

**THE EFFECT OF *PEPEROMIA PELLUCIDA* EXTRACT TO REDUCE
TRANSFORMING GROWTH FACTOR- β EXPRESSION IN THE RENAL OF
MALE WISTAR RAT THAT BEING EXPOSED TO CIGARETTE SMOKE**

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Abstract

Purpose : *Peperomia Pellucida* extract can effect the reduction of Transforming Growth Factor – β expression in the renal of male wistar rat that being exposed with cigarette smoke. Cigarette smoke

contains dangerous substance that can be toxic to the body and cause fibrosis in the renal. The main fibrosis mediator in the renal is Transforming Growth Factor – β . *Peperomia Pellucida* is one of the plant species in tropical areas as "erva de jabuti" which has been used in traditional medicine. This plant extract can inhibit Transforming Growth Factor – β expression which cause by cigarette smoke.

Method : True experimental, post test only control group design, simple random sampling. Sample: 21 male wistar rat 8 - 12 weeks with 180-200 gr of weight. This research divided to 3 groups. These groups are again divided to 1 control group and 2 treatment groups. The control group (K0) was fed with standard meals for 5 weeks then, the first treatment group (P1) were fed with standard meals for 1 week and exposed with cigarette smoke for 4 weeks (1 cigarette /1 rat/ day) with the same standard meals. The second (P2) treatment group were fed with standard meals and given with *Peperomia Pellucida* extract (400mg/kg/body weight from a nasogastric tube) for 1 week and being exposed to cigarette smoke for 4 weeks (1 cigarette /1 rat/ day) and fed with standard meals and *Peperomia Pellucida* extract (400mg/kg/body weight from a nasogastric tube). The research's data result was proceed with statistic computer program (SPSS 27.0) with one way annova test.

Result : This research shows a significant effect of Transforming Growth Factor- β expression to the renal of male wistar rat that being exposed with cigarette smoke.

Conclusion : The given *Peperomia pellucida* extract can reduce Transforming Growth Factor- β expression in the renal of male wistar rat that being exposed with cigarette smoke.

KeyWords : Cigarette smoke, Male wistar rat, *Peperomia Pellucida* extract, Transforming Growth Factor- β .

Introduction

Epidemiological research shows that smoking is linked to Chronic Kidney Disease (CKD). The Multiple Risk Factor Intervention Trial (MRFIT) in 2003 investigated 332,544 men and found that smoking was associated with an increased risk of end-stage renal disease (ESRD). Other research studied by Halimi et al (2000) which included 28,409 volunteers showed that active smokers and former smokers had a risk of having macroalbuminuria (Setyawan, 2021).

Cigarette smoke contains toxic substances such as tar, nicotine, benzo(a)pyrene, carcinogenic hydrocarbon compounds, and cadmium (Rahayuningsih et al., 2022), which can lead to coronary heart disease, chronic lung disease, chronic kidney disease, hair loss, and infertility in passive smokers (Genchi et al., 2020).

Chronic Kidney Disease (CKD) due to passive smoking is a global health problem with a continuing increase of prevalence and incidence each year (Syamsi et al., 2021). The pathological features of chronic kidney disease range from inflammation to fibrosis (Gu et al., 2020). Transforming Growth Factor- β (TGF- β) is the main mediator in kidney fibrosis, so the blocking of TGF- β can inhibit kidney fibrosis. The TGF- β superfamily includes members from TGF- β s, activin, inhibin, growth and differentiation factors (GDFs), bone morphogenetic proteins (BMPs), and glial-derived neurotrophic factors (GDNFs). It is known that mammals have

three isoforms of TGF- β , namely TGF 1, 2, and 3. *Transforming Growth Factor – β 1* (TGF- β) has been considered a profibrotic mediator in various kidney diseases (Gu et al., 2020).

Peperomia Pellucida is one of the plant species in tropical areas as "erva de jabuti" which has been used in traditional medicine, some for the treatment of asthma, coughs, fever, conjunctivitis, inflammation, and cholesterol, and also has a function as diuretic, analgesic, and antibiotic (Gomes et al., 2022).

