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Curative Properties of Kencur (*Kaempferia galanga* L.) Extract on Mice (*Mus musculus*) Kidney Histopathology Exposed to Cigarette Smoke

Intan Diah Safitri Arianto[1], Firman Lastoaji[2*], Sri Agus Sudjarwo[3], Thomas Widiyatno Valentinus[4], Eka Pramyrtha Hestiana[5], Iwan Sahrial Hamid[3], Eduardus Bimo Aksono Herupradoto[3], Aprinda Ratna Lovela[6]

Corresponding email: lastoaji12@gmail.com

[1]Magister Program of Veterinary Agribusiness, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
[2]Bachelor Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
[3]Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
[4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
[5]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
[6]Department of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Pendidikan Mandalika, Mataram 83125, Indonesia.

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Abstract

The aim of this research was to investigate the therapeutic effects of Kencur (*Kaempferia galanga* L.) extract on kidney histopathological damage in male mice exposed to cigarette smoke. Twenty 8-week-old male mice were randomly divided into five treatment groups (n=5). The negative control group (C-) consisted of mice not given kencur extract or exposed to cigarette smoke, while the positive control group (C+) comprised mice exposed to cigarette smoke without kencur extract. Treatment groups included Treatment 1 (T1) administered 150 mg/kg BW of kencur extract, Treatment 2 (T2) administered 300 mg/kg BW, and Treatment 3 (T3) administered 600 mg/kg BW. Mice were exposed to cigarette smoke for 14 days. The data were analyzed using the Kruskal-Wallis test to evaluate overall differences among the treatment groups. Where significant differences were found ($p<0.05$), further pairwise comparisons were conducted using the Mann-Whitney test, confirming statistically significant differences between specific treatment groups. Treatment 3 (T3) was found to be the most effective in reducing histopathological damage in the kidneys of male mice. This study demonstrates that kencur extract effectively reduces histopathological damage in the kidneys of mice exposed to cigarette smoke, highlighting its potential as a protective agent against smoke-induced tissue injury.

Keywords

Cigarette smoke, *Kaempferia galanga* L. extract, *Mus musculus*, Renal tubular cell

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Introduction

Smoking cigarettes is a longstanding habit among Indonesian citizens. In 2020, approximately 225,700 deaths in Indonesia were attributed to smoking-related diseases (World Health Organization, 2021). A single cigarette contains over 4,000 hazardous chemical substances, including arsenic, carbon monoxide, ammonia, formaldehyde, cadmium, acetone, methane, urea, nitrosamines, biphenyls, and arylamines (Yamamoto *et al.*, 2015; Yershova *et al.*, 2016). These chemicals are known to be extremely harmful to the human body. About 85% of the chemicals emitted from cigarette smoke are in the form of gases, with the remaining 15% consisting of particulate matter (Riady, 2014). Cigarette smoke contains organic free radicals in its gaseous phase, and the toxic substances such as nicotine, lead (Pb), carbon monoxide (CO), tar, polycyclic aromatic hydrocarbons (PAHs), and metals can penetrate mitochondria, leading to increased free radical production within cells and causing oxidative stress (Fayang, 2022).

Oxidative stress, as defined by Adwas *et al.* (2019), occurs when the balance between reactive oxygen species (ROS) formation and detoxietfication favors an increase in ROS levels, leading to disturbed cellular function. ROS formation leads to DNA transport damage, potentially resulting in cellular injury or death. This can impede oxygen delivery and constrict blood vessels in organs such as the kidneys. Vascular constriction in the kidneys may impair glomerular filtration of toxic substances. Additionally, the renal tubules are susceptible to damage due to direct exposure of their epithelial cells to absorbed substances, leading to nuclear degeneration and necrosis (Hertika and Putra, 2019).

