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Effects of Red Coconut *Tembuluk* (*Cocos nucifera* var. *rubescens*) Extract on Creatinine Level Reduction in Mice (*Mus musculus*)

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Abstract

Creatinine is a key biochemical indicator of renal function, and elevated creatinine levels reflect impaired kidney performance. Carbon tetrachloride (CCl_4) is a known nephrotoxic agent that induces oxidative stress and renal damage. This study evaluate the effect of red coconut *tembuluk* (*Cocos nucifera* var. *rubescens*) extract on creatinine in CCl_4-induced mice (*Mus musculus*). The study employed an experimental laboratory design using 24 male mice, which were divided into six groups: normal control, negative control (CCl_4-induced), and four treatment groups administered red coconut tembuluk extract at concentrations of 10%, 20%, 40%, and 80%. Renal damage was induced by intraperitoneal injection of CCl_4 at a dose of 1 mL/kg body weight. The extract was administered orally for eight consecutive days following induction. Serum creatinine levels were measured before and after treatment. The results showed that CCl_4 induction significantly increased creatinine levels in the negative control group compared to the normal control, indicating impaired renal function. Administration of red coconut tembuluk extract resulted in a significant reduction in creatinine levels across all treatment groups, with the most pronounced effect observed at the 80% concentration. Statistical analysis using one-way ANOVA demonstrated a significant effect of the extract on creatinine reduction ($p < 0.05$), followed by a Least Significant Difference (LSD) test confirming differences among treatment groups. The nephroprotective effect of the extract is likely associated with its bioactive compounds, including flavonoids, antioxidants, and L-arginine, which contribute to the reduction of oxidative stress and support renal tissue recovery. In conclusion, red coconut tembuluk extract effectively reduces serum creatinine levels in CCl_4-induced mice, with the 80% concentration showing the greatest protective effect. These findings suggest that red coconut *tembuluk* has potential as a natural nephroprotective agent.

Keywords: Creatinin; *Cocos nucifera* var. *rubescens*; *Mus musculus*; *Tembuluk*.

INTRODUCTION

The red coconut plant (*Cocos nucifera* var. *rubescens*) is widely known as a “plant of a thousand uses,” as almost every part of the coconut can be utilized as a source of livelihood for communities in Indonesia (Lawalata & Imimpia, 2020). Coconut plants are also widely used in traditional medicine because they contain various bioactive compounds with therapeutic properties (Mat et al., 2022). The type of coconut most commonly used for medicinal purposes is characterized by a pink-colored mesocarp (fiber), commonly known as *kelapa wulung* (Prabowo et al., 2021).

Red coconut water has a slightly bitter taste, unlike the sweet taste of coconuts in general. This characteristic underlies its designation as a medicinal coconut, as *kelapa wulung* contains numerous essential nutrients and bioactive compounds beneficial to health. These compounds include flavonoids, sucrose, glucose, tannins, minerals, amino acids such as alanine, cystine, arginine, and alliin, as well as pantothenate, biotin, riboflavin, fructose, and others (Ramadhani et al., 2023). In addition, red coconut contains L-arginine, an amino acid significant role in reducing free radical levels and supporting immune function. Its antioxidant properties also help combat free radicals and repair cellular damage (Makaruku et al., 2024).

The tembuluk part of red coconut is known for its health benefits due to its antioxidant and flavonoid content. Antioxidants are compounds that protect cells against damage caused by free radicals. They stabilize free radicals by donating electrons, thereby preventing oxidative stress (Tarigan, 2020). Flavonoids function as antioxidants by donating hydrogen atoms from their hydroxyl groups. These hydroxyl groups bind to free radicals, thereby rendering them neutral and less harmful (Wilujeng & Anggarani, 2021).

Creatinine is a metabolic waste product generated from muscle metabolism and serves as an important indicator for assessing kidney function. Creatinine is filtered in the glomerulus and partially reabsorbed in the renal tubules. The initial process of creatinine biosynthesis occurs in the kidneys and involves the amino acids arginine and glycine. Creatinine is produced from normal muscle contraction, released into the bloodstream, and subsequently excreted through the kidneys (Febrianti et al., 2023). Variations in blood creatinine levels may be caused by several factors, including dehydration, excessive fatigue, overuse of medications, exposure to nephrotoxic chemicals, uncontrolled hypertension, and other kidney-related diseases.

