The Effect of Telang Flower Extract on the Hepatic Catalyst Enzyme of Mice Exposed to Tobacco Smoke

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Abstract. A tobacco contains more than 4700 chemical compounds and 200 of them can be detrimental to health. These compounds include CO, CO2, HCN, NOx, nicotine, phenol, tar and cadmium which have the potential to become free radicals. Continuous exposure to chemical compounds in tobacco can result in a decrease in the body's antioxidant levels and cause oxidative stress. Telang flower extract has antioxidant properties which can reduce the number of free radicals formed from the lipid peroxidation process. The catalase enzyme is an antioxidant enzyme used to determine antioxidant levels in mice exposed to tobacco smoke. This research aims to analyze the effect of telang flowers extract on mice hepatic catalase enzyme that exposed to tobacco smoke. This was experimental research which used post-test only controlled group design. Sample of this research was 20 mice divided into 4 groups. Normale one group (N) was given standard feed, the control negative group (K0) given tobacco smoke, K1 group given tobacco smoke and telang flowers extract 2 00 mg/kgBW, and K2 group given tobacco smoke and telang flowers extract 4 00 mg/kgBW Treatment was carried out for 10 days and continued with measuring hepatic catalase enzyme levels. Statistical tests use the one-way Anova test. All samples given tobacco smoke responded to increased oxidation and when given telang extract was able to have a significant effect. The results of the Anava test showed significant differences between all groups. Telang flowers extract decreased hepatic catalase enzyme level of mice that were exposed cigarette smoke. So, preclinically, telang flower extract can act as an antioxidant due to exposure to tobacco smoke and reduce the risk of respiratory tract infections.

Keywords: Tobacco Smoke; Telang Flowers Extract; Catalase Enzyme.

1 Introduction

Exposure to cigarette smoke is one of the risk factors for the onset of various diseases, both acute and chronic exposure. Acute exposure such as respiratory tract infections and chronic exposure such as malignancy, coronary heart disease, stroke and chronic obstructive pulmonary disease (COPD) [23].

Some studies show that exposure to acute secondhand smoke has an effect on both animals and humans. Exposure to cigarette smoke increases the secretion of inflammatory mediators from a variety of different cell types including epithelial cells, macro-

Z. B. Pambuko et al. (eds.), *Proceedings of 5th Borobudur International Symposium on Humanities and Social Science (BISHSS 2023)*, Advances in Social Science, Education and Humanities Research 856,
https://doi.org/10.2991/978-2-38476-273-6_45

phages and neutrophils [22],[25]. Research by Lymperaki et al (2015) shows that there is an increase in the number of leukocytes in smokers, where a significant increase occurs in acute smokers. The study also showed that there was a significant increase in neutrophils in acute cigarette exposure while in chronic cigarette exposure was not significant [19]. Exposure to acute cigarette smoke in mice as much as 2 cigarettes 2 times a day for 3 days was shown to cause acute inflammation characterized by an increase in neutrophils [9].

Oxidative stress due to exposure to cigarette smoke has the potential to be prevented by antioxidants. Based on the source, antioxidants are divided into endogenous antioxidants, namely enzymes that are antioxidants, such as Superoxide Dismutase (SOD), Catalase (Cat), Glutathione Peroxidase (Gpx), and exogenous antioxidants, which are obtained from outside the body, such as vitamins E, C, pro vitamin A, organosulfur, flavonoids, thymoquinone and others. Exposure to cigarette smoke causes a decrease in endogenous antioxidants, so additional antioxidants from outside the body are needed to prevent oxidative stress [30].

Tobacco smoke contains 1014 free radicals in the tar phase and 1015 free radicals in the gas phase and 4700 complex chemical compounds [1], some of which are nicotine, tar and carbon monoxide. If these compounds enter the body, they can trigger the formation and increase of Reactive Oxygen Species (ROS) which act as free radicals. Cigarettes are a source of free radicals which can increase free radicals and reduce TAC in the body [2]. TAC is the total amount of antioxidant compounds in serum and blood plasma that can inhibit the formation of free radicals, exposure to cigarette smoke can reduce TAC levels [5],[12],[13],[14],[19],[30],[32].

To overcome oxidative stress, the body synthesizes an endogenous antioxidant such as catalase. Catalase is an antioxidant enzyme found in almost all living organisms that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide (H_2O_2) is produced during cellular respiration in all living cells. (H_2O_2) is dangerous and must be disposed of as soon as possible. The cells containing a small amount of catalase are very susceptible to being oxidized by (H_2O_2). Therefore, catalase plays an important role in the cell's defense mechanism against the oxidation attack of (H_2O_2) [14].