Degeneration and necrosis occur when cellular stress exceeds the cell's adaptive capacity, leading to pathological changes and eventual cell death (Sazonosa *et al.*, 2021). Degeneration represents an initial stage of toxin-induced damage, characterized by transient or reversible cellular injury, during which cells exposed to toxins can potentially heal and return to normal function. In renal tubular cells, degeneration manifests as fluid influx, vacuole formation, and cellular enlargement. Acute cellular swelling, a common reversible injury, occurs early when cells fail to maintain ionic and fluid homeostasis (Wallig and Janovitz, 2022). Necrosis signifies an advanced stage of degeneration resulting from prolonged exposure of tubular cells to substances that induce morphological cell death. Necrotic changes in renal tubular cells include karyolysis (pale and faded nucleus), pyknosis (shrinkage and increased density of the nucleus), and karyorrhexis (fragmentation of the nucleus) (Miller and Zachary, 2022).

In the realm of plant biology, numerous chemical components serve as potential sources of alternative medicine and supplementary compounds. According to phytochemical screening, one such plant reputed for its antioxidant properties is kencur. Chemical analysis indicates that kencur contains antioxidants, cytotoxic agents, anti-inflammatory compounds, sedatives, vasorelaxants, anti-angiogenic substances, anti-nociceptives, and agents that accelerate wound healing (Wahyuni *et al*., 2022). The antioxidants present in kencur extract are known to counteract free radicals that form within the body (Kiptiyah *et al*., 2021). Oxidative stress arises from an imbalance between oxidant and antioxidant molecules, leading to increased

production of reactive oxygen species (ROS) (Ikrima *et al.*, 2020). Consequently, a higher concentration of antioxidants is required to mitigate the detrimental effects of free radicals, thereby enhancing the body's antioxidant requirements.

No research has yet investigated the therapeutic properties of kencur extract in assessing its efficacy on kidney cells exposed to cigarette smoke and evaluated through histopathological imaging. This study aimed to determine the potential therapeutic effects of kencur extract on kidneys damaged by exposure to cigarette smoke.

Materials and Methods

Experimental Design

This research employed a complete randomized design (CRD). The treatment of samples in this study was carried out randomly, while the age of the mice and environmental conditions were kept uniform. The sample size for the experimental study was determined using Federer's formula (Al-Arif, 2016). Each group was required to have a minimum of four repetitions. The mice were randomly selected and divided into five groups. This study used 20 male mice, with two control groups and three treatment groups. Each group consisted of four mice.

Experimental Animal Preparation

The study utilized 20 healthy male mice (*Mus musculus*), aged eight weeks and weighing between 30 to 40 grams. The mice were selected based on criteria of clear eyes, normal behavior, and smooth fur. All animal treatments and adaptations were conducted in the experimental laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya.

Kencur Extract Preparation

Kencur extract preparation was conducted at UPT Medica Media, Batu. The kencur was peeled, washed, sliced, dried, ground, and sieved using a mesh sieve. Three kilograms of kencur powder were then macerated in five liters of 96% ethanol. The maceration process was carried out over a period of 3x24 hours, with stirring performed daily. On the third day, the macerate was filtered using a flannel cloth. The macerate was then evaporated using a rotary evaporator at 50 rpm and 45°C until a thick extract was obtained (Agustina *et al.*, 2021).

Treatment

The experimental animals underwent a seven-day adaptation period and were then randomly assigned to five groups, each with at least four repetitions.

1. **Negative Control Group (C-)**: Received 0.5 mL of distilled water orally for 14 days.
2. **Positive Control Group (C+)**: Exposed to cigarette smoke (1 cigarette/day) for 14 days.
3. **Treatment Group 1 (T1)**: Exposed to cigarette smoke (1 cigarette/day) for 14 days, followed by daily oral administration of kencur extract at 150 mg/kg BW/day in a 1% suspension of CMCNa and 0.5 mL of distilled water from day 15 to day 28.
4. **Treatment Group 2 (T2)**: Underwent the same smoke exposure regimen as T1 and received kencur extract at 300 mg/kg BW/day from day 15 to day 28.
5. **Treatment Group 3 (T3)**: Underwent the same smoke exposure and received kencur extract at 600 mg/kg BW/day from day 15 to day 28.

