CHARACTERIZATION AND ANTIOXIDANT POTENTIAL ALBUMIN PROTEIN of *Canna striata* FISH TO *Rattus novergicus* INDUCED by ISONIAZID TOXIC DOSE

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Abstract: The *Canna Striata* fish is a potent source of albumin as an antioxidant. Albumin is a protein capable of binding to free radicals in plasma and has 17 disulfide bonds linking sulfur-containing amino acids. The existence of thiol group bonds in albumin extract of *Canna Striata* fish is possible to bind to diphenylphycril hydrazyl (DPPH), causing the antioxidant capacity of *Canna Striata* fish extract to be high. The accumulation of free radicals produced by the administration of antituberculosis drugs from the results of isoniazid metabolism can cause hepatic necrosis. So it requires antioxidants that can reduce free radical levels, which in this study was tested in white rats induced with isoniazid and then treated with albumin protein extract *Canna Striata* fish. This research method is the first experimental method done is the characterization of albumin protein extract *Canna Striata* fish with temperature 25˚C and 40˚C by Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE). Then the antioxidant level was tested by measuring the levels of Malondialdehyde (MDA) after 2 last week treatment period in 5 groups of white rats are positive control group, negative control group and 3 treatment groups induced Isoniazid toxic dose are 37.8 mg accompanied by giving of albumin protein extract Canna Striata fish with content of 20ml/kg, 40 ml/kg and 60 ml/kg. The result of this study showed that there were ten albumin protein profiles with size 15,41 kDa, 19,74 kDa 28,6 kDa, 38,87 kDa, 46,08 kDa, 72,45 kDa, 94,42 kDa , 120.82 kDa, 142.80 kDa, and 155.97 kDa. Based on One Way ANOVA and Post Hoc Tukey statistic test, it were found that there was the significant influence and differentiation of Malondialdehyde (MDA) level on giving of albumin protein extract in white rats induced by Isoniazid with p-value 0,002. The most effective decrease in MDA levels is high levels of albumin protein extract of 60 ml/kg.

Keywords: Albumin Protein Extract of *Canna Striata*, Malondialdehyde, Isoniazid

INTRODUCTION

Global tuberculosis control in the WHO in 2009 reported that in Indonesia, Pulmonary TB became a major public health topic. Pulmonary TB patients are ranked the 3rd largest in the world after India and China with patients accounting for about 10% of the total number of Pulmonary TB patients in the world. While five countries with the highest number of Pulmonary TB patients with the following sequence; India has 2 million people, China 1.3 million people, Indonesia 0.53 million people, Nigeria 0.46 million people and South Africa 0.46 million people. The 1995 Household Health Survey claimed that Pulmonary TB was the leading cause of death after cardiovascular disease and respiratory disease in all age groups but was the more significant number one in the infectious disease group.[1]

The principle of TB treatment uses at least two kinds of drugs and takes place in the long term.[2] Antituberculosis (OAT) drugs may be used in tuberculosis therapy but still have a potentially toxic effect, especially in elderly and small adults. Antituberculosis drugs commonly used are Isoniazid (INH), Rifampicin, Pirazinamide, Ethambutol, and Streptomycin.[3]

Risks that can be caused by the administration of Antituberculosis Drug is a disorder of liver function, from mild to severe form of hepatic tissue necrosis. In general Antituberculosis drugs have hepatotoxic effects except for streptomycin.[4]

The type of antituberculosis drug that has a hepatotoxic effect is Isoniazid. In drug biotransformation, the hydrazide groups of Isoniazid are known to form an N-acetyl conjugate in an acetylation reaction catalysed by the enzyme N-acetyl transferase to acetyl-isoniazid. This conjugate is a substrate for the result of hydrolysis to isonicotinic acid and acetyl hydrazine which will then be converted by cytochrome P450 to Mono-Acetyl-Hydrazine (MAH) reactive metabolite. Mono Acetyl Hydrazine will stimulate acetylation of macromolecules and have hepatotoxic effects.[5]

The cause of this hepatotoxic effect is due to the accumulation of free radicals from Isoniazid metabolism. Excessive free radicals will cause oxidative stress that triggers the lipid peroxidation process that causes hepatic tissue necrosis. Therefore the body needs antioxidants to neutralise free radicals produced by the metabolism of Antituberculosis Drugs and free radicals that come from outside the body.

