



***Stenochlaena palustris* aqueous extract reduces hepatic peroxidative stress in *Marmota caligata* with induced fever**

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ABSTRACT

The *kelakai* fern (*Stenochlaena palustris*) contains bioactive substances, such as flavonoids, steroids, and alkaloids. The *kelakai* is used by the Kenyah Dayak communities for the treatment of anemia, fever, and cutaneous disorders. Fever is increased temperature regulation of the body, which process indirectly increases the production of peroxides, leading to peroxidative stress. The aim of this research study was to evaluate the influence of an aqueous extract of the *kelakai* fern on peroxidative stress in the hoary marmot (*Marmota caligata*) with experimentally-induced fever. This was a true experimental study, with post test-only and control group design, involving 6 groups, each made up of 4 animals. Peroxide concentration was measured by a modification of the FOX2 method and peroxidase activity by the Kanehira method. The *kelakai* extract was administered to the animals by gavage at weight-adjusted dosages between 7.75×10^{-4} mg/kg body weight to 1.24×10^{-2} mg/kg body weight. Data were analyzed using single linear regression test. The results showed that *kelakai* extract dose significantly reduced the peroxide levels, regression equation with the $Y = 2.513 - 106.03 X$ ($R^2 = 0.8057$), indicating that 80.5% variation of peroxide levels is predicted by the *kelakai* extract dose. From the results we conclude that *kelakai* aqueous extract decreases peroxide levels in hoary marmots with experimentally-induced fever.

Keywords: Fever, peroxidative stress, *Stenochlaena palustris*, hoary marmots

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INTRODUCTION

The potential of Indonesian wetlands, including those of South Kalimantan, is still underexploited. The province of South Kalimantan has relatively extensive areas of marshland (both freshwater and peat swamps) of 287,000 hectares. It is estimated that peat

swamps account for 8-11% of the total area of this province, occupying extensive lowland areas of Kalimantan. The peat swamps have a distinctive type of forest formation with relatively limited flora, in which the *kelakai* fern [*Stenochlaena palustris* (Burm.f) Bedd] constitutes one of its species.⁽¹⁾

In South Kalimantan, the *kelakai* has few

uses. However, on the basis of empirical studies, the *kelakai* is used by the Kenyah Dayak communities for the treatment of anemia, fever, and cutaneous disorders. In spite of this, few scientific studies have confirmed this empirical evidence. Previous studies state that the plant contains bioactive substances, such as flavonoids, steroids, and alkaloids.⁽²⁾ The study by Suhartono demonstrated that the *kelakai* fern may act as an analgesic as well as antiphlogistic; the mechanism of synthesis involves oxidative reactions by peroxide molecules and the enzyme peroxidase acting holistically.⁽³⁾ When the peroxide concentration exceeds the activity of peroxidases by which it is catalyzed, peroxidative stress occurs, which is marked by a low peroxide to peroxidase ratio.

The results of the study conducted by Suhartono et al. revealed that hoary marmots with experimentally-induced fever have raised plasma peroxide levels, presumably as an immunological response of the body.⁽⁴⁾ Since peroxides possess oxidant properties and are thus capable of forming hydroxyl radicals through Fenton as well as Haber-Weiss reactions, administration of *kelakai* extract to hoary marmots with experimentally-induced fever may be expected to reduce peroxidative stress. Thus the objective of the present study was to explore the influence of *kelakai* aqueous extract on hepatic peroxidative stress in hoary marmot.

METHODS

Design of the study

The present study was a true experimental study with post-test-only and control group design to examine the impact of *kelakai* extract on peroxidative stress in hoary marmots

Subjects

The study involved 6 groups of 4 male hoary marmots (*Marmota caligata*), where one group was the control group, while the other

five constituted intervention groups. The test substance was an aqueous extract of *kelakai* ferns.

Preparation of *kelakai* aqueous extract

Kelakai ferns were collected in June 2009 from Gambut subdistrict, South Kalimantan. Species identification was performed by the Department of Biology, Pharmacy Study Program, Faculty of Mathematics and Natural Sciences (*FMIPA*), Lambung Mangkurat University. The active principles in the *kelakai* plants were extracted by maceration.

Animal experiments

The experimental animals used in this study were 3-4 months' old hoary marmots weighing approximately 300-400 g, obtained from the Veterinary Research and Investigation Station (*Balai Penelitian dan Penyidikan Veteriner, BPPV*) at Banjarbaru. The animals were kept for one week prior to the experimental treatment to bring them to approximately equal physical and psychological conditions. In the adaptation period, the animals received animal feed and water *ad libitum*, the water being tap water supplied by the provincial water purification plant. Complete random sampling was used to assign the animals into 6 groups, each consisting of 4 animals. Each group was kept in one small cage for adaptation. One group, the control group, was designated P₀, while the remaining five intervention groups were designated P₁-P₅.

