THE EFFECT OF BLACK TEA (Camellia sinensis) INFUSION ADMINISTRATION ON RENAL HISTOPATHOLOGY OF Sprague Dawley MALE RATS INDUCED BY USED COOKING OIL

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ABSTRACT

Used cooking oil is cooking oil that has been heated repeatedly so that it can cause the formation of free radical compounds. Free radicals are toxic kidneys that generate cell injury via lipid peroxidation, DNA damage, and protein peroxidation. Black tea (Camellia sinensis) infusion has a strong polyphenol group, namely epigallocatechin gallate, thearubigin, theaflavin, the amino acid L-theanine, flavonols, and catechins that can inhibit free radicals and protect organs from oxidative stress. This study was a post-only control group design experiment. The research subjects used were 24 Sprague Dawley male rats which were divided into six groups. The first group was the control group (K0). Treatment group 1 was given distilled water and used cooking oil after 12 times heating (K1), treatment group 2 was given black tea at a dose of 2.5g/kg bw (body weight) and distilled water (K2), and treatment group 3 was given black tea at a dose of 3.75 g/kg bw and distilled water (K3). Treatment group 4 was given used cooking oil after 12 times heating and black tea with a dose of 0.50 gr/200 gr bw (K4), treatment group 5 was given used cooking oil after 12 times heating and black tea with a dose of 3.75 g/kg bw (K5). All groups were treated for 42 days. Then, the rats' kidney was collected, and data were analyzed by Kruskal Wallis and One Way ANOVA test. The results of the Kruskal Wallis and One Way ANOVA test showed a decrease in glomerular and tubular damage after the rats orally induced with black tea were given 12x heating used cooking oil (p<0.05). The mean histopathological score in the administration of black tea at a dose of 3.75 g/kg bw was lower than at a dose of 2.5 g/kg bw. This study shows that infusion of black tea (Camellia sinensis) can prevent glomerular and tubular damage induced by used cooking oil.

Keywords: Used cooking oil; Camellia sinensis; Renal histopathology.

INTRODUCTION

Indonesian people still use the deep frying method in frying food by soaking all food ingredients in hot oil. This cooking oil will be reused to fry other food ingredients or without adding a little new cooking oil to the used cooking oil.¹ Cooking oil repeatedly used (more than two times) adding new cooking oil is usually called used cooking oil². This used cooking oil is still often used by households, fried food traders because it can save costs. In addition, there is still a lack of knowledge about the dangers of using used cooking oil.³-⁵

Repeated heating of the oil causes degradation, oxidation, and dehydration.² The oxidation process will produce hydroperoxide compounds which are free radicals.⁶,⁷ Research conducted by Shastry et al. regarding the effect of giving palm oil with 6-8 times heating on Wistar strain rats for eight weeks showed an inflammatory
reaction in the lumen of the rat kidney tubules caused by free radicals. Free radicals can induce cell injury through inflammation, lipid peroxidation, DNA damage, and protein peroxidation.\textsuperscript{6,7} Oxidative stress caused by excess free radicals that react with cellular lipids, proteins, and nucleic acids can cause local damage, kidney dysfunction, and necrosis of kidney cells. Chemical compounds can reduce the negative effects of free radicals in the form of antioxidants which are contained in several types of plants, one of which is tea.

The black tea (\textit{Camellia sinensis}) plant has been cultivated for thousands of years, and its leaves have been used for medicinal purposes. Three types of tea known to have this benefit are unfermented green tea, partially fermented oolong tea, and fully fermented black tea.\textsuperscript{11} The type of tea widely consumed, especially in Indonesia, is black tea.\textsuperscript{12} The bioactive compound of black tea are thearubigins, theaflavins, a strong group of polyphenols, namely epigallocatechin gallate, the amino acid L-theanine, flavonols, and catechins.\textsuperscript{10,11,13} The ingredients in this tea can inhibit free radicals and protect organs from oxidative stress.\textsuperscript{10,13,14}

Black tea is also useful as an anticancer and antimicrobial, prevents diabetes, dental caries, and obesity, and can increase bone mass density.\textsuperscript{15} Research conducted by Jean regarding the effect of giving black tea on the histopathological property of the kidneys of rats with various doses of black tea found that the amount of damage to the kidney tubules of rats decreased according to the increase in the dose of black tea. Based on this description, research on the effect of giving black tea on kidney histopathology induced by used cooking oil has not been done much. Therefore, this study aimed to determine the effect of black tea infusion on the histopathological changes in the kidney of rats that had been induced by used cooking oil.