Staged doses of kencur extract that administered according to the mice groups with the dosages are 150, 300, and 600 mg/Kg BW (Sulaiman *et al*., 2008). Cigarette smoke exposure was administered using a smoking chamber consisting of a plastic box with two designated holes. The first hole served for inserting the lit cigarette into a container to collect smoke, while the second hole facilitated smoke release and air exchange within the box. A 20cc syringe attached to a rubber hose was used to draw smoke from the container and simulate cigarette smoke exposure (Riady, 2014).

Slide Preparation and Histopathological Examination

On the 29th day, the experimental animals were euthanized, followed by abdominal surgery to retrieve the kidneys for histopathological examination. The kidney tissues were processed into histopathological slides and observed under a microscope at 400x magnification.

Data Collection

The assessment of kidney cell damage was conducted by evaluating the degree of tubular epithelial cell degeneration and necrosis in the interstitial space using the modified Klopfleisch (2013) method. The result of each sample is the average number of all types of lesions that occur in the parameter based on Table 1.

Table 1. Scoring parameter for kidney cell damage (Klopfleisch, 2013).

Lesion	Score	Description
Tubulus Epithelial Cell Degeneration	0	No degenerative changes
	1	If degenerative cell <25% from Field of View (FoV)
	2	If degenerative cell is between 26-50% from FoV
	3	If degenerative cell is between 51-75% from FoV
	4	If degenerative cell >76% from FoV
Tubulus Epithelial Cell Necroses	0	No necrotic changes
	2	If necrotic cell <25% from FoV
	4	If necrotic cell is between 26-50% from FoV
	6	If necrotic cell is between 51-75% from FoV
	8	If necrotic cell >76% from FoV

Data Analysis

Prior to the statistical analysis, a normality test was conducted to determine the distribution of the data. If the data were normally distributed, an ANOVA test was performed followed by a post hoc test to

identify specific group differences. However, if the data were not normally distributed, the Kruskal-Wallis test was used to assess differences between groups, followed by the Mann-Whitney test for pairwise comparisons between specific groups (Al-Arif, 2016). Statistical significance was determined at a p-value of less than 0.05.

Results and Discussion

Based on the research findings, the administration of kencur extract at doses of 150 mg/kg BW/day, 300 mg/kg BW/day, and 600 mg/kg BW/day provided protection against histopathological damage caused by exposure to cigarette smoke. The data from the research were analyzed using ANOVA to assess the normality of the data. Since the data distribution was not normal, the Kruskal-Wallis test was applied, followed by the Mann-Whitney test, which indicated significant differences between groups ($p<0.05$). The mean rank table and histopathological images are presented in Table 2.

Table 2. The average number of degeneration and necrosis rates of renal tubular cells in male mice.

Treatments	Mean ± SD	
	Degeneration	Necrosis
C (-)	$0.70^{a} \pm 0.22$	$1.40^{a} \pm 0.45$
C (+)	$2.80^{bc} \pm 0.24$	$6.00^{b} \pm 0.79$
T1	$2.70^{c} \pm 0.31$	$5.50^{c} \pm 0.49$
T2	$2.20^{c} \pm 0.13$	$4.50^{c} \pm 0.32$
T3	$1.80^{d} \pm 0.57$	$3.60^{d} \pm 1.23$

Note: ^{a,b,c,d} Different superscripts in the same column show significant differences ($P<0.05$)

Based on the results, the levels of degeneration and necrosis in renal tubular cells of mice were analyzed using the Kruskal-Wallis test, revealing a significant effect between the control group and the treatment groups ($p<0.05$). As indicated in Table 2, the C+ group exhibited the highest damage due to cigarette smoke exposure, followed by the T1 and T2 groups. The T3 group demonstrated significantly lower damage levels, approaching those of the C- group, suggesting that a dosage of 600 mg/kg BW/day provides substantial protective effects.