Materials and Methods

This research is a true experimental research with posttest only control group design. Population used in this research were from adult male wistar rats from the age of 8 to 12 weeks with 180 to 220 grams of weight. From this population, 20 adult male wistar rats were taken through simple random sampling. These wistar rats were divided into 3 groups. The first group was the control group (K0) which used 6 male wistar rats to be fed with standard meals for 5 weeks. The second and last group were the first and second treatment group (P1 and P2) which each used 7 male wistar rats. The first treatment group was fed with standard meals for 1 week before being exposed to cigarette smoke for 4 weeks (1 cigarette/1 rat/day) with the same standard meals as food. The second treatment group was also fed with standard meals for 1 week before being exposed to cigarette smoke (1 cigarette/1 rat/day) with the same standard meals as food and also given *Peperomia pelucida* extract (400 mg/kg/body weight through a nasogastric tube) for 4 weeks. All wistar

rats will be given Ketamine-Xylazine before making the renal Immunohistochemistry sample. The result will be seen from microscope.

Results of this research will be analyze using one way anova to analyze about the effect of *Peperomia pellucida* in decreasing TGF- β expression and multiple comparison with least significant difference method to analyze the differences between and inside of each treatment or control groups.

Ethical Clearance

This research permit has been given by Wijaya Kusuma University Surabaya ethical clearance team with ethical clearance number 79/SLE/FK/UWKS/2024. The permit includes research preparation (disposable mask, disposable handscoon, laboratory coat, laboratory sandal, sock, and gloves), *Peperomia pellucida* plant as sample's treatment was collected in Surabaya and the species determination, wet sortation process, powder making of *Peperomia pellucida* were done by technical unit of herbal Materia Medica Batu laboratory health department of East Java with analytic-grade ingredients.

The dimension of the sample's cage that was used in this research is 33 cm x 27,5 cm x 13 cm for a maximum of 3 rats per cage as the reference given by the experimental animal laboratory of medical faculty Wijaya Kusuma University Surabaya. The standard meals that were given to the rats are chicken meals with the code 511. Cocktail Ketamine-Xylazine which is delivered as deep anaesthesia was used to numb the samples. The supportive tools used during the sample's treatment

phase are nasogastric tube (NGT), smoke chamber, scissors, clamp, 60 ml urine container as organ container, needle, and animal fixation board. All meals and drinks for the samples were given *ad libitum* with 1 week of adaptation phase and 5 weeks of caring-treatment phase.

Research Period and Location

This research began on 24th of April and ended on 22nd of May 2024. The samples were taken from the animal experimental laboratory of Wijaya Kusuma University Surabaya and pathological anatomy laboratory of Brawijaya University. The research took place in animal experimental laboratory of Wijaya Kusuma University Surabaya.

Result and Discussion

Table 1. Immunoreactive Score of Transforming Growth Factor- β Expression

No.	Group	Mean
1.	K0	0,171428571
2.	P1	0,171428571
3.	P2	4,257142857

Based on the table 1 the highest average value for TGF- β was found in the control group (K0) with a value of 0.4 and the lowest expression for TGF- β expression was in the control group with a value of 0. The highest average value for TGF- β expression β was found in treatment group 1 (P1) at 8.8 and the lowest average value for TGF- β expression was in treatment group 1 with a value of 3.4. In Treatment Group 2 (P2) who received the extract *Peperomia Pellucida* with a dose of 400 mg/KgBW there was a decrease in TGF- β with the highest value of 6.8 and the lowest value of 1.4.

The immunohistochemistry result of the kidney can be seen below:

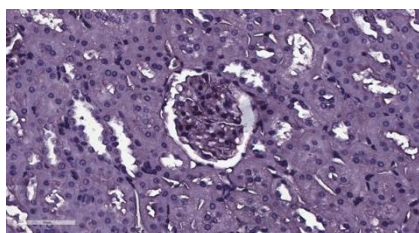


Figure 1. Picture From Wistar Rat Control Group, No Transforming Growth Factor- β

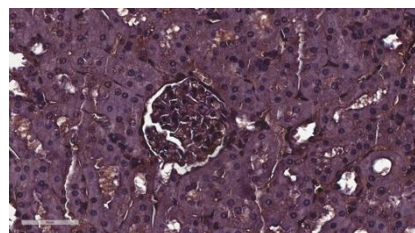


Figure 2. Picture From Wistar Rat First Treatment Group, With Transforming Growth Factor- β Expression (Fibrotic Tissue In Brown Staining).

