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Transaminase Enzyme and Liver Histopatological Structure of Mice Facing to Smoke Cigarettes After Administerred with *Enhalus acoroides* Peel Extract

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Abstract. The objective of this research were aimed to determine the level of transaminase enzymes and the histopathological structure of mice liver exposed to cigarette smoke after treatment of *E. acoroides* peel extract. Completely randomized design was conducted with 3 doses treatment groups (P1, P2, P3) and a control group (K+, K-) with 3 times repeated. The test was carried out during 14 days for cigarette smoke, and 14 days for seagrass peel extract tretment. The data measured were the levels of SGOT, SGPT and were analyzed using the Anova test. Liver histopathology (necrosis, parenchymatous, hydropic degeneration and normal) were described descriptively. The results showed that the administration of seagrass *E. acoroides* peel extract had a significant effect on decreasing levels of SGOT, SGPT (p value <0.05) when compared with K+ $\underline{K^+}$. The P_3 treatment had lower levels of SGOT (80.07±3.1 IU/L) and SGPT (88.3±2.1 IU/L). Liver histopathology consist of 34 necrosis cells less than the positive control group (K^+) and normal cells were 47, higher than the positive control group (K+). It can be concluded that *E. acoroides* peel extract had a significant effect on SGOT, SGPT levels in mice exposed to cigarette smoke. Peel extract of *E. acoroides* 75 mg/kgBW (P3) was an effective dose to reduce the levels of SGOT, SGPT and improve the histopathological structure of mice liver exposed to cigarette smoke. This research is expected to be useful for the community, especially in the coastal areas regarding the benefits of using *E. acoroides* as a therapeutic agent against free radicals originating from cigarette smoke.

Keywords: cigarette smoke, transaminase enzyme, histopathological structure, *E. acoroides* peel.

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INTRODUCTION

The liver is the largest organ in the body. The liver is also an immunologically complex organ because it contains several inflammatory cells such as lymphocytes and Kupffer cells (Machado-Junior et al. 2020). The liver works in detoxifying substances that enter the body such as some drugs, alcohol, and toxins. Despite its work in detoxification, the liver can be damaged if exposed to toxic compounds continuously (Lang and Beier, 2018).

One of the compounds that can cause liver toxicity is cigarette smoke (Park et al., 2016; Zong et al., 2019). The mixture of cigarette smoke (CS) is very complex. There are more than 5000 hazardous chemical compounds that can induce damage and dysfunction of biological tissues (Wang et al., 2015). The content of carbon monoxide (CO) in CS will bind to hemoglobin (Hb), leading to hypoxia in tissue. The occurrence of tissue hypoxia will increase erythropoiesis, and increase the absorption of iron (Fe), causing deposition in the liver (Rose et al., 2017).

Iron accelerates the conversion of hydrogen peroxide (H2O2) into hydroxyl free radicals (HO-) which will oxidize fats, proteins, and Deoxyribose Nucleic Acid (DNA) through the chemical reactions of Fenton and Haber-Weiss, resulting in damage to cell membranes and even necrosis of the liver parenchyma (Pietrangelo, 2016). Damage to hepatocyte membranes will cause a decrease in the function of hepatocytes which is characterized by increased levels of liver marker enzymes, namely alanine transaminase (ALT) and aspartate transaminase (AST) (Alvina, 2016).

AST and ALT were previously called serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Saputro et al., 2022). The increasing level of SGOT or SGPT levels is the first way to diagnose liver disease. However, elevated levels of both enzymes do not always indicate liver damage.

Therefore, histopathological examination is also very important to ensure liver tissue damage due to exposure to free radicals, especially those from CS (Conteras-Centella & Hernandez-Munoz, 2016).