**MATERIAL AND METHODS**

This study used a Completely Random Design (CRD) experimental research design with a post-test-only control group design pattern. The research samples were 24 white male rats (\textit{Rattus norvegicus}) of the Sprague Dawley strain aged 8-10 weeks with a body weight of 200-300 grams, obtained from STIH ITB (School of Technology and Life Sciences, Bandung Institute of Technology). The number of rats used in this study was determined based on the minimum and maximum sample sizes for three ANOVA Design formulas.\textsuperscript{16} Ethical approval number for this study was 086/UN.30.14.9/LT/2020.

The black tea infusion used in this study was obtained from Kabawetan, Kepahiyang, Bengkulu Province. Black tea infusion was collected with heating 10 grams black tea leaves and 100 mL aquadest at 90\textdegree C temperature in 15 minutes. The boiled black tea is then filtered to separate the infusion from the black tea powder. Filtrate in the process, which is then used in this study. Black tea infusion was treated to the rats orally. As much as 1 mL of black tea infusion (2.5 g/kg bw and 3.75 g/kg bw concentration) was given to the rats.

The experimental animals were divided into six groups, each consisting of 4 rats. The control group (K0) was the group without treatment of heating used cooking or black tea infusion. Treatment group 1 (K1), which was given 12x heating, used cooking oil and distilled water. Treatment group 2 (K2) was given black tea with a dose of 2.5 g/kg bw and distilled water. Treatment group 3 (K3) was given black tea at a dose of 3.75 g/kg bw. Therefore, K1 and K2 were the groups that were treated with black tea infusion without used cooking oil.

Group K4 and K5 was the groups that treated with used cooking oil and black tea infusion. Treatment group 4 (K4) which was given used cooking oil and black tea at a dose of 2.5 g/kg bw, and treatment group 5 (K5) which was given used cooking oil and black tea at a dose of 3.75 g/kg bw every day for 42 days. On the 43\textsuperscript{rd} day, the laparotomy property and kidney organs of these rats were collected to be analyzed.

Observations on kidney histopathology preparations were carried out using a light

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{Histopathological Changes in Kidney Tissues}
\end{figure}
microscope with 400x magnification in five different fields of view. The level of damage to the glomerulus and kidney tubules was seen in each section. The degree of kidney damage was categorized according to Mitchell's scoring.

The data were the score of changes in kidney histopathology, which was analyzed using the One Way ANOVA statistical test if the distribution was normal. If the p-value of the One Way ANOVA test obtained was less than 0.05, it would be continued with the Post Hoc test to see the difference in the histopathological property of the rats' kidneys among the treatment groups. If the distribution was not normal, then the Kruskal Wallis test would be used and followed by the Mann-Whitney test.

RESULTS
During the six weeks of treatment, there were several rats experiencing weight loss in the first week of treatment, but the weight of the rats increased again in the second week after the rats were able to adapt to the given treatment. There were also two rats that died, namely two rats in treatment group 2 (K2). The death of rats in treatment group 2 (K2) was caused by an injury during treatment, so that only 24 rats were harvested kidney organs. This numbers were still accepted for minimum sample size for this study.

The statistical tests on glomerular histopathological scores showed that the mean histopathological property of K1 given used cooking oil with 12x heating and distilled water had a significant difference with K0 (p : 0.013). This revealed that the administration of used cooking oil with 12x heating could damage the glomerular appearance of the white rat kidney seen from the mean histopathological score. The mean histopathological picture of K2 and K3 had a significant difference with K0 (p : 0.013 and p : 0.046), meaning that giving black tea to K2 at a dose of 2.5 g/kg bw and K3 at a dose of 3.75 g/kg bw could increase the mean score of rats' glomerular histopathological score.