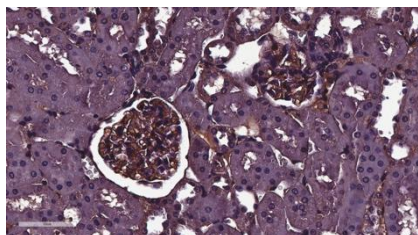


Figure 3. Picture From Wistar Rat Second Treatment Group, With The Reduction of Transforming Growth Factor- β Expression (Fibrotic Tissue In Brown Staining)

This histopathology examination is done to analyze the TGF- β expression in the kidney of the wistar rats. TGF- β expression of each sample was assessed with immunoreactive score in a semiquantitative way. Each data from the sample is the IRS mean score which was observed in 5 different field of view with 400 times magnification. This TGF- β expression stadium result can be seen from the intensity of the color that is stronger that shows more positive TGF- β cells.

The statistical analysis of this research used One-way ANOVA (Analysis Of Variance) normality and homogeneity test.

Table 2. Normality Test of Transforming Growth Factor- β Expression

<i>Shapiro-Wilk</i>			
Group	statistic	df	P
K0	.833	7	.086
P1	.926	7	.518
P2	.920	7	.467

Normality test done in this research used shapiro-wilk test. Based on the table 2, the data from TGF- β expression is distributed normally with the P-value above 0,05 (0,086

Table 3 Homogeneity Test of Transforming Growth Factor- β Expression Data

	P.
Based on Mean	.089
Based on Median	.171
Based on determined df Median	.180
Based on regulated Mean	.087

Based on table 3, the levene homogeneity test based on table 3 shows that the TGF- β expression P-value is 0,089 which mean this data is homogeneous.

Table 4. ANOVA Test of Transforming Growth Factor- β Expression Data

	df	mean	P.
Between group	2	66.688	.000
Within group	18	.126	
Total	20		

Based on table 4 ANOVA test, there's an effect of giving peperomia pellucida extract on reducing TGF- β expression in the renal of adult male wistar rat which being induced with cigarette smoke because the P value result is not more than 0,05.

Table 5. Multiple Comparison Test of Transforming Growth Factor- β Expression Data

Group (I)	Group (J)	P.
	P1	.000
K0	P2	.000

	K0	.000
P1		
	P2	.000
	K0	.000
P2		
	P1	.000

Based on table 5, this post hoc test comparison compared the control group (K0) with the first treatment group (P1) and second group treatment (P2) which resulted with a significant difference. When the two treatment groups were compared, there's also found a significant difference.

Non-hemodynamic mechanisms produce toxic effects on endothelial cells, which cause endothelial dysfunction through oxidative stress mechanisms, reactive oxygen species(ROS) and decreased production of nitric oxide (nitric oxide, NO), the hemodynamic mechanism involved in activating the sympathetic nervous system through the increasing of systemic blood pressure and intraglomerular pressure (Setyawan, 2021).

Transforming Growth Factor – β (TGF- β) is considered a profibrotic mediator in various kidney diseases (Gu et al., 2020). TGF- β has the ability to activate interstitial fibroblasts, induce apoptosis causing intrinsic kidney cells to be lost and replaced with fibrotic ones, high TGF- β can cause hardening of the glomerulus and trigger the growth of scar tissue in the kidney, through increasing

the synthesis of extracellular matrix (Isaka, 2018). Based on previous research, exposure to 1 cigarette of kretek cigarette smoke/day can damage organs, because the contents of kretek cigarettes in the form of tobacco and cloves are toxic if consumed excessively (Mayyas et al., 2019). In the research results for the first treatment group (P1), there was a significant increase with a value of 8.8. The second treatment group (P2) had a significant decrease with a value of 1.4.

Peperomia pellucida can be used for the treatment of various diseases, including kidney infections. This plant also shows pharmacological activity as antimicrobial, antioxidant, anti-angiogenic, anti-inflammatory, analgesic, antipyretic, neuropharmacological, anti-cancer, enzyme inhibitor, antiulcer, lowering blood pressure, immunostimulant, healing bones, and antidiabetic (Islamiyah, 2020). From the previous research. *Peperomia pellucida* extract acts as an anti-inflammatory, antidiuretic, anti-cholesterol and analgesic (Ivan Charles Seran1, 2023).

This research shows that *Peperomia pellucida* extract can reduce TGF- β because *peperomia pellucida* extract has antioxidant and anti-inflammatory pharmacological activity.

Conclusion

The research result shows a significant effect of giving *Peperomia Pellucida* extract on reducing Transforming Growth Factor- β expression in the renal of adult male wistar rats which being induced to cigarette smoke.

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Author's Contribution

1. Conceptualization

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2. Methodology

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5. Formal analysis

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