Normal plasma creatinine levels range from 0.7 to 1.5 mg/dL. When kidney function is normal, blood creatinine levels remain within this range. Lower creatinine levels are generally observed in women, while higher levels are more common in men due to greater muscle mass. The kidneys are vital organs responsible for excreting metabolic waste products as urine (Putri et al., 2023). Kidney disease is a significant global public health issue, and one of its indicators is elevated levels of waste products such as urea in the blood, which may progress to kidney failure (Wiliyanarti & Muhith, 2019). Impaired kidney function can be identified by increased creatinine levels in the blood (Ambarwati et al., 2020). This study aimed to determine the effect of red coconut tembuluk extract (*Cocos nucifera* var. *rubescens*) on reducing creatinine levels in mice (*Mus musculus*).

MATERIALS AND METHODS

This study was conducted at the Biology Education Laboratory, Universitas Tadulako, from September to October 2025. The research employed an experimental research design, in which samples were randomly assigned into two main groups: a control group and a treatment group. A total of 24 male mice (*Mus musculus*), aged 2–6 weeks and weighing 20–40 g, were used in this study. The mice were divided into several groups, including a normal control group (KN) and a negative control group (N). The treatment groups consisted of treatment group 1 (PI) receiving red coconut tembuluk extract at a concentration of 10%, treatment group 2 (PII) receiving 20%, treatment group 3 (PIII) receiving 40%, and treatment group 4 (PIV) receiving 80% extract. Each group consisted of four mice.

The research procedure began with acclimatization, followed by sample preparation and extraction of red coconut tembuluk. Acclimatization was conducted to allow the mice to adapt to their new environment and minimize stress. Prior to experimentation, the mice were acclimatized for seven days in cages, during which they were maintained under standard conditions and provided with food and water ad libitum.

Procedures

Preparation of Induction Material

The Toxic induction was performed using carbon tetrachloride (CCl_4), which was administered to mice by intraperitoneal injection at a dose of 1 mL/kg body weight. This dose was selected because it is considered optimal for inducing kidney damage. Lower doses of CCl_4 solution, typically ranging from 0.08 mL/kg to 0.2 mL/kg, are commonly used for long-term or chronic exposure, whereas higher doses generally range from 1 mL/kg to 2.6 mL/kg.

Red Coconut Tembuluk Extract

Red coconut (*Cocos nucifera* var. *rubescens*) *tembuluk* (young coconut endosperm) was collected from a community garden in Taat Village, Gadung District, Buol Regency. The collected *tembuluk* was washed thoroughly with water to remove impurities. It was then thinly sliced and air-dried at room temperature in a shaded area, protected from direct sunlight, for one week until it turned brownish and completely dried. The dried material was weighed (1 kg) and ground into powder using a blender.

The resulting powder was subjected to maceration with 96% ethanol (2.5 L) for 3 × 24 hours to extract bioactive compounds. The maceration process aimed to extract all bioactive chemical constituents present in the red coconut *tembuluk*. The macerate was then filtered using a Büchner funnel lined with filter paper. The filtrate was concentrated using a rotary evaporator equipped with a water bath and vacuum pump at a temperature of 50°C and a rotation speed of 96 rpm.

Administration of Test Materials

Prior to treatment, the mice were acclimatized at the Biology Education Laboratory, Universitas Tadulako, for seven days. On days 8–10, the normal control group (KN) was provided with food and water *ad libitum*, while the negative control group (K−) was induced with CCl_4 for three consecutive days. On day 10, creatinine levels were measured in the treatment groups (PI, PII, PIII, and PIV) to confirm an increase in creatinine levels following induction.

From days 11–19, the normal control (KN) and negative control (K−) groups continued to receive food and water *ad libitum*, while the treatment groups were administered red coconut tembuluk extract at different concentrations. Treatment group 1 (PI) received 10% extract, treatment group 2 (PII) received 20% extract, treatment group 3 (PIII) received 40% extract, and treatment group 4 (PIV) received 80% extract for eight days. On day 20, blood sampling was performed in mice that had received red coconut tembuluk extract. On day 21, final blood samples were collected from all groups (KN, K−, PI, PII, PIII, and PIV) to determine changes in creatinine levels. Prior to blood collection, the mice were fasted for eight hours to ensure accurate measurement.

RESULTS AND DISCUSSION

The mean creatinine levels in each group of mice including the normal control group (KN), negative control group (K−), treatment group 1 (PI) receiving red coconut tembuluk extract at a concentration of 10%, treatment group 2 (PII) at 20%, treatment group 3 (PIII) at 40%, and treatment group 4 (PIV) at 80% (Table 1 and Figure 1).