Treatment group 4 (K4) compared to K2 did not have a significant difference (p : 0.137). Meanwhile, K4 compared to K1 had a significant difference (p : 0.020). This showed that black tea infusion at a dose of 2.5 g/kg bw could reduce the level of glomerular damage in white rats that had been induced by used cooking oil with 12x heating. The comparison of the mean histopathological scores between K5 and K3 did not have a significant difference (p: 0.741). However, when K5 was compared with K1, the mean of the histopathological
score had a significant difference (p: 0.017). This means that giving black tea infusion at a dose of 3.75 g/kg bw in rats that had been induced by used cooking oil with 12x heating could reduce the level of glomerular damage.

The results of the histopathological picture of each treatment can be seen in Figure 2.

![Figure 2](image1.png)

***Figure 2.*** Histopathological description of the white rats’ (*Rattus norvegicus*) glomerulus at 400x magnification. Control group, score 0 (K0) with normal histology; treatment group 1, score 2 (K1) with a picture of glomerular enlargement, capsular space narrowing, and erythrocyte grains reaching 50%; treatment group 2, score 0 (K2) with normal histology; treatment group 3, score 0 (K3) with normal histology; treatment group 4, score 1 (K4) with a description of glomerular enlargement, capsular space narrowing, and erythrocyte grains reaching 25%; treatment group 5, score 1 (K5) with a description of glomerular enlargement, capsular space narrowing, and erythrocyte grains reaching 25%; glomerulus; B. capsular space narrowing; C. erythrocyte grains.

**Analysis of Experimental Animals' Mean Tubular Histopathology Score**

![Figure 3](image2.png)

***Figure 3.*** Analysis of One Way ANOVA Test and Post Hoc Test on the Comparison of Histopathological Scores of *Rattus norvegicus*’ Tubular Damage After Treatment; "a" = significant difference to K0 with p value < 0.001; "b" = significant difference to K1 with p value < 0.05; K0 = normal control; K1 = treatment group 1 (used cooking oil with 12x heating + distilled water); K2 = treatment group 2 (black tea dose of 2.5 g/kg bw + distilled water); K3 = treatment group 3 (black tea dose of 3.75 g/kg bw + distilled water); K4 = treatment group 4 (used cooking oil with 12x heating + black tea dose of 0.50 gr/200 gr bw); K5 = treatment group 5 (used cooking oil with 12x heating + black tea at a dose of 0.75 g/200 gr bw).

In Figure 3, the statistical test results show that the mean of histopathological score of *Rattus norvegicus* tubular damage in K0 compared to K1 had a significant difference (p : 0.000). This means that the administration of used cooking oil with 12x
heating could damage the kidney tubules of white rats seen from the mean of histopathological score. The mean of histopathological scores of K2 and K3 did not have a significant difference when compared with K0 (p : 0.698 and p : 0.958), meaning that the administration of black tea with a dose of 2.5 g/kg BW in K2 and 3.75 g/kg BW in K3 did not significantly improve the renal tubular histopathological score of rats.

There was no significant difference between K4 and K2 (p : 0.998). While K4 compared to K1 had a significant difference (p : 0.001), so that the administration of black tea with a dose of 2.5 g/kg bw could reduce the tubular histopathological score of rats that had been induced by used cooking oil with 12x heating.

In K5 compared to K3, there was no significant difference (p : 1.000). However, the mean of histopathological score in K5 compared to K1 had a significant difference (p : 0.001). This shows that black tea infusion with a dose of 0.75 gr/200 g bw could reduce the level of tubular damage in rats after being induced by used cooking oil with 12x heating.

The results of observations of histopathological preparations K0 and K1 can be seen in Figure 4.

Figure 4. Histopathological description of the kidney proximal tubule of white rats (Rattus norvegicus) with 400x magnification; control group, score 0 (K0) with the appearance of not swelling cells, round cell nuclei, and clear tubular lumen; treatment group 1, score 2 (K1) with degeneration of cloudy swelling, hydropic degeneration, and unclear tubular lumen reaching 50%; A, proximal tubule.

