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THE EFFECT OF SWALLOW'S BIRD NEST EXTRACTS (*COLLOCALIA FUCIPHAGA THUNBERG*) ON LEVEL OF OXIDATIVE STRESS AND FUNCTION IN LIVER

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Abstract – Sprague Dawley mice were given swallow's nest extract at a dose of 10, 20 and 40 mg/kg BW per oral previously induced H_2O_2 on the 31st and 32nd days. In addition, there was also a normal group and a positive control group for vitamin E. After being turned off, the liver was taken and measured the GSH, MDA and AST/ALT activity of the liver tissue. The results showed a MDA level: control (+), normal, 10, 20, 40 mg/KgBW dose of swallow's nest extract: 0,065; 0.050; 0.043; 0.051; 0.070 ng/mg tissues. Then GSH levels: control (+), normal, 10, 20, 40 mg/KgBW dose of swallow's nest extract: 0.081; 0.077; 0.33; 0.057; 0.077 μg/mg tissues (ANOVA p <0.05), Pearson correlation test results showed a strong positive relationship, whereas the higher the MDA level the higher the GSH level (R = 0,960). AST/ALT ratio: control (+), normal, 10, 20, 40 mg/KgBW dose of swallow's nest extract: 0.391; 0.377; 0.323; 0.398; 0.42 (ANOVA p >0.05). The histological image of Swallow's Nest extract doses 10 and 40 shows normal cells. Swallow's nest extract can reduce the level of oxidative stress as evidenced by increased GSH and decreased MDA levels, and can also reduce liver damage by reducing AST/ALT activity. Swallow's nest extract does not cause damage to liver cells or tissue.

INTRODUCTION

Swallow birds are insectivorous birds that are scattered in almost all provinces in Indonesia. The specialty of the swallow is that it has a pair of salivary glands located under its tongue. These salivary glands have the ability to produce a lot of saliva for use in making nests. This nest is a place to rest and breed swallows which are found in caves and dark and damp houses. One example of a bird's nest is the nest of white swallow birds (*Collocalia fuciphaga* Thunberg) (Babji *et al.,* 2015). Indonesia is one of the main exporters of swallow's nests, which accounts for around 38% of the world's total swallow nest exports. This puts Indonesia in the first place as an exporter of swallow's nest in the world. The main export markets for Indonesia's swallow's nest are Hong Kong, Singapore, the United States, and Vietnam (Sandi *et al.,* 2019).

Since hundreds of years ago, swallow's nest has been widely known and consumed because it is believed to have high nutritional content such as carbohydrates, protein, fat, calcium, phosphorus, vitamins and mineral. Amino acids contained in white swallow's nest are classified as complete ranging from essential amino acids to non-essential amino acids. Apart from being a food ingredient, white swallow's nest is also believed to have medicinal properties that can treat asthma, malnutrition, increase metabolism, and increase stamina (Babji *et al*., 2015; Norhayati *et al.,* 2010; Hamzah *et al.,* 2013).

Swallow's nest contains high amino acids so that it can be used as stimulation of endogenous antioxidants such as the enzyme superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), non ezymatic or tripeptides is a glutathione (GSH). These enzymes can inhibit lipid oxidation through biological mechanisms such as antioxidant enzymes and iron binding protein as well as non-specific mechanisms. Overall, enzyme activity as an antioxidant has the ability to activate Reactive Oxygen Species, free radical scavenger, chelating reactions, and reduction of hydroperoxides. can decrease (Valko *et al*., 2006).

Free radicals in excess amounts in the body can cause oxidative stress. This situation can cause oxidative damage starting from the level of cells,

tissues, to organs which can accelerate the aging process and the appearance of various diseases (Lobo *et al.,* 2010). The formation of free radicals is triggered by an oxidation reaction that can occur at any time. However, these free radicals can be inhibited through antioxidant defense mechanisms (Lu *et al.,* 2010).