Antioxidants are compounding that function to ward off radicals that enter the body, and the catalase enzyme (CAT) is one of them catalase enzyme functions to ward off free radicals by converting hydrogen peroxide (H2O2) into H2O and O2. This catalase enzyme is produced by cell organelles, namely peroxisomes, and is mostly found in the liver. [5] [6], [8], [30], [32].

Antioxidants are also found in many herbal plants, whose use as medicine to ward off free radicals has been widely researched and applied. Indonesia itself, as a tropical country, is rich in herbal plants which have properties as antioxidants. One of them is telang flower which contains flavonoids and anthocyanins which function as antioxidants [11], [16], [17], [20] [21],[27], [28], [30].

Apart from being an ornamental plant, since a long time ago this plant was known traditionally as a medicine for the eyes, and food coloring that gives it its blue color. Judging from the phytochemical review, the flower has a number of active ingredients that have pharmacological potential. The pharmacological potential of other flowers is

as an antioxidant, antibacterial, anti-inflammatory and analgesic, antiparasitic and anticidal, antidiabetic, anti-cancer, antihistamine, immunomodulator, and potential role in the arrangement of central nerve, Central Nervous System (CNS). Other parts of this plant, namely leaves and roots also have their own potential [24].

Clitoria ternatea has been observed for its anti-oxidant activity through the DPPH method. *Clitoria ternatea* containing all phenols and flavonoids showed significant inhibition compared to standard gallic acid and quercetin. This shows that the leaves and flowers of the eagle have antioxidant activity against free radicals such as DPPH, hydroxyl radicals, and hydrogen peroxide. This result is a potential source of antioxidants from biological ingredients [18].

2 Methods

2.1 Extraction Flavonoid from *Clitoria ternatea*

A total of 100 grams (5 x replicates) of *Clitoria ternatea* was macerated with 1 liter mixture of ethanol solvent. Maceration was carried out in a place protected from light and at room temperature. The resulting macerate had its solvent evaporated using a rotary evaporator at 50 ºC to yield a thick extract. The thick extract of containing the target compound flavonoid was tested for compound confirmation using the TLC method with a mobile phase of chloroform : methanol: aquadest (64:50:10).

2.2 Preparations

This research is true experimental with a post test only controlled group design. The research was carried out at the Animal Testing Laboratory of the Pharmacy Science Foundation College of Pharmacy Semarang in the period June-October 2023. The samples used were male mice with the inclusion criteria of body weight 200-220 grams, 12 weeks of age, healthy mice, looking active, and not (Fig. 1). there is an anatomical abnormality. The exclusion criteria in this research were that the mice did not want to eat and were weak, and the mice died [8].

Fig. 1. Smoke mice (private document).

Acclimatization of Experimental Animals. Experimental animals were made into cages, given a drinker and one feed con-tainer per cage. Mice were fasted for 20 hours. Mice were placed in collective cages at a temperature of 20 - 25oC. Mice were divided randomly into 4 groups with 5 animals each. The mice were weighed and marked by coloring the mice's tails. At the top of the tub, there is a wire ram to prevent the mice from getting out of the cage. Feed is given in the morning at 07.00 WIB and in the afternoon at 16.00 WIB. Sufficient drinking water is provided. The cage is cleaned every 3 days by changing the husks. The purpose of acclimatization is so that animals can adapt to their environment. Mice were given standard food and water adlibitum.

Treatment of experimental animals. Samples were obtained using the random allocation sampling method, and divided into 4 groups, namely normal control (N), negative control given cigarette smoke (K0), treatment one given cigarette smoke and telang flower extract 200 mg/kgBW (K1) and the second treatment given was given cigarette smoke and 400 mg/kgBW (K2) telang flower extract. The number of samples used was 20 mice with 5 mice for each group. The independent variable in this research was the treatment: cigarette smoke and telang flower extract with respective doses of 200 mg/kgBW for mice and 400 mg/kgBW for mice. Meanwhile, the dependent variable is antioxidant activity. Data collection was carried out after being given treatment for 10 days.

Observing of Hearts. First, the mice were given anesthesia by injecting ketamine intramuscularly, waiting until the mice became limp. After that, surgery is performed and the hepar organ is weighed.

$$\text{index organ} = \frac{\text{Whepar x 100}}{\text{Wmice}}$$

Where Whepar as Weighed hepar and Wmice as Weighed mice

Measurement catalase enzyme level. Second, the mice were given anesthesia by injecting ketamine intramuscularly, waiting until the mice became limp. After that, surgery is performed and the hepar organ is removed, then hepar juice is made from the hepar organ. After making hepar juice, immediately measure catalase enzyme levels by adding 100 µL of 10% H2O2, into a test tube containing 100 µL of hepar juice. Then wait for 5 minutes, and measure the height of the bubbles that appear on the surface of the test tube with a ruler twice and record the results.