Table 1. Mean creatinine levels (mg/dL).

Treatment	Replication			Average (mg/dl)	Standard Deviation (SD)
	1	2	3		
K Normal	0,204	0,321	0,500	0,342	0,149
K-	1,500	2,500	1,000	1,667	0,764
PI	1,205	1,200	1,225	1,210	0,013
PII	1,141	1,000	1,284	1,142	0,142
PIII	0,830	0,607	0,770	0,736	0,115
PIV	0,633	0,603	0,,656	0,631	0,027

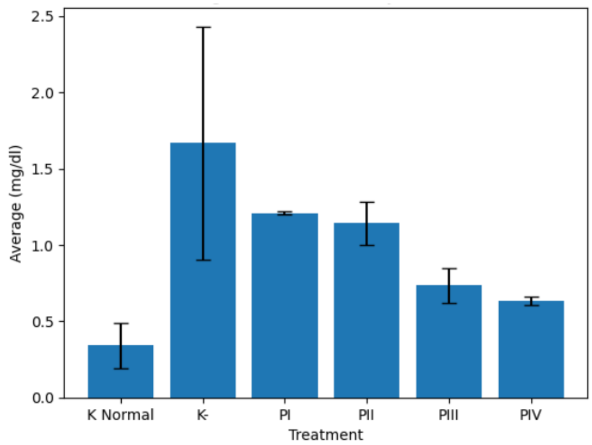

Figure 1. Average glucose levels by treatment.

Based on Table 1 and Figure 1, the mean creatinine level of normal control mice (KN), which were only provided food and water ad libitum, remained within the normal range at 0.342 mg/dL. In contrast, the creatinine level in the negative control group (K−), consisting of mice induced with CCl_4, showed a marked increase to 1.667 mg/dL. The mean creatinine levels in the treatment groups decreased following administration of red coconut tembuluk extract. Treatment group 1 (PI), which received a 10% extract concentration, showed a mean creatinine level of 1.210 mg/dL, while treatment group 2 (PII) receiving 20% extract had a level of 1.142 mg/dL. Further reductions were observed in treatment group 3 (PIII) administered 40% extract, with a mean creatinine level of 0.736 mg/dL, and in treatment group 4 (PIV) administered 80% extract, which exhibited a mean creatinine level of 0.631 mg/dL.

The obtained data were subsequently analyzed using analysis of variance (ANOVA) to determine the effect of red coconut *tembuluk* (*Cocos nucifera* var. *rubescens*) extract on the reduction of creatinine levels in mice (*Mus musculus*). The results of the ANOVA are presented in Table 2.

Table 2. Analysis of Variance (ANOVA) of Creatinine Levels in Mice Administered Red Coconut Tembuluk Extract.

Source of Variation	db	Sum of Squares (SS)	Mean Square (MS)	F_{hitung}	F_{tabel} 0,05
Treatment	5	3,407	0,681	17,03	3,11
Galat	12	0,477	0,040		
Total	17	3,884			

Note: $F_{hit} > F_{tab}$

Based on the results of the analysis of variance (ANOVA) of creatinine levels in mice (*Mus musculus*), the null hypothesis (H_0) was rejected and the alternative hypothesis (H_1) was accepted. These results indicate that administration of red coconut tembuluk extract (*Cocos nucifera* var. *rubescens*) had a significant effect on reducing creatinine levels. Therefore, the analysis was further continued using the Least Significant Difference (LSD) test at a significance level of 0.05. The results of the LSD test are presented in Table 3.

Table 3. Least Significant Difference (LSD) Test Results on Creatinine Levels.

Treatment	Treatment Average	Average					LSD 0.05
K Normal	0,342						
K-	1,667	1,325*					
PI	1,210	0,868*	0,457*				0,356
PII	1,142	0,800*	0,525*	0,068^			
PIII	0,736	0,394*	0,931*	0,474*	0,406*		
PIV	0,631	0,289^	1,036*	O,579*	0,511*	0,105^	

Note: An asterisk (*) indicates a significant difference, whereas the caret (^) indicates a non-significant difference.

Based on the results of the Least Significant Difference (LSD) analysis, there were significant differences in creatinine levels among mice induced with CCl_4 after administration of red coconut tembuluk extract. These findings indicate that the reduction in creatinine levels in mice occurred as a result of treatment with red coconut tembuluk extract. Therefore, the second hypothesis (H_1) was accepted, indicating that certain concentrations of red coconut tembuluk extract are effective in reducing creatinine levels in mice.