Free radicals are molecules that have unpaired electrons in their outer orbitals and are therefore highly reactive with other unpaired electrons to fill their orbital vacancies. Under normal conditions, the body produces free radicals which are indispensable in some physiological processes but in limited numbers. which are produced in the body comes from aerobic metabolic processes, enzymatic oxidation, and phagocytosis by phagocytic cells that use large amounts of oxygen (Hirage *et al.,* 2011). In addition, free radicals can also come from outside the body, namely ionizing radiation (X-ray and UV), fastfood, cigarette smoke, and drugs (Lobo *et al.,* 2010; Lu *et al.,* 2010).

Antioxidants are factors that protect cells from free radicals. The mechanism of free radical inhibition consists of endogenous and exogenous antioxidants. When there is an increase in free radical levels, the body will produce endogenous antioxidants as a resistance mechanism against free radicals. However, if the number of free radicals is too much, additional antioxidants that come from outside the body are needed, called exogenous antioxidants. Endogenous antioxidants consist of superoxide dismutase, glutathione peroxidase, and catalase. Whereas exogenous antioxidants consist of Vitamin C, vitamin E, selenium, β-carotene (Hiragi *et al.,* 211).

Antioxidants can neutralize free radicals by breaking the chain reaction of free radicals. The body can produce antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Hiragi *et al.,* 211; Mahjoub *et al.,* 2012). GSH as the body's natural antioxidant has a role in counteracting free radicals. GSH levels in the blood range from 5-8 mM/l or 2-20 μmol/L with the highest concentration in the liver which is the most important organ in the detoxification process (Mitchell *et al.,* 2016; Zhengtou *et al.,* 2014). GSH is a multifunctional molecule that influences cellular processes. GSH levels in the body are an important aspect that must be considered, because decreased GSH levels can affect oxidative stress, resulting in the emergence of various diseases such as fatty liver, cancer, cystic fibrosis, and others (Huang *et al.,* 2020). As an important organ that has a detoxification function, the liver plays a major role in the balance of GSH between organs (Mescher *et al.,* 2018).

In some conditions, there can be an imbalance in the amount of free radicals and antioxidants in the body. If the number of free radicals is higher, this can cause oxidative stress (Mahjoub *et al.,* 2012). Oxidative stress will cause damage to cells that can attack nucleic acids, proteins, and lipids in the cell membrane and plasma lipoproteins. This cell damage can directly lead to disease, aging, and death (Mahjoub and Roudsari, 2012). The liver is the largest gland and the most important organ in the body for metabolic homeostasis and detoxification of toxic substances. Detocfication of the liver is done by neutralizing and removing metabolic waste and xenobiotic substances. The liver is very hard work, therefore it is vulnerable to damage and is prone to interference. This disorder is usually caused by disorders of the metabolic system, toxic substances, infections, circulatory disorders, and neoplasms. The liver will release GOT and GPT enzymes as a parameter of hepatocellular damage. Therefore, there is an increase in the activity of these enzymes in liver damage (Winkler *et al.,* 1993).

One that can cause oxidative stress is lipid peroxidation. Lipid peroxidation will produce an aldehyde compound, namely malondialdehyde (MDA). MDA acts as a free radical indicator marker. The higher the MDA value correlates with the lower antioxidant activity so that it can produce ROS which causes oxidative stress (Liou and Storz, 2010). The liver has many roles for the body, one of which detoxifies and breaks down waste products, hormones, drugs and other foreign compounds (Sherwood, 2015). All nutrients that enter the body will be absorbed by the small intestine and carried to the liver for processing in the liver (Moore *et al.,* 2013). Therefore, this study was conducted to determine the effect of giving swallow's nest extract which is believed to be an antioxidant that affects MDA and GSH levels which is used as a marker of lipid peroxidation in the liver. The parameters of liver damage can be measured by measuring the activity of the transaminase enzymes (AST/ALT). This study also to see cell damage due to oxidative stress, an observation of the histology image of liver cells was also carried out

MATERIALS AND METHODS

Collection and treatments swallow's nest

The design used in this study is an experimental design. The research was conducted at the Faculty of Medicine, Syarif Hidayatullah State Islamic University, Jakarta, measuring levels of MDA, GSH and AST/ALT activity in the Biochemistry Laboratory and making preparations for liver tissue images in the histology laboratory. This study used an extract of white swallow's nest (Collocalia fuchipaga Thunberg.) Obtained from Painan, West Sumatra and then determined in the Ornithology Laboratory, Biological Research Center in the field of Zoology, LIPI Bogor.This study used a healthy male white rat Sprague Dawley strain aged 5-6 weeks with a weight of 150-200 grams.