Currently, the use of plants as anti-inflammatory, anti-cancer, and various types of diseases has been widely reported. One type of aquatic plant that can also be used as a therapeutic agent is *Enhalus acoroides* belonging to the Hydrocharitaceae family. *E. acoroides* is one type of flowering plant that lives in sea water (Supattra et al., 2018). The potential of *E. acoroides* as a therapeutic agent for various diseases has been reported by many researchers. Widiastuti et al., (2021) reported that the methanolic extract of the leaves of *E. acoroides* has cytotoxic activity with an IC50 value of 122 ppm, and the antiproliferative effect on HeLa cells is higher so that it has potential as an anticancer. According to Dewi et al., (2012) leaves of *E. acoroides* contain phytochemical compounds such as flavonoids, alkaloids, and steroids groups. Parthasarathy et al., (2017) also reported about the content of alkaloids, phenolics, flavonoids, steroids, and tannins as well as the antioxidant activity of *E. acoroides* leaves. Although several bioactive compounds and antioxidant activity of *E. acoroides* have been found, the reported part is the leaves. One part of *E. acoroides* that can be used is the peel of the fruit. Seagrass fruit was consumed as food by coastal communities in some areas of Maluku, and the peel of the fruit is only disposed of as a by-product. With the presence of bioactive components in the plant parts of *E. acoroides*, it is not impossible that the peel of the fruit also contains bioactive compounds that can be used for therapy, especially for liver function disorders. The objective of this study was to determine the effect of *E. acoroides* peel extract on the transaminase enzyme and the histopathological structure of mice exposed to CS.

This research may prove to be useful for the community, especially in coastal areas in deciding the utilization of seagrass peel extract as a therapeutic agent to treat impaired liver function due to exposure of free radicals, especially those that come from cigarette smoke. For instance, this result was pertinent for further development as a new medicinal sources originating from marine ecosystem.

METHOD

This study used 15 male mices (*M. musculus*) with a body weight of ± 20-30g, 2-3 months of age. Peels of *E. acoroides* were obtained from the coastal waters of Suli Village, with variations in doses of 25, 50 and 75 mg/kg of body weight (bw). The study used a completely randomized design with 5 groups including: K- (negative control: normal group without cigarette smoke); K+ (positive control: mice exposed to cigarette smoke given Hepa-Q 0.3 g), and the treatment group consisting of P1: extract dose 25 mg/kg bw, P2; extract dose 50 mg/kg bw, and P3: extract dose 75 mg/kg bw (P3).

Research Procedure

Aclimatitation of Mice

Prior to carrying out the experiment, the mice were put into cages that had been coated with husks. Each cage contains 3 mices. The cage I (group I) was a negative control, cage II (group II) was a positive control, and cages III, IV, and V (groups III, IV, V) were treatment groups. All mice were adapted for 7 days and standard feed was given until the weight reached the range of 20-30 g.

Collecting of *E. acoroides*

The fruits of *E. acoroides* taken were healthy, whole, and green ripe seagrasses. Then peel and take the peel of the fruit. The peel of the fruit was cleaned using running water and dried in the shade for ± 24 hours. The peel of the seagrass was cut into small pieces and re-dried at room temperature for 3 days. After drying, the peel of the seagrass is crushed to become a powder.

Extraction of *E. acoroides*

Extraction of *E. acoroides* was conducted according to the method from Santoso et al., (2012) with slight modification. As much as 100g peel powder of *E. acoroides* was put into Erlenmeyer 1000 ml, and 500 ml of methanol was added, then left for 24 hours.

After 24 hours, the solution was filtered with Whatman-filtered paper. The residue was filtered 3 times. The extract was then evaporated by using a rotary vacuum evaporator for 3 hours until concentrated extract was obtained. The dosage was made for each treatment and dissolved using 50 ml of distilled water.