2.3 Analysis Data

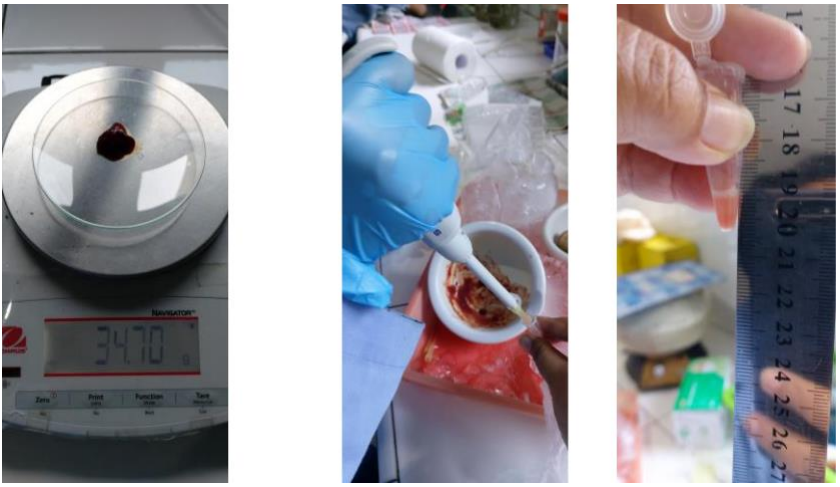
In both groups, the data normality test was carried out used the Shapiro-Wilk test. Hepatic catalase enzyme levels showed a normal distribution used the Shapiro-Wilk test. After transformation, hepatic catalase enzyme levels still showed a normal distribution,

so parametric tests were then carried out, namely one-way ANOVA. After the test is carried out, the *Post hoc* test continues

3 Results

Approximately 100 grams (5 x replicates) of *Clitoria ternatea* produced extract yields averaging 48.85%, with Rf values 0.86 for each spot.

Based on the test results obtained (Fig. 2), it is concluded that *Clitoria ternatea* contain flavonoid. The presence of a single stain in some of these TLC tests indicates that a compound with a high level of purity has been obtained.



(a) (b) (c)

Fig. 2. (a) Weighed hepar, (b) juiced hepar and (c) measured catalase enzyme level.

Table 1. Index organ hepar

Group Mice	Average Weighed Mice (gram)	Average Weighed Hepar (gram)	Index Organ
Normal (N)	39.8678	2.6400	6.62
Control negative (K0)	36.6703	2.3650	6.45
Dosage 200mg (K1)	41.6395	2.0525	4.93
Dosage 400mg (K2)	39.7611	2.0800	5.23

This research was conducted on male mice that met the inclusion criteria and did not meet the exclusion criteria (Table 1). The selection method is random allocation sampling. In this research, 2 mice dropped out, so the number of samples used was 18 samples. The research was carried out for 10 days and ended by terminating the mice used ketamine by intramuscular injection, waiting for their consciousness to decrease and surgery was carried out, make hepar extracts and ending with measuring levels of the hepatic enzyme catalase.

Table 2. Descriptive presentation of data on hepatic catalase enzyme levels

Group	N	K0	K1	K2
Average hepatic catalase enzyme levels	0.1	2.30	1.75	1.00

Table 2 shows the average levels of the mice hepatic enzyme catalase. There was the highest level of hepatic catalase enzyme in the K0 group. After carrying out the distribution test, it was continued with the Shapiro-Wilk test to determine the normality of the data, and the data obtained were normally distributed. So a one-way ANOVA parametric test was carried out and a p value of 0.000 was obtained. This shows that there are significant differences between at least the two groups. Then proceed with the *Post hoc* test. Based on the results of *Post hoc* analysis in Table 3, significant differences were found between all groups, the hepatic catalase enzyme was lowest in the N group.

Table 3. *Post hoc* analysis

Group	N	K0	K1	K2
N	-	0.000	0.000	0.000
K0	0.000	-	0.000	0.011
K1	0.000	0.000	-	0.001
K2	0.000	0.011	0.001	-

4 Discussion

The results of the research carried out showed that almost all human body organs that are exposed to cigarette smoke experience lung disorders which will experience detrimental deterioration due to Chronic Obstructive Pulmonary Disease (COPD), obstruction of the heart's function, and can reduce the capacity of regenerative organs as a result of the inability of the reproductive organs in the form of impotence.