The number of tested animals was 25 animals divided into 5 groups, namely the control only gave 0.5% NaCMC; positive control by giving vitamin E (1000 IU 4.08 ml/g); three treatments by administering swallow's nest extract at a dose of 10 mg/kg, 20 mg/kg and 40 mg/kg. All of these treatments lasted for 32 days and then induced H2O2 solution at a dose of 0.1 mg/kgBW on days 31 and 32. This study used a healthy male white rat Sprague Dawley strain aged 5-6 weeks with a weight of 150-200 g. The number of tested animals was 25 animals divided into 5 groups, namely the control only gave 0.5% NaCMC; positive control by giving vitamin E (1000 IU 4.08 mL/g); three treatments by administering swallow's nest extract at a dose of 10, 20 and 40 mg/kg. All of these treatments lasted for 32 days and then induced H_2O_2 solution at a dose of 0.1 mg/kgBW on days 31.

Test animals that have been given treatment for 32 days, were terminated by inhalation anesthesia using ether. The rats were put into a container covered with cotton that had been moistened with ether. The dead mice were then necropsed and the liver organs were weighed, part of it was taken for making histological preparations and the rest was stored in a freezer at -80R° C to be made homogenate. The data were analyzed using the SPSS version 22 application with a one-way ANOVA statistical test, because this study included a comparative analysis of more than two groups and a normal and homogeneous distribution.

Measurement of MDA Levels

The prepared 250 µL of liver homogenate was then put into a test tube. Add 500 µL of 10% TCA and centrifuged at 4,000 rpm for 10 minutes. The supernatant was taken and added with TBA 0.67% as much as 750 µL, incubated for 10 minutes. The solution was read at a wavelength of 532 nm using a spectrophotometer.

Glutathione (GSH) Level Test

The prepared 50 µL of liver homogenate was included in the microtube. Then 200 µL of 5% TCA was added and 1750 µL of phosphate buffer solution of pH 8.0 was added. After that it was centrifuged at 3500 rpm for 10 minutes. Take the supernatant, then add 25 µL of DTNB and let stand for 1 hour. Solutions were measured with a spectophotometer at 412 nm waves and compared with a standard GSH curve.

AST test activity

The examination was carried out using the DiaSys Kit, there was reagent 1: containing TRIS buffer pH 7.15, L-Alanine, and lactate dehydrogenase; and reagent 2: containing 2-Oxoglutarate and NADH. The liver homogenate solution of 100 µL was added to the microtube. Add 1000 µL of reagent 1 and let stand for 5 minutes at room temperature. Then the solution was added with reagent 2 as much as 250 µL. The absorbance of the solution was read using a spectrophotometer with a wavelength of 340 nm at 1, 2 and 3 minutes. AST activity is calculated using the existing formula in the kit procedure.

ALT activity test

The examination was carried out using the DiaSys Kit, namely reagent 1 containing TRIS buffer pH 7.5, L-Alanine and lactate dehydrogenase; whereas reagent 2 contains 2-Oxoglutarate and NADH. 100 µL of hepatic homogenate was added with 1 1000 µL of reagent. Mix gently and let stand for 5 minutes at room temperature. Add 250 µL of reagent 2 and let stand for 1 minute at room temperature. Then the solution was read absorbance with a wavelength of 340 nm minutes 1, 2 and 3. AST activity was calculated using the formula in the kit procedure.