Calculation of the dosage

- **Extract dosage**

Dosage conversion from human to mice as much as 0.0026 g (Anggara, 2009). The average weight of mice was ± 25 g. extract dose of *E.*

acoroides peel was 25 mg, 50 mg dan 75 mg. The calculation of dosage conversion from human with 70 kgBW to mice was as follows :

Dossage	Convertion with 0.0026	Convertion to mice body weight (mg/kgBW of mice)	Dose for 1 mice	Final dose of Extract for mice treatment (mg)
I. 25 mg	0.0026 x 25 mg	0.065	0.065 x 1000/25	2.6
II. 50 mg	0.0026 x 50 mg	0.13	0.13 x 1000/25	5.2
III. 75 mg	0.0026 x 75 mg	0.195	0.195 x 1000/25	7.8

- **Dosage of liver medicine (Hepa-Q)**

Dosage for human was 10 mg. Conversion of human dosage to mice 20 mg was 0.0026. dosage for mice 20g was 10 mg x 0.0026 = 0.026 g. Dosage for mice ± 25 g was : 25/20 x 0.026 = 0.03 g

Cigarette Smoke (CS) treatment

Before treatment, mice fasted for 3-4 hours. After that, the body weight was weighed. Then the mice were given CS according to the group. Exposure to CS was carried out once a day for 14 days, each as many as half a cigarette using a smoking chamber, namely a 10cc syringe as a CS pump. The exposure time of half a CS was about ± 15 minutes.

Treatment of *E. acorides* peel extract

After exposure to CS, the extract treatment was conducted for 14 days in 3 treatment groups, namely P1, P2, and P3 with a dose of 25, 50, and 75 mg/kgBW respectively, and a dose of 0.3 g of the liver medicine namely Hepa-Q in the K+ group. Methods of administering *E. acoroides* peel extract and medicine were given orally. The mice were clamped at the back of the neck, the tail was pulled open until the mouth was open, then the extract dose was taken with a 1.0 ml syringe and inserted into the mouth to the esophagus.

Histopathological examination

One day after the treatment of CS and the extract, the mice were dissected and their livers were taken. The liver was then washed with a physiological saline solution. After washing, the liver was put into bottles containing 10% formaldehyde solution for preservation and fixation processes and histological preparations using the paraffin method (Widiastuti et al., 2019).

Liver fixation was carried out by soaking in 10% formaldehyde phosphate buffered for 24 hours, then sliced in agar and put in a box for processing in a tissue processor. Then, the tissue was introduced into the 70%, 80%, 90%, and 96% alcohol series, then continued with toluene twice for 2 hours. Then the tissue was put into liquid paraffin at a temperature of 56°C for 2 hours 2 times. The tissue was then taken with tweezers, followed by blocking using paraffin blocks. Cutting was carried out by a microtome with a thickness of 4-5 µm. The cut tissue was developed over the water in a water bath, then captured with an object glass. Then it was dried at room temperature and the slide were ready to be stained with Hematoxylin Eosin (HE).

The slides were immersed in xylol I for 5 minutes, followed by xylol II, III for 5 minutes. Then the slide were immersed in 100% alcohol I and II for 5 minutes, then in distilled water and then immersed in Harris Hematoxylin for 15 minutes. The object were then dipped in 1% alcohol for 7-10 dips, soaked in distilled water for 15 minutes, and in eosin for 2 minutes. Then, the slide were immersed in 96% alcohol I and II for 3 minutes, 100% alcohol I and II for 3 minutes, and in xylol IV and V for 5 minutes. The objects were dried and covered with a cover glass. The slide were placed under a microscope for histopathological observation which was conducted in 5 microscopic fields of view at 100 and 400 times of magnification.

Determination of SGOT and SGPT Level

Blood was taken intracardially using a 1cc syringe inserted into the cool box. Blood samples were centrifuged at 3000 rpm for 15 minutes to separate serum from blood. The separate serum is taken and put in a clean and dry tube. SGOT and SGPT calculations were performed by pipetting 100 l or 1 mL of serum samples into a cuvette using a sterile micropipette. SGOT and SGPT kit reagents were added to the cuvette and incubated for 5 minutes at room temperature. After incubation, the absorbance of the serum and

reagent mixture was measured using a spectrophotometer at a wavelength of 340 nm (Suparmi et al., 2011; Setyawati & Anggraeni, 2018).

Data Analysis

Data from the measurement of SGOT-SGPT levels were analyzed by analysis of variance. If there was a significant effect, then continued with Duncan's multiple range test (DMRT). The data about the number of cells undergoing necrosis, parenchymatous degeneration, hydropic degeneration, and normal cells were presented in form of a table and described based on the results obtained.

