

THE EFFECT OF ETHANOL EXTRACT OF *Plectranthusamboinicus* (Lour.) SPRENG AS PREVENTIVE AND CURATIFTO TOXIC EFFECT OF RHODAMINE B TO BODY WEIGHT AND LIVER WEIGHT OF RATS (*Rattusnorvegicus*)

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Abstract

The aim of this research is to know the effect of ethanolic extract of *Plectranthusamboinicus* (EEDB) as preventive and curative to toxic effect of rhodamine B to body weight and ratio of organ weight to body weight. This is experimental research that used non factorial Complete Random Designed (CRD) using 40 rats. EEDB was given orally with graded doses 350, 700 and 1050 mg/kg bw whereas rhodamine B was given as antigen with doses 980 mg/kg bw. Preventive treatment was done by giving EEDB from the 1st day to 42nd day then rhodamine B was given from 21st to 42nd day. Whereas in curative treatment was done by first inducing rhodamine B from the first day to the 21st day then given EEDB from the 22nd day until the 42nd day. Rats were given food and drink *ad libitum*. Parameters which observed are body weight and ratio organ weight to body weight. Data analysis results showed in preventive and curative treatment EEDB dose 700 mg/kg bw increased body weight, 1050 mg/kg bw increased ratio of organ weight to body significantly ($p < 0,05$).

Keywords : *Plectranthusamboinicus* (EEDB), body weight, ratio of organ weight to body weight

A. INTRODUCTION

In order to make food looks more attractive, the traders usually add some food colors to their foods. Lately, due to the price of food colors get more expensive and has expiry date, some traders replace it with synthetic dye known as rhodamine B. Rhodamine B includes to xanthene dyes which usually used for textile and papers industry as a dye for fabrics, cosmetics, oral cleaning products, and soap. Rhodamine B contains Cl a halogen compound which is radical if it's found in the body because it can bind to proteins, lipids and DNA, potentially causes damage to the function of organs in the body. Rhodamine B toxicity tests conducted on mice have proved the existence of a carcinogenic effect with the onset of local sarcoma in LD50 dose of 890 mg/kg. Therefore, consumption of rhodamine B in the long term can accumulate in the body and cause liver and kidney enlargement symptoms, impaired liver function, liver damage, physiological disorders of the body, and even cause liver cancer (BBPOM, 2012). In Mayoriat *al.* (2013) argued that continuously consumption of rhodamine B can cause liver and kidney damage. According to Junqueira (1991), liver is the largest organ and most useful in the body. Liver cells have functions as endocrine and exocrine, synthesize, store the substances, detoxify and transport substances. Associated with liver function as digestive organ, of course, the liver is also vulnerable in contact with foreign substances that enter the body whether toxicant or carcinogenic materials. Toxic and carcinogen materials that come into liver are processed in the liver, if found in excessive amounts and expose the liver in a long period can cause liver damage. In Frank (1995) some liver damages that often occur such as fatty liver, cholestasis, necrosis, caused by viral hepatitis, cirrhosis

that leads to liver cancer (hepatocellular carcinoma). The worst effects of induction of carcinogens which in this case is arhodamine B can lead to liver cancer.

Hepatocellular carcinoma (HCC) is the third leading cause cancer-related death and accounts for as many as 600.000 deaths worldwide annually. While HCC is common in Southeast Asia and sub-Saharan Africa, its incidence has continued to increase in the United State and Europe in the past 25 years. Incidence and mortality rates are expected to double in the next two decades (Tomuleasa *et al.*, 2009). The treatment cost of patients with liver cancer either in early or advance stage are still expensive, especially for chemotherapy, which gives negative side effects as well. Therefore the use of traditional medicines derived from plants as an alternative cure against cancer should be developed. Traditional plant hat can be developed as medicine for cancer treatment is *Plectranthusamboinicus*, known as aromatic plants typical of North Sumatera. In Santosa and Hertiani (2005) *Plectranthusamboinicus* phytochemical analysis showed the main compound contained in the leaves are polyphenols, saponins, glycosides flavonoids and essential oils. Phytochemical Database (Duke, 2004) reported that in these leaves are also vitamin C, vitamin B12, vitamin B1, beta carotene, niacin, carvacrol, calcium, fatty acid, oxalic acid, and fiber. The compounds have wide range potential of biological activities, such as antioxidant, diuretic, analgesic, to prevent cancer, antitumor, antivertygo, immunostimulant, anti-inflammatory, anti infertility ,hypocholesterol, hypotensive, and other properties that need to be investigated further. The ethanol extract of this plant contains a variety of flavonoids, they are quercetin, apigenin, luteolin, salvigenin and genkwain which have been proven as an anti-inflammatory because it inhibits the inflammatory response induced by cyclooxygenase, also tested as anti-cancer and anti-tumor (Kaliappan, 2008). Therefore, the potential of *Plectranthusamboinicus* for antitoxic and anticancer supports the background of this research.

