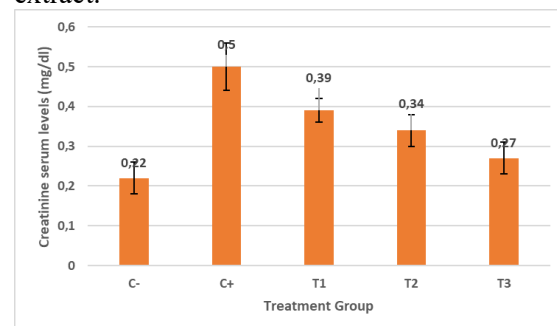


Figure 2. Creatinine serum levels in mice (*Mus musculus*) exposed to cigarette smoke and treated with different doses of green tea extract.

In the negative control group (C-), which hadn't been exposed to cigarette smoke, serum BUN levels averaged 17.14 ± 2.38 mg/dl well within the normal range for healthy kidney function. In contrast, the positive control group (C+), exposed to smoke but without any green tea extract, showed a sharp rise to 30.42 ± 4.15 mg/dl. That jump alone suggests substantial kidney strain caused by the smoke. Once green tea extract entered the picture, however, the pattern began to shift. BUN levels declined gradually with each increasing dose: 23.62 ± 3.71 mg/dl for the lowest dose (T1), 23.69 ± 3.68 mg/dl for the middle (T2), and 18.95 ± 3.09 mg/dl for the highest (T3). By the highest dose, the values were nearly indistinguishable from the healthy controls,

implying that the extract provided considerable relief from smoke-induced damage.

A similar trend was observed for serum creatinine levels. The C⁻ group maintained normal levels at 0.22 ± 0.04 mg/dl, whereas C⁺ nearly doubled to 0.50 ± 0.06 mg/dl, signaling clear kidney distress. Again, treatment groups showed a steady improvement as the green tea dose increased: 0.39 ± 0.03 mg/dl for T1, 0.34 ± 0.04 mg/dl for T2, and 0.27 ± 0.04 mg/dl for T3. The highest-dose results came remarkably close to the baseline, reinforcing the idea that green tea's bioactive compounds may help protect renal function under oxidative stress.

DISCUSSION

Our findings suggest that green tea extract (*Camellia sinensis*) offers substantial protection against kidney stress caused by cigarette smoke exposure in *Mus musculus* mice. This protective effect was most evident in the dose-dependent decreases in serum BUN and creatinine levels. The positive control group (C⁺), which was exposed to smoke without any extract treatment, showed markedly elevated BUN (30.42 ± 4.15 mg/dl) and creatinine (0.50 ± 0.06 mg/dl) compared with the negative control (C⁻), which maintained normal levels of 17.14 ± 2.38 mg/dl and 0.22 ± 0.04 mg/dl, respectively. These findings align with previous research showing how cigarette smoke generates oxidative stress and damages renal tissue, thereby disrupting kidney function (Mayyas & Alzoubi, 2019). The nephrotoxic nature of cigarette smoke stems largely from its bioactive compounds—nicotine, polycyclic aromatic hydrocarbons, and reactive oxygen species (ROS)—which interfere with glomerular filtration and tubular reabsorption (Raja et al., 2022).

When green tea extract was administered at increasing doses (20 mg/kg BW for T1, 40 mg/kg BW for T2, and 60 mg/kg BW for T3), there was a clear downward trend in both biomarkers. The highest dose (T3) nearly restored values to those of the healthy control group (BUN: 18.95 ± 3.09 mg/dl; creatinine: 0.27 ± 0.04 mg/dl). This dose-dependent response implies that the nephroprotective effect strengthens with higher concentrations of the extract, likely due to its polyphenolic

compounds, particularly epigallocatechin gallate (EGCG). Several studies have described EGCG's ability to neutralize free radicals, suppress lipid peroxidation, and boost endogenous antioxidant enzymes such as superoxide dismutase and catalase, which collectively reduce oxidative damage in kidney tissue (Mao et al., 2017). These antioxidant processes counteract the oxidative burden imposed by cigarette smoke, which is known to impair cellular integrity and renal microarchitecture (Rahajeng et al., 2024).

The near-normalization of renal biomarkers in the treated mice suggests that green tea extract may have preserved both glomerular filtration rate (GFR) and tubular reabsorption efficiency—two cornerstones of kidney homeostasis (Elsawi et al., 2024). This finding supports earlier evidence of green tea's protective role in other models of renal injury, such as cisplatin-induced nephrotoxicity and diabetic nephropathy, where it mitigated oxidative stress, inflammation, and apoptosis (Mohib et al., 2016). Although green tea extract has been studied in several renal injury models, the present study adds evidence regarding its potential protective effect in cigarette smoke induced renal stress. It's worth acknowledging, however, that the extract's protective mechanisms are likely multifaceted. Beyond its antioxidant role, green tea is known to temper inflammation by inhibiting NF- κ B activation and reducing levels of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β (Ohishi et al., 2016).

Several limitations should be acknowledged. Renal function was assessed exclusively through serum BUN and creatinine concentrations, which, while established biochemical markers of nephrotoxicity, provide limited resolution of the underlying structural pathology. Kidney histopathological examination, kidney weight index, and urinalysis including assessment of proteinuria were not performed in the present study and would have substantially strengthened the nephroprotective claim. Then, the small sample size of five animals per group, adopted in accordance with the 3Rs framework for preliminary animal research, was not preceded by a formal a priori power calculation; this limits the statistical power of the study and the generalizability of the findings. The green tea extract also was not chemically standardized

for EGCG or total catechin content, which introduces variability that may affect reproducibility. Therefore, although the present findings provide preliminary evidence of a protective effect of green tea extract against smoking-induced renal dysfunction, further studies incorporating histopathological evaluation, molecular biomarkers of oxidative stress and inflammation, and standardized phytochemical characterization are warranted to elucidate the precise mechanisms underlying these observations.

CONCLUSION

This study provides compelling evidence that green tea extract (*Camellia sinensis*) exerts significant nephroprotective effects in mice exposed to cigarette smoke, as demonstrated by dose-dependent reductions in serum BUN and creatinine levels. The highest dose of 60 mg/kg BW was particularly effective, nearly restoring renal biomarkers to baseline levels, likely due to the antioxidant and anti-inflammatory properties of GTE's polyphenolic compounds. These findings highlight the potential of GTE as a natural therapeutic agent for mitigating smoking-related renal dysfunction.

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