RESULT AND DISCUSSION

SGOT and SGPT are intracellular enzymes that will be released into the blood when there is interference or damage of organs such as the liver, heart, lungs and muscles (Ramadhani et al., 2017). SGOT and SGPT are also always used as early indicators in the diagnosis of liver function disorders (Conteras-Centella & Hernandez-Munoz, 2016). Normal levels of SGOT and SGPT vary between laboratories and the methods were used, but generally range from 4-36 IU/L (ALT/GOT), and 5-30 IU/L (AST/GPT) (Lala et al., 2022).

The results of this study showed that the transaminase enzyme (SGOT-SGPT) levels of mice in the normal group were still within the standard range, while in the group of mice exposed to CS, the values were highest more than the standard range, but tended to decrease with the increasing of dose extract. The results of the analysis of variance showed that there was a significant effect and significant difference between each treatment of *E. acoroides* peel extract on SGOT and SGPT levels of mice ($p < 0.005$) (Table 1).

Table 1. Level of SGOT-SGPT of mice

Groups	SGOT (µ/L)	p-value (sig < 0,005)	SGPT (µ/L)	p-value (sig < 0.005)
Negative control (K-)	30.03 ± 7.5[a]	0.000	30.7 ±6.1[a]	0.000
Positive control (K+)	113.07 ±3.1[b]	0.000	133.7 ± 6.7[b]	0.000
Dose 25 mg/kgBW (P1)	117.03 ±2.0[c]	0.000	131.0 ± 8.7[c]	0.000
Dose 50 mg/kgBW (P2)	104.00 ±3.0[d]	0.000	118.0 ± 2.0[c]	0.000
Dose 75 mg/kgBW (P3)	80.07 ± 3,1[e]	0.000	88.3 ± 2.1[d]	0.000

In addition to measuring SGOT-SGPT levels, histopathological structure (Figure 1) was also carried out on the mice liver for normal, exposed to CS and treated with *E. acoroides* peel extract. Histopathological observation was also accompanied by the calculation of the number of normal cells, necrosis, parenchymatous, and hydropic degeneration (Table 2)

Table 2. Amount of parechyimatose, hydrophic degeneration, necrosis and normall cells

Groups	Cell Amount			
	Parenchymatose (cell)	Hydrophic (cell)	Necrosis (cell)	Normal (cell)
Negative control (K-)	6	6	11	27
Positive control (K^+)	21	17	39	32
Dose 25 mg/kgBW (P1)	23	16	51	9
Dose 50 mg/kgBW (P2)	24	26	48	31
Dose 75 mg/kgBW (P3)	23	24	34	47

Observation of the histopathological structure found that the number of hepatocytes with necrosis, parenchymatous degeneration, and hydrophic degeneration, as well as normal cells, was lower in the negative control group (K-) compared to the positive control group (K+) and the treatment group. This shows that cigarette smoke causes impaired liver function in the positive control group as well as the treatment group.

In the positive control group, the number of cells undergoing necrosis, parenchymatous degeneration, and hydropic degeneration was smaller than the P1 and P2 treatments and the number of normal cells was also higher than P1 and P2. This shows that Hepa-Q works better in improving the structure of liver cells than the extract doses of 25 and 50 mg/kgBW of mice. On

the other hand, the P3 treatment showed that the number of cells undergoing necrosis, parenchymatous degeneration, and hydropic degeneration was smaller than P1 and P2, and the normal number of cells was greater than P1 and P2. This indicates an improvement in liver cells after the administration of the extract. Thus, if the dose of the extract is greater, there is a possibility of improvement in the liver cells of mice exposed to cigarette smoke. The histopathological structure of normal mice and the treatment of *E. acoroides* extract are shown in Figure 1.