B. OBJECTIVE

According to researches and opinions above, this research conducted to know the potential of antitoxic and anticancer by administration of ethanolic extract of *Plectranthusamboinicus* to the body weight and ration of organ weight to body weight as the parameters.

C. MATERIAL & METHODS

Animals

Wistar male rats (2 months between 110 – 200 g in body weight) were used in the study. The animals were maintained at room temperature on 12 h light – 12 h dark cycle. They were fed with PC 202 C pellet diet and tap water *ad libitum*. Rats were placed in plastic cages measuring 40 x 20 x 15 cm, at the top of the cages were equipped with a wire cover. Each cage was filled with chaff as the base and then placed one rat per cage. Acclimatization was done for 7 days.

Ethanol Extract of *Plectranthusamboinicus* (EEDB)

Ethanol extract from the plant leaves were processed using maceration method (Shenoy, 2012). The extract was mixed with CMC 1% before given orally to the rats. the ethanol extract was given with graded doses, 350, 700 and 1050 mg/kg bw according to Samosir (2014) which had converted from mice to rat by using Lawrence and Bacharach (1964) conversion table.

Rhodamin B solution

Rhodamine B in this study served as an antigen. Rhodamine B powder 6 g dissolved in 20 ml of distilled water with a concentration of 30%. Dose of rhodamineB was given based on the research of Mayoriet *al.* (2013) was 890 mg / kg bw.

Experiment Design

This research consisted of preventive and curative treatment. Each was divided into five groups they are, control (CMC 1%), rhodamine B (980 mg/kg bw), EEDB with graded doses 350, 700 and 1050 mg/kg bw. Preventive treatment was done by giving EEDB from the 1st day to 42nd day then rhodamine B was given from 21st to 42nd day. Whereas in curative treatment was done by first inducing rhodamine B from the first day to the 21st day then given EEDB from the 22nd day until the 42nd day.

Body Weight and Liver Measurement

Body weight of rats were weighed daily using OHAUS scale to the nearest 0.1 g. Liver weight measurements performed on day 42 after treatment. Rat liver was dissected and then lifted from the body and weighed with Tanika digital scale and calculated by the ratio of liver weight to body weight of rats.

Data Analysis

The data were analyzed using SPSS 17.0 software and statistically analyzed using ANOVA program and the means evaluation was done using LSD test. A value of $p < 0.05$ was considered as statistically significant. Results were presented as mean \pm SD.

C. RESULT AND DISCUSSION

Control treatment had the highest body weight, while rhodamine B had the lowest weight. In preventive treatment group, EEDB provided preventive effect on rats body weight. In curative treatment, giving EEDB proven to give effect to the weight gain of rats. It's seen from the value of the highest body weight of rats sequentially EEDB 700, EEDB 1050 mg/kg and EEDB 350 mg/kg bw compared to rhodamine B were significant ($p < 0.05$).