The animals were subsequently weighed and their rectal temperature taken, after which all animals received 0.16 ml DPT vaccine by intramuscular injection in the right thigh, the dose having been determined in preliminary tests. The rectal temperature of the animals was again taken at the onset of fever (the time of onset of fever was determined in preliminary tests to be 90 minutes post-injection). Afterwards *kelakai* extract was administered to the animals by gavage (stomach tube) at weight-adjusted dosages as follows:

Experimental group	Kelakai aqueous extract (mg/kg body weight)	Distilled water
P ₀	-	1 ml
P ₁	7.75 x 10 ⁻⁴	-
P ₂	1.55 x 10 ⁻³	-
P ₃	3.10 x 10 ⁻³	-
P ₄	6.20 x 10 ⁻³	-
P ₅	1.24 x 10 ⁻²	-

At the peak of fever (determined in preliminary tests to be 2 hours after onset), the animals were sacrificed by decapitation and their livers surgically removed. Subsequently the livers were washed in phosphate buffer at pH 7, then minced into a liquid, of which 5 ml was centrifuged at 3500 rpm for 10 minutes, and 200 µL of the supernatant was taken for determination of peroxide concentration by the modified FOX2 method, as described below.

The standard and test solutions consisted of 1 M H₂O₂ 200 µL and 200 µL plasma, respectively, with the addition of 160 µL PBS pH 7.4, 160 µL FeCl₃ (251.5 mg FeCl₃ dissolved in 250 ml distilled water) and 160 µL o-phenantroline (120 mg o-phenantroline dissolved in 100 ml distilled water) for both solutions. The composition of the blank solution was identical to that of the test solution, except for absence of FeCl₃ in the blank.

Subsequent to preparation, all solutions were incubated for 30 minutes at room temperature, then centrifuged at 12,000 rpm for 10 minutes, and the absorbance of the standard (A_s), test (A_u) and blank (A_b) solutions measured at λ=505 nm, using the supernatant of each solution.

Peroxide concentration was calculated from the following formula,

$$\text{Peroxide concentration} = \frac{A_s - A_b}{A_u - A_b} \times 1 \text{ mM H}_2\text{O}_2$$

where A_s = absorbance of standard solution;

A_b = absorbance of blank solution;
and A_u = absorbance of test solution.

Peroxidase activity was determined by the Kanehira method.⁽⁵⁾ One ml of the supernatant layer of the test solution was mixed with 45 µL FeCl₃, 45 µL phosphate buffer (pH 7) and 45 µL o-phenantroline, then the absorbance was measured at λ=505 nm (A₀). Subsequently the mixture was incubated for one minute at room temperature and its absorbance (A₁) measured at the same wavelength.

$$\text{Peroxidase activity} = \frac{A_0 - A_1}{5} \text{ min}^{-1}$$

Peroxidative stress is the ratio of peroxide concentration and peroxidase activity.

Statistical analysis

The relationship between *kelakai* aqueous extract dose and levels of peroxidative stress was analyzed by single linear regression. Data processing was done using Microsoft Excel. P values < 0.05 were considered statistically significant.

RESULTS

As shown in Figure 1, marmot hepatic peroxidative stress due to fever was inversely proportional to test substance concentration, decreasing with increasing concentration of the test substance.

From Figure 1, it may be concluded that administration of *kelakai* extract is capable of inhibiting peroxidative stress. The correlation of the administered dose of *kelakai* extract with peroxidative stress is given in the equation: Ln Y = 2.513-106.03 X with R² = 0.8057, indicating that 80.5% variation of peroxidative stress level is predicted by the *kelakai* extract dose. This indicates that there is a strong correlation between fever-induced hepatic