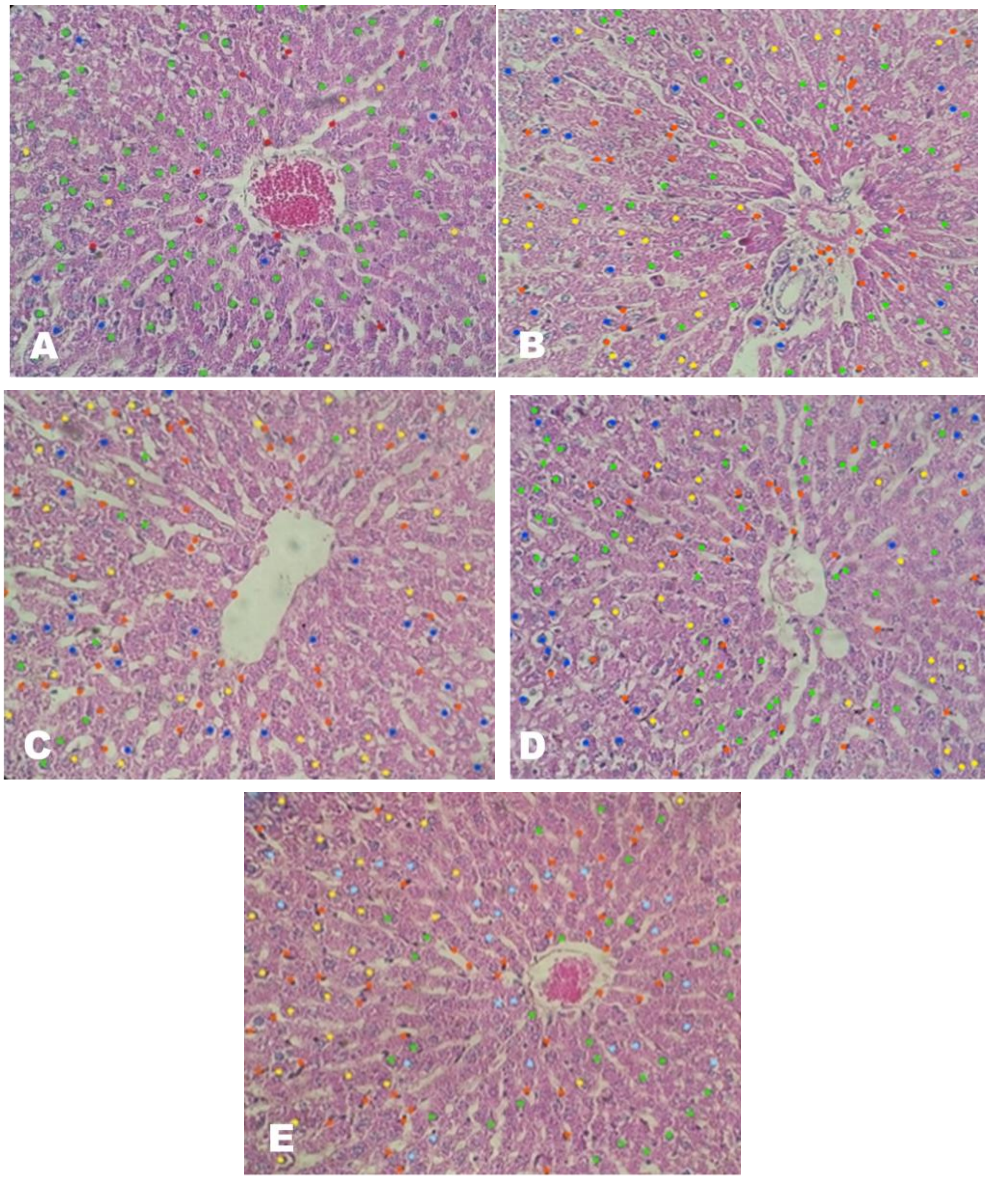

Figure 1. Histophatological structure of mice normal and treatment of *E. acoroides*.
Note: A. Negative control (K+), B. positive control (K-), C. P1 ; D. P2; E. P3; red color: necrosis; yellow: parenchymatose; blue: hydrophic; green : normal

The results of this study found that high doses of *E. acoroides* peel extract were able to protect against liver damage, whereas low doses were less powerful in protecting against liver damage. In the negative control group, SGOT-SGPT levels were normal, and also the histopathological structure showed a large number of normal cells. This means that mice that were not exposed to CS have healthy hepatocytes. Although there were necrosis, parenchymatous degeneration, and hydropic degeneration, in low numbers. This is presumably due to environmental stresses (feed, cage conditions, and endurance) experienced by mice during testing.