Preparation of Liver histological image

The network that is included in the 10% formalin buffer will then go through the following stages: network fixation with PBS 7.4; dehydration with graded alcohol; clearing with toluel-alkohon ratio; embedding with paraffin at 60 °C; then a network block is made with paraffin; cutting with a microtome; and carry out the hematosillin-eosin

(HE) staining process. Histologic images of the liver were observed at the microscope.

RESULTS AND DISCUSSION

The results of measuring MDA and GSH levels after giving swallow's nest extract are seen in Figure 1. The results showed that the lowest MDA levels occurred in the group given swallow's nest extract at a dose of 10 mg/Kg compared to the normal group. The highest levels of MDA occurred in the provision of bird's nest extract at a dose of 40 mg/kgBW. Figure 1 shows that the lowest levels of GSH occurred at the dose of 10 mg/kgBW of bird's nest extract. The highest GSH levels were given swallow's nest extract at a dose of 40 mg/kgBW compared to normal controls. Pearson correlation test results showed a strong positive relationship, whereas the higher the MDA level the higher the GSH level (R = 0.960).

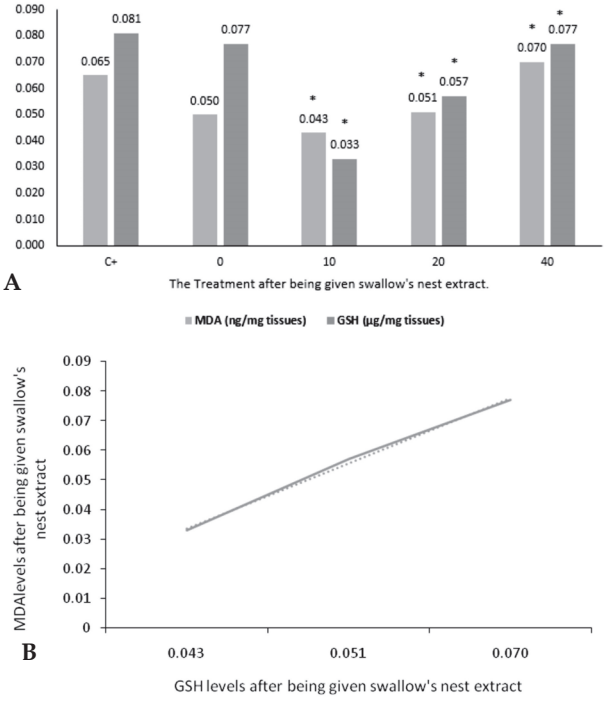

Fig. 1. MDA and GSH levels (A) and their regression (B) after being given swallow's nest extract (ANOVA, p<0,05; Corelation Pearson R = 0,960)

This study, in the treatment 10 and 20 mg/KgBW of swallow's nest extract MDA levels were lower than the positive control. This shows that swallow's nest extract acts as an antioxidant that can ward off free radicals so that free radical levels through the MDA indicator decrease. Swallow's nest has a function as an endogenous antioxidant and stimulant to compile endogenous antioxidant components such as superoxide dismutase enzymes, glutathione peroxidase, and catalase. Swallow's nests contain various components, including carbohydrates, protein, and fat as proven by qualitative tests in the form of biuret reaction, molish reaction and xanthoprotein reactions with positive results (Triawanti *et al.*, 2019).

The antioxidants contained in swallow's nest extract are thought to be due to swallow's nest having high protein content. with the active ingredient in the form of amino acids.Swallow's nest has a protein content of 55.62% and contains 7 types of essential amino acids and 9 types of non-essential amino acids (Kanerva, 2014).

Proteins can inhibit lipid oxidation through biological mechanisms such as antioxidant enzymes and iron binding protein as well as non-specific mechanisms. The mechanism of inhibition of lipid oxidation can occur through antioxidant enzymes, namely the enzyme superoxide dismutase, glutathione peroxidase, and catalase (Hiragi *et al.*, 2011). Enzymes are proteins, so bird's nest extract containing the amino acids that make up the protein may reduce MDA levels. The activity of the catalase enzyme in 2017, concluding that swallow's nest extract can increase the activity of the enzyme catalase.[19] the extract swallow's nest can increase the activity of the enzyme superoxide dismutase (Kanerva, 2014). The results of these two studies can be concluded that swallow's nest extract can be used as an antioxidant through the mechanism of antioxidant enzymes.