Discussion

The elevation of creatinine levels observed in this study is consistent with findings from previous experimental studies involving CCl_4-induced renal damage. Ramadhan et al. (2022) reported increased creatinine levels (1.133 mg/dL) in male rats following oral administration of CCl_4 at a dose of 0.2 mL for seven days. Similarly, Ammar et al. (2022) demonstrated a more pronounced increase (2.005 mg/dL) in Wistar rats treated with higher doses of CCl_4 (2.8 mL/kg). These variations may be attributed to differences in dosage, duration of exposure, animal species, and routes of administration. Collectively, these findings reinforce the notion that CCl_4 is a potent nephrotoxic agent and support its use as an experimental model for studying kidney dysfunction and nephroprotective interventions (Lau et al., 2025).

Creatinine is a metabolic byproduct of creatine phosphate degradation in muscle tissue and is released into the bloodstream at a relatively constant rate, depending on muscle mass. Under normal physiological conditions, creatinine is efficiently filtered by the glomeruli and excreted in urine, making it a reliable biomarker for assessing renal function. Elevated serum creatinine levels indicate a reduction in the glomerular filtration rate (GFR), reflecting impaired kidney function (Purnawinadi, 2021). Therefore, the significant increase observed in the CCl_4-induced group strongly suggests compromised renal filtration and structural damage to renal tissues, particularly the glomerular basement membrane and tubular epithelium (Septiani et al., 2025).

Renal dysfunction characterized by elevated creatinine levels may result from various pathological conditions, including chronic kidney disease, acute kidney injury, and severe dehydration. Normal creatinine values vary according to age, sex, and muscle mass, with males generally exhibiting higher levels due to greater muscle mass (Leriyanti et al., 2021). In clinical practice, creatinine measurements are frequently combined with other parameters, such as blood urea nitrogen (BUN) and estimated glomerular filtration rate (eGFR), to provide a more comprehensive evaluation of kidney health. Persistent elevation beyond normal reference ranges indicates progressive renal impairment and highlights the importance of early intervention to prevent irreversible kidney damage (Khairiyah & Sulisma, 2025).

The observed reduction in creatinine levels across treatment groups (PI–PIV) suggests that red coconut tembuluk extract exerts a protective effect against CCl_4-induced renal damage. This nephroprotective activity is likely associated with the rich composition of bioactive compounds present in the extract, including flavonoids, tannins, sugars, minerals, amino acids, and fructose (Ramadhan et al., 2023). These compounds are known to enhance antioxidant defense mechanisms, reduce lipid peroxidation, and improve cellular resilience against oxidative stress. The dose-dependent decrease further indicates that higher extract concentrations may confer greater renal protection.

In addition to flavonoids, red coconut tembuluk extract contains L-arginine, an amino acid that plays a crucial role in nitric oxide (NO) synthesis. Nitric oxide is essential for maintaining renal blood flow, regulating vascular tone, and modulating inflammatory responses in renal tissues. L-arginine supplementation has been shown to mitigate oxidative stress by enhancing endogenous antioxidant enzyme activity and reducing free radical accumulation (Pedrazini et al., 2024). Furthermore, the antioxidant properties of the extract contribute to cellular repair processes, facilitating the recovery of damaged renal cells and improving overall kidney function (Bratovcic, 2020).

Previous studies have demonstrated that coconut-derived extracts, including kentos and tembuluk, possess significant antioxidant and antimicrobial properties due to their flavonoid and ascorbic acid content (Kurniawati, 2024). Antioxidants play a critical role in neutralizing free radicals by donating electrons, thereby preventing oxidative damage to lipids, proteins, and DNA. Flavonoids, in particular, act as effective radical scavengers by donating hydrogen atoms from their hydroxyl groups, resulting in the stabilization of reactive oxygen species (ROS). This mechanism reduces oxidative stress, preserves cellular integrity, and prevents further deterioration of renal tissues (Wibawa et al., 2020; Oktavia & Sutoyo, 2021).

CONCLUSIONS

Based on the results of the study, it can be concluded that the administration of red coconut *tembuluk* extract significantly reduces creatinine levels in CCl_4-induced mice, with the 80% extract concentration being the most effective, as indicated by an average creatinine level of 0.631 mg/dL.

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