In the group of mice exposed to CS, the level of SGOT-SGPT was higher than normal, and the histopathological structure showed liver damage. This shows that CS causes impaired liver function.

This is following the statement of Rutledge & Asgharpour (2020) that CS is an agent that is very harmful to health because it causes tissue and organ damage. When exposed to CS for a long time, there will be a risk of experiencing impaired organ function, including the liver.

The P3 treatment (dose 75mg/kgBW) tended to decrease SGOT-SGPT levels and repair hepatocyte damage. This is due to the antioxidant effect of *E. acoroides* peel extract. As mentioned in the introduction, *E. acoroides* contains flavonoids, phenolics, saponins, tannins, steroids, and alkaloids (Dewi et al., 2012; Parthasarathy et al., 2017; Gono et al., 2022). These compounds were recognized as antioxidant compounds because it acts as hydrogen donor on free radicals originating from CS or other toxic agents.

Free radicals consist of unpaired electrons of atoms or molecules which were usually unstable and reactive with other atoms or molecules. One type of free radicals is oxygen based radicals which consist of superoxide, hydroxyl radicals, and peroxyl radicals and those were known as reactive oxygen species (ROS). In fact, at the certain level ROS are required in the human body since they preserve important physiological functions. It were generated from aerobic process and play important role in maintaining cellular functions such as signaling pathways, defense mechanisms as a barrier to invading microorganisms, and regulation of gene expression including cell growth and death (Li et al., 2015).

The evidence of oxidative stress was also usefull in specific physiological conditions. According to Simioni et al., (2018) during exercise, or ischemia oxidative stress could induced to strengthen biological defense mechanisms. However, in particular situations, and in most other cases, high levels of ROS and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury (Li et al., 2015).

Parenchymal cells are primary cells which subjected to oxidative stress and lead the induced of injury in the liver. Other type of liver injury also expressed in form of the hidrofic degeneration. Parenchymatose degeneration, and hydrofic degeneration were characterizised by lobular inflammation, ballooning hepatocytes, toxic cell swelling, vacuolar degeneration, or hydropic changes (Mumtazah et al., 2021). Although parenchymatous degeneration and hydropic degeneration are less lethal, they can serve as a precursors to necrosis (Mumtazah et al., 2021). According to Shojaie et al., (2020) the mechanism of liver damage is very complex. Molecularly, it involves various genes and proteins. Morphologically, it can be seen in the form of parenchymatous degeneration, hydropic degeneration and necrosis. The presence of necrosis was characterized by the presence of damaged cells in their membranes, uclear condensation, cell swelling or shrinkage, loss of plasma membrane integrity and blebbing. The final step of necrosis will followed by apoptosis (Shojaie et al., 2020).

In this result, the levels of SGOT and SGPT increased and were followed by an increase in the number of liver cells undergoing parenchymatous degeneration, hydrophic degeneration, and necrosis in mice exposed to CS. However, the levels of SGOT-SGPT began to decrease and were followed by an improvement in the structure of liver cells in line with the administration of *E. acoroides* peel extract. This shows that *E. acoroides* peel extract has the ability to inhibit liver damage in mice exposed to cigarette smoke (CS).

CONCLUSIONS

Seagrass peel extract had a significant effect on the transaminase enzyme (SGOT-SGPT) levels in mice exposed to cigarette smoke. The treatment of *E. acoroides* fruit peel extracts 75 mg/kgBW (P3) was an effective dose to reduce the levels of SGOT, and SGPT and improve the histopathological structure of the liver of mice exposed to cigarette smoke. It is necessary to carry out further research regarding the antioxidant capacity and the content of bioactive compounds in seagrass peel which support its potential to be developed as a new plant source to overcome the risk of disease exposure to free radicals originating from cigarette smoke.

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