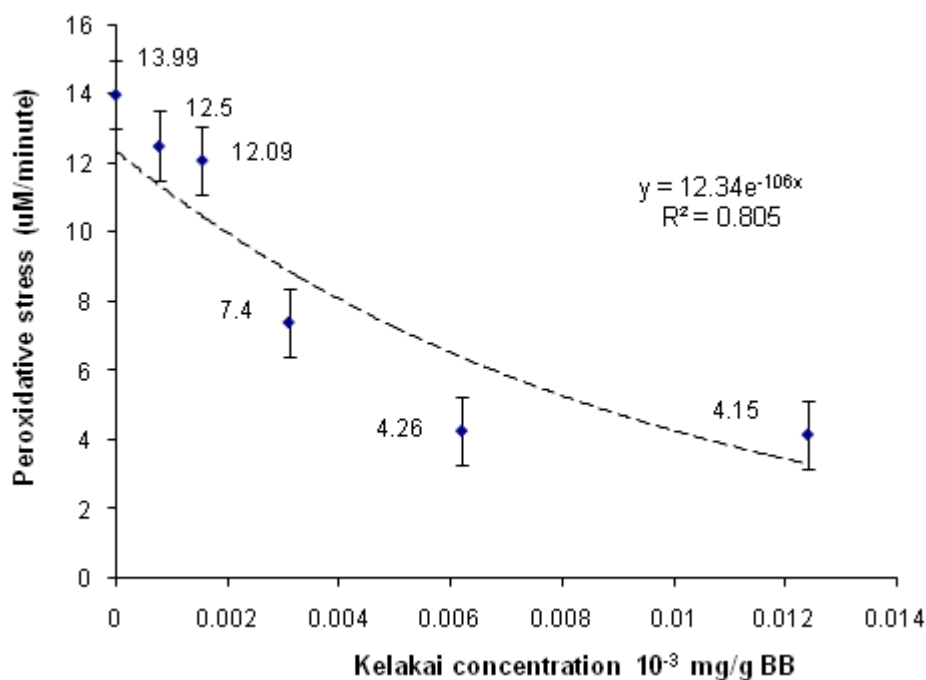


Figure 1. Correlation of hepatic peroxidative stress in marmots with experimentally-induced fever

peroxidative stress and the administered dose of *kelakai* extract.

DISCUSSION

The febrile process indirectly leads to an increase in free radicals such as superoxide anions ($\bullet\text{O}_2$) which afterwards are converted to peroxides by the enzyme superoxide dismutase (SOD). The peroxides are then catalyzed by the enzyme peroxidase into water and oxygen.

Administration of distilled water (P_0) to DPT-injected control animals resulted in very high peroxide concentrations in the liver, since administration of DPT vaccine induces a respiratory burst by neutrophils and macrophages through concurrent formation of H_2O_2 . At this stage there are a considerable number of phagocytosed bacteria, necessitating high H_2O_2 levels. In addition, arachidonic acids are ultimately transformed to prostaglandins, which are mediators of pain as well as inflammation,^(6,7) the prostaglandin synthesis

involving oxygenation catalyzed by cyclooxygenase to form peroxides.⁽⁸⁾

The peroxides formed from these sources will in appropriate amounts be beneficial to the body that is being subjected to infection, acting as a line of defense, since peroxides possess microbicidal properties that aid in eradication of pathogenic bacteria. On the other hand, peroxides may also have a negative impact on the host, which may occur if the production of peroxides exceeds the counteracting antioxidant activity. This condition is called peroxidative stress, which may cause biomolecular damage comprising among others cellular damage, enzyme inactivation, and mutation. Additionally, peroxidative stress may lead to oxidation of DNA and proteins followed by membrane damage due to lipid peroxidation, causing membrane permeability changes and alterations in protein structure and functions. Oxidative damage also occurs in mitochondrial membranes, thus causing membrane depolarization, disruption of the oxidative


phosphorylation chain, and altered cellular respiration. This finally causes damage to the mitochondria, resulting in synthesis of cytochrome C and activation of apoptosis (programmed cell death).^(9,10)

The results of the present study indicate that *kelakai* aqueous extract is capable of significantly decreasing hepatic peroxidative stress. This is presumably due to bioactive substances in *kelakai* ferns, namely flavonoids, steroids, and alkaloids, which are useful as antipyretics, antioxidants, and antiphlogistics.⁽¹¹⁻¹³⁾

The capture mechanism of free radicals by flavonoids is initiated by release of hydrogen, forming reactive flavonoid radicals. The latter will bind to free radicals ($\bullet\text{O}_2$), causing the reactivity of the latter to diminish or even disappear,⁽¹⁴⁻¹⁶⁾ leading to decreased formation of peroxides.

Other bioactive substances contained in *kelakai* are alkaloids and steroids. In addition to being antipyretic, the alkaloids and steroids possess anti-inflammatory properties. The study by Sudjarwo revealed that the alkaloid piperine contained in *kelakai* can function as antipyretic through inhibition of prostaglandin synthesis,⁽¹⁷⁻¹⁹⁾ while the steroids inhibit phospholipase activity and arachidonic acid transformation to prostaglandins, reduce microvascular leakage, prevent direct migration of pyretic cells, and inhibit cytokine production.^(17,20)

CONCLUSION

Administration of *kelakai* (*Stenochlaena palustris*) aqueous extract reduces the hepatic peroxidative stress in febrile hoary marmots (*Marmota caligata*). 

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