Figure 5. Histopathological description of the kidney proximal tubule of white rats (Rattus norvegicus) with 400x magnification; treatment group 2, score 0 (K2) with the appearance of not swelling cells, round cell nuclei, and clear tubular cell lumen; treatment group 3, score 0 (K3) with a picture of not swollen cells, round cell nuclei, and clear tubular cell lumen; A, proximal tubule.

Figure 6. Histopathological description of the kidney proximal tubule of white rats (Rattus norvegicus) with 400x magnification; treatment group 4, score 0 (K4) with a picture of not swollen cells, round cell nuclei, and clear tubular cell lumen; treatment group 5, score 1 with degeneration of cloudy swelling, hydropic degeneration, and unclear tubular lumen reaching 25%; A, proximal tubule.

DISCUSSION

The initial sample in this study was 26 rats, but during the study, two rats died in treatment group 2 (K2) due to an error in the treatment procedure. In this study, it was also found that the entire treatment group of rats
experienced an increase in body weight, especially in the treatment groups that were given used cooking oil. This is in accordance with the study of Shastry et al., 2011 which showed that there was a significant increase in body weight in rats given used cooking oil for 8 weeks. This can be caused by the repeated use of cooking oil, affecting the deposition of fat cells in various organs of the body.9

Analysis of the Mean Glomerulus Histopathological Score of Experimental Animal

The statistical test results showed that the mean histopathological score of K1 given used cooking oil with 12 times heating and distilled water had a significant difference with K0 (p : 0.013) (Figure 1), which means that the administration of used cooking oil with 12 times heating can cause damage to the glomerulus of white rats. This is because repeated heating of the oil causes the process of degradation, oxidation, and dehydration2. The oxidation process will produce hydroperoxide compounds which are free radicals.6,7 Hydroperoxide compounds in the body will react with unsaturated fatty acids on the membrane through lipid peroxidation reactions. Lipid peroxidation in cell membranes can change the membrane's fluidity, structure, and function, so that the cell membrane becomes damaged and causes the cell to lose the ability to protect its internal structure.20 The dehydration process due to repeated heating of the oil will also produce an unsaturated aldehyde compound, namely acrolein, a toxic substance that can damage DNA so that cells can experience mutations, transcription errors, and apoptosis.21

In K2 and K3, each given black tea at a dose of 2.5 g/kg bw and 3.75 g/kg bw compared to K0 revealed no significant difference (p : 0.013 and p : 0.046). This means that giving black tea to K2 and K3 can increase the mean glomerular histopathological score of rats. According to Chacko et al., black tea and green tea have side effects when consumed in excess. The side effects of black tea and green tea can be caused by the caffeine, aluminum, and polyphenols contained in both teas. In this study, the increase in the mean glomerular histopathology score can be due to the high caffeine content in black tea. Caffeine increases renal glomerular filtration and inhibits sodium reabsorption, leading to increased sodium and water excretion. In addition, caffeine is also an antagonist of the adenosine A2a receptor which causes increased activation of polymorphonuclear cells (PMN), so that it can develop the risk of inflammation which will decline kidney function, causing proteinuria, and result in changes in renal histology.

The mean histopathological scores of K4 and K5 which were compared with K1 had a significant difference (p : 0.020 and p : 0.017), meaning that the administration of black tea infusion at a dose of 2.5 g/kg bw and 3.75 g/kg bw could reduce the mean histopathological score in white rats that had been induced by used cooking oil with 12x heating. This is because black tea contains strong polyphenols such as epigallocatechin gallate, the amino acid L-theanine, flavonols, catechins, theaflavins, and thearubigin which can inhibit free radicals and protect organs from oxidative stress.10,13,14 In addition, the theaflavin and thearubigin content contained in black tea can also reduce the inflammatory process and reduce the number of damaged cells in the kidneys.16 The antioxidant content in black tea will automatically donate one of its electrons to free radicals, thereby reducing the damaging power of free radicals and be able to inhibit cell damage.