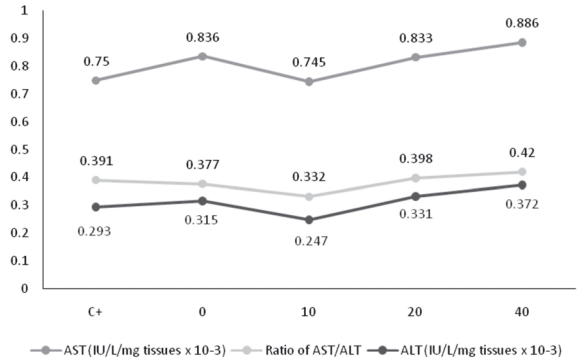

Fig. 2. AST, ALT and their ratio activities after giving swallow's nest extract (ANOVA test; p > 0.05)

Overall, the antioxidant activity of proteins goes through a variety of complex pathways, including their ability to activate ROS, free radical scavenger,

chelating reactions, and reduction of hydroperoxides. The free formed can decrease (Valko *et al.*, 2006; Lobo *et al.*, 2010). So that in treatment giving swallow's nest extract 10 and 20 mg/Kg BW obtained low levels of MDA. In giving swallow's nest extract 40 mg/KgBW high levels of MDA. This is probably because the bird's nest follows a non-linear pharmacological model, that is, by increasing the dose, it is inversely proportional to the pharmacological effect. So it can be concluded that at high doses, swallow's nest is thought to be hepatotoxic.

Antioxidants are substances that can prevent the formation of free radical reactions. The body has a natural (endogenous) antioxidant, namely glutathione (GSH). Glutathione is a tripeptide with three amino acids as its main constituents, namely glycine, glutamic acid, and cysteine. GSH can be found in almost all organs, but the highest concentration is in the liver, which is an important organ in detoxification function (Hiragi *et al.*, 2011). Apart from endogenous antioxidants, the body also needs antioxidants from outside the body (exogenous) such as vitamin C, vitamin A, and vitamin E to deal with excessive oxidative stress. Antioxidant GSH is the main endogenous protection system, because GSH is directly involved and actively participates in the destruction of oxygen reactive compounds (ROS) and also maintains the reduced (active) form of vitamins C and E (Pzzorno, 2014; Olugbami *et al.*, 2015). Free radical is a compound that contains one or more more unpaired electrons, so it becomes reactive to find a partner by binding to electrons from other compounds. If the number of free radicals in the body increases, it will result in oxidative stress which can cause damage to cell death which causes various organ damage, one of which is the liver (Valko *et al.*, 2006; Lobo *et al.*, 2010).

Amino acids and vitamins A, C, and D contained in swallow's nest have high oxidant activity. Amino acids act as tertiary antioxidants which are responsible for repairing damage to biomolecules caused by free radicals and work in increasing enzymes that deactivate free radicals such as glutathione peroxidase, superoxide dismutase, and catalase. Glutathione peroxidase and catalase are known to be abundant in the liver, the way this enzyme works by converting hydrogen peroxide and peroxide into water and vitamins as secondary antioxidants that play a role in capturing (scavenger free radicals) so that free radicals do not act with cellular components (Roh *et al.*, 2012). During a balance between oxidants and antioxidants in the liver will not cause damage to the liver. This swallow's nest has a high antioxidant fraction which has antioxidant properties as a scavenger free radical.

The data show that AST activity is lowest after administration of swallow's nest extract dose of 10 mg/KgBW. While the highest ALT activity was at a dose of bird's nest extract 40 mg/KgBW (One Way Anova test; $p > 0.05$). Data from the measurement of AST, ALT and the ratio of both can be seen in Figure 2. The lowest ALT activity was at a dose of 10 mg / KgBB of swallow's nest extract. While the highest ALT activity was the dose of swallow extract at 40 mg / KgBB. The lowest GOT / GPT activity ratio was at a dose of 10 mg/KgBB of swallow's nest extract. Meanwhile, the highest AST/ALT activity was at the bird's nest extract dose of 40 mg / KgBW (ANOVA test; $p > 0.05$).