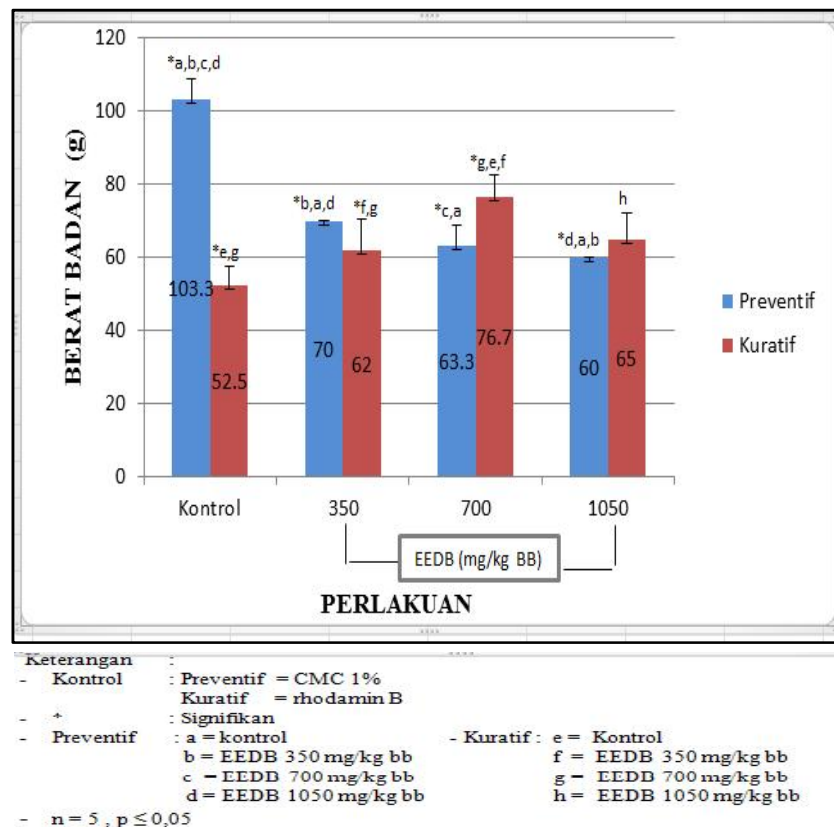


Figure 1. The Effect of EEDB to Body Weight

The liver weight in every group increased compared with control. EEDB affected significantly to increase the liverweight in EEDB 350 mg/kg bw and EEDB 1050 mg/kg bw. The lowest liver weight was in the control then the rhodamine B treatment. In curative, the effect of EEDB not significantly improved in EEDB 350 mg/kg bw and EEDB 700 mg/kg bw. LSD test showed that the treatment EEDB 1050 mg/kg bw increased the weight of the liver significantly compared to control $p < 0.05$.

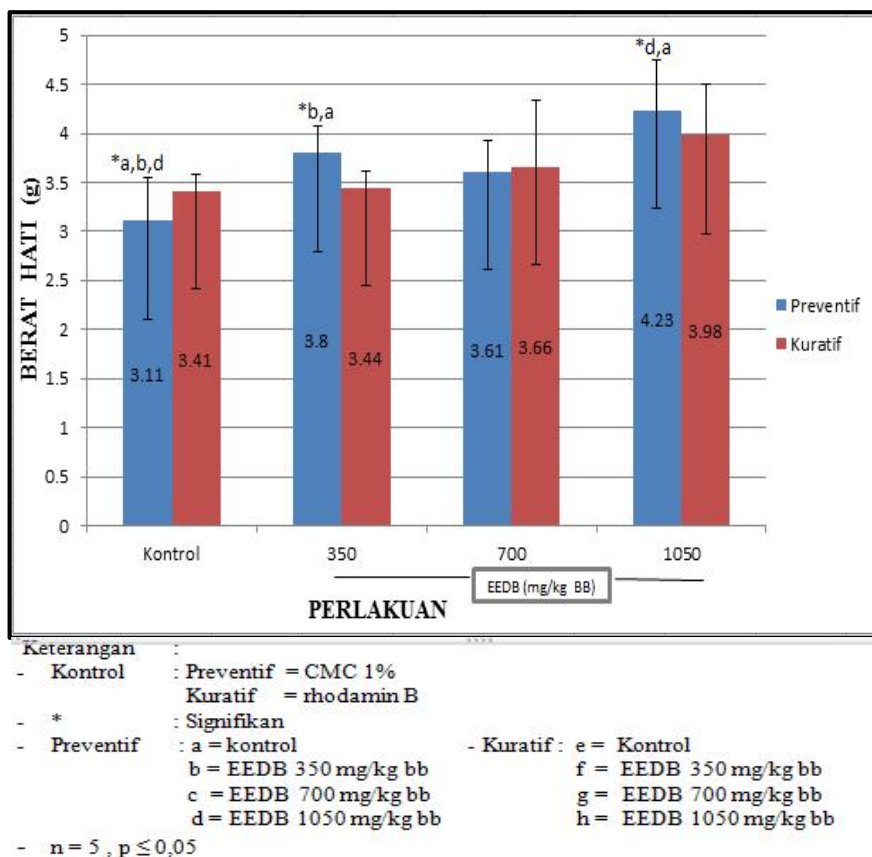


Figure 2. The Effect of EEDB to Ratio of Liver Weight to Body

D. DISCUSSION

The Effect of Ethanol Extract of *Plectranthusamboinicus* to Body Weight

Measurement of body weight is one of the research parameters that normally used to determine the physical condition. According to Sellers *et al.* (2007) weight measurement is required as an evaluation of the effects of toxicant in the body. Changes in body weight are often associated with the effects of the treatment. In toxicological studies changes in body weight associated with the mechanism of action, metabolism and toxicokinetic in the body. In this study, weight loss is the result of the difference in body weight of rats in early treatment and end of the treatment.