The K4 and K5 groups which were given used cooking oil and black tea at a dose of 2.5 g/kg bw and 3.75 g/kg bw had a non-significant difference in the level of damage (p : 0.180). However, when viewed from the mean glomerular histopathological score, K5 rats had a lower histopathological score (0.55 ± 0.100) when compared to K4 (0.85 ± 0.341). This shows that giving black tea infusion at a dose of 3.75 g/kg bw has the best effectiveness in preventing glomerular
damage in white rats that have been induced by used cooking oil with 12x heating.

**Analysis of Mean Tubular Histopathology Score of Experimental Animals**

One Way ANOVA test analysis carried out on the average tubular damage data for all groups found that there were significant differences in tubular damage among groups (p : 0.000). The histopathological picture of tubular damage of K0 and K1 had a significant difference (p : 0.000) (Figure 3), meaning that the administration of used cooking oil with 12x heating can damage the kidney tubules of white rats seen from the mean histopathological score. In a study conducted by Shastry et al., on the effect of giving oil with 6x-8x heating on the kidney appearance of wistar strain rats, it was found that the administration of used cooking oil caused damage to the kidney tubules. Free radicals generated by repeated heating of the oil cause oxidative stress and are toxic to the proximal renal tubular cells so that they can cause damage.

The mean histopathological scores of K2 and K3 increased when compared to K0, but the results of statistical analysis tests showed that there was no significant difference between groups (p : 0.698 and p : 0.958). This can be caused by the fact that black tea has a high caffeine content. Caffeine itself is an adenosine A1 receptor antagonist that can cause increased angiotensin secretion. An increase in angiotensin will trigger arteriolar vasoconstriction, post glomerular peritubular capillary hypoperfusion, and tubulointerstitial hypoxia which will reduce the distribution of oxygen and nutrients to the tubules, so that it can cause tubular damage. In addition, caffeine is also an antagonist of the adenosine A2a receptor which causes increased activation of polymorphonuclear cells (PMN), so that it can increase the risk of inflammation which will decrease kidney function, causing proteinuria, and changes in renal histology.

The level of damage in K4 and K5 compared to K1 had a significant difference (p : 0.001), meaning that the administration of black tea infusion at a dose of 2.5 g/kg bw and 3.75 g/kg bw can reduce the mean histopathological score of the proximal renal tubule of rats, induced by used cooking oil with 12x heating. Theaflavin and epigallocatechin gallate those which are widely contained in black tea acts as intermediary in the protection mechanism of renal tubular epithelial cells, so that the tubules can be protected from free radicals and oxidative stress. The antioxidant content in black tea will automatically donate one of its electrons to free radicals, thereby reducing the damaging power of free radicals and can inhibit cell damage.

In the analysis of the mean histopathological score of tubular damage, it showed that K5, in which the rats were given black tea at a dose of 0.75 g/200 g bw, had a lower histopathological mean score of 0.20 ± 0.163. On the other hand, K4 which was given a dose of 0.50 gram black tea/200 g bw had a mean histopathological score of 0.25 ± 0.191. It can be concluded that the administration of black tea (Camellia sinensis) at a dose of 3.75 g/kg bw has the best effectiveness in preventing damage to the kidney tubules of white rats induced by used cooking oil with 12x heating.

**CONCLUSION**

The conclusions obtained from this study is giving black tea (Camellia sinensis) infusion for 6 weeks causes changes in the histopathological picture of the kidneys of white rats seen by the increasing of the mean histopathological score. Beside that, induction of used cooking oil with 12x heating for 6 weeks causes changes in the histopathological picture of the kidneys of rats in the form of damage to the glomerulus and tubules of rats.

Giving infusion of black tea (Camellia sinensis) in graded doses of 2.5 g/kg bw and 3.75 g/kg bw are able to prevent damage to the kidneys of white rats seen from the histopathological description of the rats' kidney glomerulus. Giving infusion of black tea (Camellia sinensis) in graded doses of 2.5
g/kg bw and 3.75 g/kg bw is able to prevent damage to the kidneys of white rats seen from the histopathological description of the rats' kidney tubules.

REFERENCES