The toxic effect of cigarettes that occurs indirectly is that when exposure to cigarette smoke occurs in large quantities, it will cause tissue hypoxia because there is an increase in carboxyhemoglobin which reduces the capacity of hemoglobin to carry oxygen. The occurrence of hypoxia will stimulate the production of erythropoietin which causes increased absorption of iron in the intestine and if it is not compensated it can cause a buildup of iron in liver tissue which leads to liver fibrosis or liver cell damage [4], [29], [31], [33].

The liver/hepar is an important organ in the body which functions as a neutralizer of toxins. The entry of toxic compounds into the liver can cause changes in liver cells, for example hemorrhage, congestion, degeneration to necrosis.

In the research shown that given butterfly pea (telang) flower extract as much as 200mg/kgBW and 400mg/kgBW has not helped much to restore liver function exposed to high doses of cigarette smoke, therefore it is necessary to increase the dose of butterfly pea flower extract to more than 400 mg/kgBW, however, this administration is statistically significant. shows that there are significant differences.

The lowest mean catalase levels were in the normal group (N), while the highest mean catalase levels were in the negative control group (K0). After knowing the catalase levels of each group, the data was analyzed and the results showed that there were significant differences. Then an inter-group test was carried out and the results showed that there were significant differences between the groups. Based on the results obtained, the average hepatic catalase levels of treatment groups 1 and 2 (K1 and K2) were higher than the average hepatic catalase levels of the normal group (N). This difference has been analyzed statistically, obtaining a p value <0.05. This can prove that administering telang flower extract can increase levels of the hepatic enzyme catalase. This increased level of the hepatic enzyme catalase shows the body's antioxidant activity in protecting against oxidative stress conditions significantly [1], [2], [5] [6], [12], [13], [15], [19], [32].

Antioxidants are compounds that can inhibit or prevent the oxidation process by inhibiting the initiation or propagation process of the oxidation chain reaction. Previous studies have shown that there are various antioxidant compounds contained in telang flowers such as flavonoids, anthocyanins, and tannins, where anthocyanins are the most abundant bioactive compounds found in telang flowers [3], [11], [16], [17], [26], [28].

The results of the research showed that there was a significant difference in the levels of the catalase enzyme between the treatment groups given cigarette smoke and the normal group and those given telang flower extract, both at a dose of 200 mg/kgBW for mice and 400 mg/kgBW for mice. Based on the results obtained, it can be said that telang flower extract has an effect on increasing catalase enzyme levels.

Telang flower extract itself contains anthocyanins which have antioxidant effects. The effect of telang flower extract can increase the activity of antioxidant enzymes, which in this study can increase catalase enzyme levels. This research shows that administering telang flower extract can increase hepar antioxidant levels in the form of the enzymes catalase (CAT) so this research shows that telang flower extract is a strong antioxidant and can also be a supplement against hepar carcinoma [11], [16], [17], [21], [27], [32].

The catalase enzyme is one of the components of the body's antioxidant defense system in the form of an enzyme that functions to prevent the formation of hydroxy radicals and protect cells from oxidative stress. The catalase enzyme works by breaking down hydrogen peroxide (H2O2) and protecting the body from reactive hydroxyl radicals. A decrease in the activity of the catalase enzyme can result in detrimental effects due to the accumulation of free radicals. This research showing that in a group of mice given high-cholesterol feed there was an increase in H2O2 levels compared to the control group given standard feed. On the other hand, catalase enzyme activity will increase if given anti-oxidants, which are antioxidants. functions to prevent oxidative stress. Telang flower extract contains antioxidant compounds, namely anthocyanins, which can increase antioxidant activity [16], [26], [32], [33].

The use of cigarette smoke in this study can increase the formation of free radicals in the body, cause oxidative stress and can cause damage at the cellular level. An increase in free radical levels in the body results in a decrease in the body's antioxidants. In this research, the mean levels of the N group catalase enzyme were lower than the

K0 group catalase enzyme levels with a significant difference. This resulted in an increase the levels of the liver enzyme catalase in rats given cigarette smoke. This occurs because cigarette smoke causes excessive formation of ROS due to lipid peroxidation [1], [2], [13], [19], [32].

5 Conclusion

Telang flower extract causes an decrease in hepatic catalase enzyme levels when exposed to cigarette smoke. This is proven by the treatment given telang flower extract which has a high average level of catalase enzyme. Further testing of liver function (SGOT/SGPT) and MDA was carried out.

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