Treatment of Rhodamine B has the lowest weight due to the exposure of rhodamine B containing toxic substances such as, arsenic and chlorine in rhodamine B cause organ damage, which affects the body's metabolism. This is supported by Guyton (1997) which stated that the animals experienced weight loss seen from the declining appetite. Decrease of appetite due to administration of large doses in animals that cause

the animal becomes limp and possible lesions in the hypothalamus intilateral (as the center of hunger). Therefore, in this study a decrease in appetite will have an impact on body weight of rats, particularly rhodamine B treatment. It can be observed during the study where rats feed consumption in treatment rhodamine B less than the other treatments in the observation day to day , In the study Nagaraja *et al.* (2006) said that the direct effect of the stress experienced by the body is a decrease in the diet in rats resulting in weight loss. Stress on the body will increase protein catabolism and impeding the use of meal consumed during stress and ultimately lead to weight loss.

Giving EEDB before exposure to the rhodamine B plays a role in maintaining the physical condition of the body to maintain body weight and physiological condition of the body. The results were consistent with research Pillai *et al.* (2011) in which the methanol extract of *P.amboinicus* at a dose of 200 mg/kg and 400 mg/kg proven to increase weight gain during treatment of 28 days but not significant compared to the control mice. consumption for 28 days was also shown to increase body weight in rats in a study conducted by Asiimweet *et al.* (2014), also said that as high as the dose of *P. amboinicus* extract is given the higher body weight of rats will gain. This plant has a high nutrient content and the content of the active compounds thymol, carvacrol and forskolin which have physiological effects that can improve the metabolic processes in the body (Santosa and Hertiani, 2005). By improving the body's metabolism, the physiological condition of the rats body, the better, especially in terms of increasing the appetite, so weight will increase. This is in line with research of Aboubaker (2011) which stated that the mechanism of carvacrol and thymol in increasing body weight in broiler chickens stimulated the increasing of secretion of endogenous digestive enzymes to enhance utilization of the digestion products so that there was also an increase in the appetite.

The Effect of Ethanol Extract of *Plectranthusamboinicus* to Liver Weight

Organ weight measurement was conducted to see the effect of the treatment in the body. The value of measuring liver organ weights obtained from the ratio of liver weight to body weight of rats. by Sellers *et al.* (2007) organ weight measurement is done by comparing the weight of organs and body weight due to a change in body weight will affect whether or not increased organ weights. The increase of liver weight in rhodamine B treatment was caused by liver cells exposed to undergo hydropic degeneration, which then caused the hydropic degeneration where fluid was accumulated and increased the volume in liver cells. It is based on the theory of Chang (1986) which said that the accumulation of toxic and other metabolites cause cell degeneration. Substances that have toxic properties will cause a disruption in mitochondria organelles that produce energy adenosine triphosphate (ATP) and ATP are needed for passage of the sodium pump (Na^+). If then no present of ATP in the cells, Na^+ would not be out of the cell. Where Na^+ has property to absorb water. It caused disruption of cell permeability and fluid in the extracellular will enter into intracellular in large numbers then lead to the formation of vacuoles clear, small and numerous. The vacuole united to form larger vacuoles or single vacuole occupies the cytoplasm and nucleus of cells then replace the swelling of the cell so that happened to hydropic degeneration (Tatukude, 2014). This is in line with research Saraswati and Goddess (2009) which induced extract stew vinca leaves to see the level of toxic substances in mice at a dose of 10% and 20% which caused hydropic degeneration in hepatocytes. In research Nagaraja *et al.* (2006) the increase in organ weight in rats can result from the stress that occurs due to the induction of toxicant or isolation. The increased secretion of stress steroids, corticosteroids are known to increase metabolic activity and levels of m-RNA in liver cells. The changes of metabolism and chronic stress will require more amount of protein to repair damage which done to the liver cells. This causes an increase in liver weight.

Giving EEDB on preventive and curative treatment increased the weight of the liver. The value of liver weight in EEDB preventive and curative treatment in line with research Nagaraja *et al.* (2006) that was equal to 3.192 ± 0.005 g in stressed rats treated for 14 days. Giving EEDB was influential to repair physical condition of the liver. Exposure rhodamine B had caused lipid peroxidation and rupture of hepatocytes, hydropic degeneration and metabolic damage which nutrients are present in infected blood and becomes toxic poisons as the result in liver damage. EEDB contains flavonoids (quercetin, tannins and saponins) that play a role in stopping the peroxidation of lipids in the membrane. This is supported by Shenoy (2012) where *Plectranthus amboinicus* by of flavonoids in fatty degeneration, improved sinusoid and activate liver regeneration. The increase of liver weight occurred because hepatocytes were being regenerated. The restoration of liver cells returned liver's ability to absorb nutrients so that nutrients were absorbed also helped hepatocytes for having cell-division. The emergence of new hepatocytes led to increase the liver weight.

E. CONCLUSION

In conclusion, the ethanol extract of *Plectranthus amboinicus* has significant influence on body weight gain especially at dose 700 mg/kg bw and liver weight gain at dose 1050 mg/kg bw.

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