The comparison of AST and ALT activity obtained shows lower AST activity than ALTT, this can occur because the location of ASTT is in the cytosol (20% of total activity) and mitoconndria (80% of total activity), while ALTT is located on the cell membrane. Therefore, if there is damage to the cell membrane due to the induction of H_2O_2 as oxygen-centered non radicals which causes fat peroxidation in the cell membrane so that the results of the ALTT test will increase because the damage starts in the cell membrane and the amount of AST is located in the cytosol only. 20%. It is known that the ALT enzyme is more effective in determining liver damage. While the results of the AST/ALT activity ratio were low after giving swallow extract at a dose of 10 mg/KgBB had the lowest activity. This was due to the possibility that the levels of antioxidants contained in swallow's nest were still high so that they could suppress free radicals and reactive compounds.

This study conducted observations on the liver preparations of tested animals which were given low and high doses of swallow's nest extract using hematoxylin and eosin staining. Observation of preparations at doses of 10 and 40 mg/KgBW of giving swallow's nest extract, can be seen in Figure 3.

In normal hepatocytes image have a large polyhedral shape with six or more surfaces. The diameter of the hepatocytes was 20-30 μm. Cytoplasm is eosinophilic with haematoxylin and eosin staining because it has very many

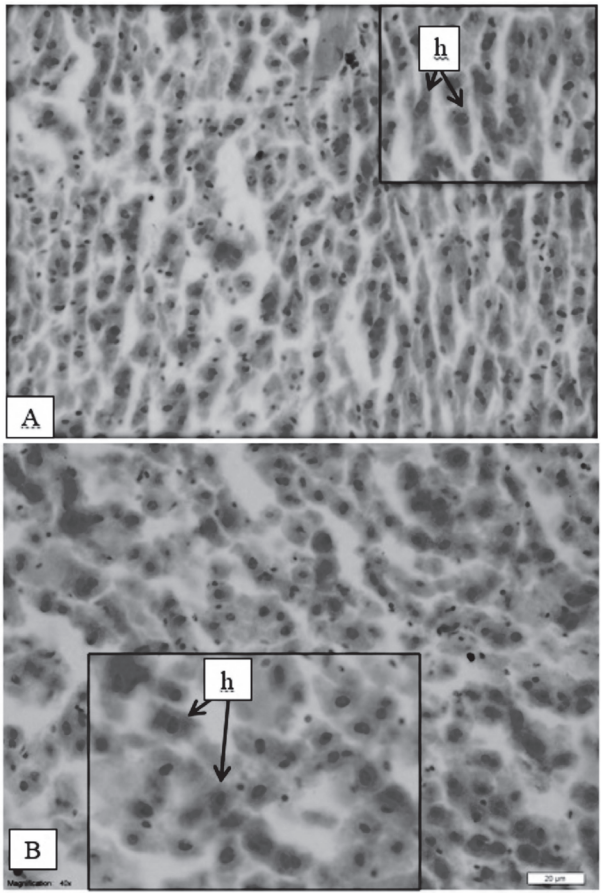

Fig. 3. Hepatocytes at 40x Magnification. (A) The swallow's nest extract at a dose of 10 mg/KgBW. (B) The swallow's nest extract at a dose of 10 mg/KgBW. (h) normal hepatocytes.

mitochondria around 2000 per cell. Hepatocytes have a large spherical nucleus with a nucleoli. Hepatocytes often have two or more nucleoli and about 50% are diploid with two, four, eight or more of the normal diploid chromosome (Mescher, 2018). The results of hepatocyte observations in the low and high dose groups qualitatively indicate that there are still good hepatocytes. This situation can support the results of research on the effect of giving swallow's nest extract on the liver so that it can be concluded that swallow's nest extract at low and high doses does not cause damage to the liver histologically.

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