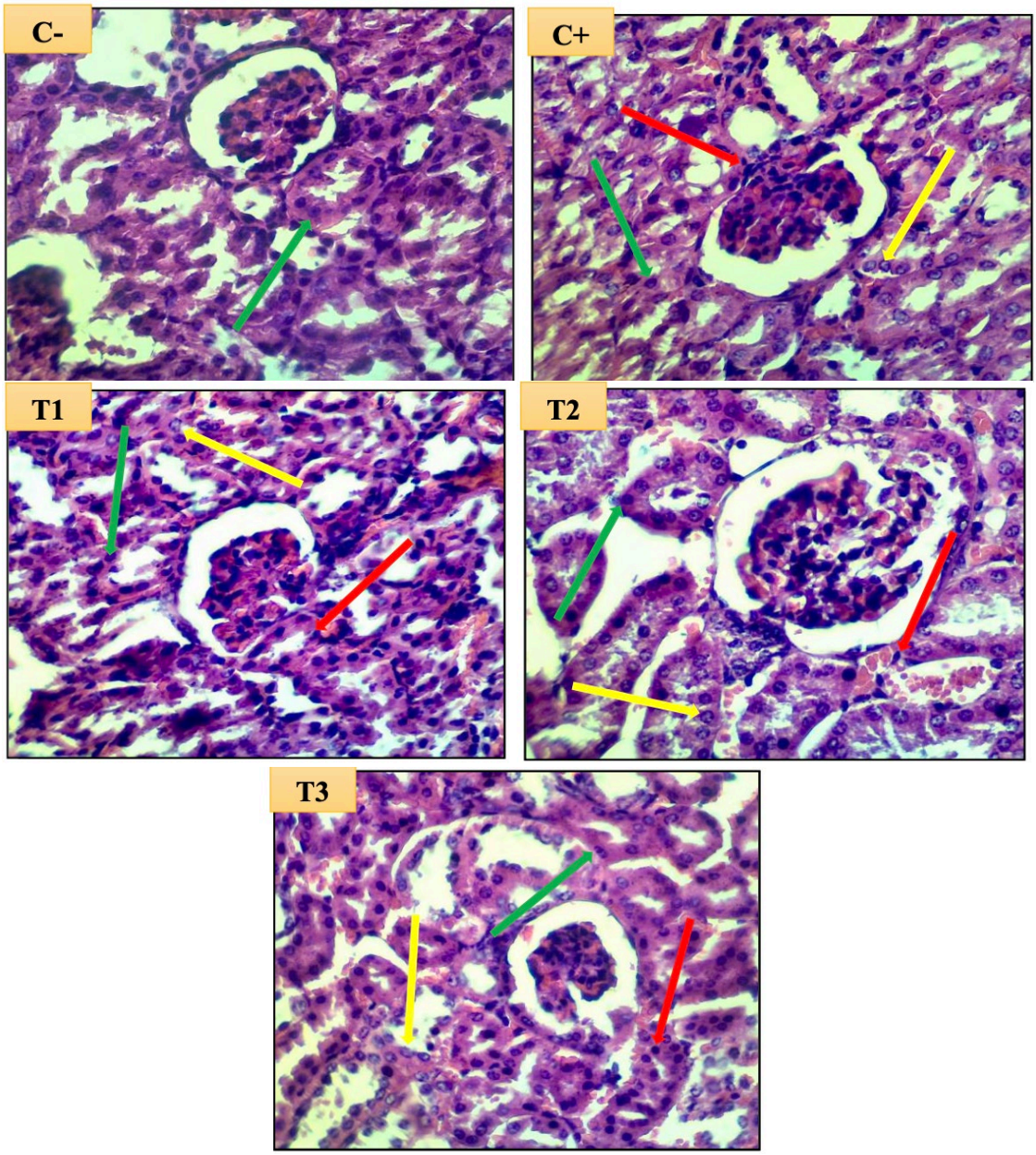

Figure 1. Histopathological slides of renal tubular cell of male mice exposed to cigarette smoke on each group (H&E stain, 400x magnification) (Green arrow: normal cell, yellow arrow: Degeneration cell, Red arrow: Necrosis cell).

Cigarette smoke exposure is a major source of free radicals, leading to oxidative stress and subsequent cellular damage. As detailed by Chaudhary *et al*. (2023), cigarette smoke is laden with harmful substances such as carbon monoxide, hydrogen cyanide, sulphur oxide, ammonia, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs), all of which contribute to the excessive production of reactive oxygen species (ROS). These ROS

overwhelm the cellular antioxidant defense resulting in oxidative stress, which is a key driver of histopathological damage, particularly in renal tissues (Ortiz *et al*., 2016).

Renal tubular cells are particularly vulnerable to such oxidative damage due to their high metabolic activity and reabsorption function, which exposes them to a concentrated load of toxins and free radicals (George *et al*., 2017). The findings from this study indicate that the administration of kencur extract significantly reduces histopathological damage in the kidneys of mice exposed to cigarette smoke. Specifically, groups treated with kencur extract exhibited fewer instances of tubular epithelial cell degeneration and necrosis compared to the control group exposed to cigarette smoke alone.

Previous studies have highlighted the ethnomedical efficacy of various plant extracts, including kencur, in mitigating organ damage caused by oxidative stress. For instance, Padmiswari *et al*. (2020) demonstrated that kencur extract has potent antioxidant properties that can neutralize free radicals, thereby protecting renal tissues from oxidative injury. Similarly, Sarungallo *et al*. (2022) and Wijayanti, (2016) reported that plant-based antioxidants, like those found in kencur, could effectively reduce oxidative damage in renal tissues exposed to toxic substances, including cigarette smoke.

The higher dose of kencur extract (600 mg/kg BW/day) in the T3 group provided the most significant protective effect, as evidenced by the minimal degeneration and necrosis observed in this group. This aligns with Wahyuni *et al*. (2022), who noted that the chemical constituents of kencur, such as phenolic compounds and flavonoids, are known for their robust antioxidant activities. These compounds scavenge free radicals, reducing oxidative stress and preventing cellular damage.

Moreover, nicotine, a major component of cigarette smoke, exacerbates oxidative stress by interacting with nicotinic acetylcholine receptors (nACHRs) on renal mesangial cells, leading to necrosis (Bertrand *et al*., 2015). The protective effects of kencur against nicotine-induced renal damage could be attributed to its ability to inhibit ROS formation and bolster the antioxidant defense system within the kidney cells (Sayuti and Rusita, 2023).

In addition to its antioxidant properties, kencur has been shown to possess anti-inflammatory, anti-apoptotic, and cytoprotective effects, which further contribute to its protective role against cigarette smoke-induced renal damage (Ali, 2018). The presence of bioactive compounds such as phthalic acid, oleic acid, and hexadecanoic acid in kencur enhances its ability to mitigate oxidative damage and support cellular health under stress conditions (Ikrima *et al*., 2020).

In conclusion, this study reaffirms the ethnomedical potential of kencur as a protective agent against cigarette smoke-induced renal damage, supported by both current findings and existing literature. Further studies are warranted to explore the mechanistic pathways through which kencur and similar botanicals exert their protective effects, as well as their potential applications in clinical settings.

Conclusion

Based on the conducted research, the administration of kencur (*Kaempferia galangal* L.) demonstrated a protective effect by significantly reducing degeneration and necrosis in the kidneys of male mice (*Mus musculus*) exposed to cigarette smoke. The most

effective dose of kencur extract in preventing histopathological damage to the kidneys of male mice exposed to cigarette smoke was found to be 600 mg/kg BW/day.

Approval of Ethical Commission

Prior to the study, ethical approval was obtained from the Animal Care and Use Committee, Faculty of Veterinary Medicine Universitas Airlangga with number 1.KE.122.10.2021.

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Author's Contribution

Conceptualization, F.L; Methodology, F.L, S.A.S, T.W.V; Software, I.D.S.A, F.L, A.R.L; Writing Original–Review & Editing, I.D.S.A, F.L, A.R.L; Project Administration, I.D.S.A, F.L, A.R.L; Formal Analysis I.D.S.A, F.L, A.R.L; Supervision, S.A.S, T.W.V, E.P.H, I.S.H, E.B.A.H; Software, I.D.S.A, F.L, A.R.L; Funding Acquisition, F.L.

Conflict of Interest

The authors have declared no conflicts of interest.

Data Availability Statement

Data presented within the article, provided in supplementary materials, or referenced throughout the article.

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