Food sources that can act as antioxidants are *Canna striata* fish. Santoso *et al*., (2009) reported in his research that administration of *Canna striata* fish extracts could withstand a decrease in serum an antioxidant activity due to paracetamol poisoning. This albumin is due to the high activity of antioxidants due to the presence of albumin and minerals in *Canna striata* fish extract. Antioxidant activity is positively correlated with albumin levels. The association of albumin levels with serum antioxidant activity may be due to the presence of thiol groups in albumin.[6]

Canna striata fish has high nutrient and albumin content than other fish, and is a source of albumin for people with hypoalbumin (low albumin) and wounds. Both postoperative wounds and burns. In fact, in rural areas in boys after circumcision is always recommended to consume this type of fish for faster healing. *Canna striata* fish contains compounds - compounds that are important for the human body such as high enough protein, fat, water and minerals.

Canna striata is a source of minerals (including Zn, Cu, Mn and Fe) supporting the process of tissue synthesis, so it is very

instrumental in the process of wound healing.[7] Mineral Zn is essential for DNA synthesis by mammalian cells. Zn mineral deficiency causes cell apoptosis. Zn minerals prevent lymphocyte cell death by inhibiting endonuclease activity. Rusjianto, 2009 states that Zinc can serve to the process of metallothioneine who is required to fight free radicals and expel poison heavy metals of the body. Zn with methallothionein significantly decreases apoptosis due to oxidative stress.[8]

To measure the presence of oxidative stress, it can be done by measuring the levels of Malondialdehyde. Malondialdehyde (MDA) is an end product of lipid peroxidation, and is commonly used as a biomarker to determine the magnitude of oxidative stress.[9]

Based on the description above, the authors are interested to examine the characterization of albumin protein profile in *Canna Striata* fish and its potential as an antioxidant in white rats induced by isoniazid toxic dose.

RESEARCH METHODS

Beaker glass, stirrer, funnel, sheker, evaporator, spectrophotometer, sonde, syringe, paraffin board, test tube, mouse cage, label, dropper, micropipette, vortex, centrifugation, SDS-PAGE tool.

Isoniazid, Malondialdehyde reagent, Acrylamide, Bis-Acrylamide, bromthymol blue, ammonium persulfate, tris phosphate buffer, tetra ethylene diamine, sodium dodecyl sulfate, coomassie blue, methanol, Filter Paper**.**

Canna striata Fish extract is the result of extraction from steamed Canna Striata fish with temperature 25oC and 40 oC then filtered with filter cloth. This *Canna striata* fish extract would use for the detection of protein albumin profile as the characterization of protein albumin *Canna striata* fish. While the extract of *Canna striata* fish used for analysis of antioxidant is the fish extract of *Canna striata* which was steamed at temperature 40 oC with content 20ml / kg BB, 40ml / kg BB, and 60ml / kg BB.

Isoniazid was obtained in tablet form obtained from Apotik Z. Giving toxic dose in humans of 30mg / kg BW. The conversion factor for humans weighing 70 kg in mice weighing 200 g was 0.018 (Kusumawati, 2004). Dose in humans weighing 70kg: 30mg x 70 = 2100 mg. Conversion in mice with BB 200g: 2100 x 0.018 = 37.8 mg / 200 g BB. So the dose is given 37.8mg / ml / 200 g BB. The dose is still in the toxic zone because the oral lethal dose for mice is 650 mg /kg. The toxic dose of 30mg / kg BW based on research Himawan (2008).

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Twenty μL protein sample of *Canna striata* fish extract mixed with five μL buffer sample. For markers, ten μL protein markers (fermentas) were obtained with two μL buffer samples. All solution is heated in boiling water for two minutes and immediately cooled. Subsequent protein and protein marker samples were incorporated in the wells at a 12% running gel. Electrophoresis at 100 volts, 40 mA in a chamber that has been filled with an electroferesis buffer, is carried out for 1.5 to 2.5 hours. Larger molecular weight protein bands will form closer to the separation site. The protein

bands formed in the gel after electrophoresis are determined by their molecular weight (kDa).

For the acclimatiom periode, a male rat (Rattus norvegicus) of 30 rats aged 12-16 weeks was inserted in a cage, with Rats in each cage. The Rats were adapted for seven days under the conditions of the cage. During the adaptation stage, rats were fed in the form of standard mouse food in the form of pellets and feeding of ad libitum Rats. Rats were randomly selected and divided into five groups, each group containing seven rats. Groups K +, K-, G1, G2, G3. Rats were treated from day 8-21.

The treatment group is divided into five groups: Positive K Group (the group of rats used as a comparison to assess the normal state of rats, in the Control group, mice were given only standard food and drink); group K Negative (mice were given isoniazid with dose 37.8ml/200 gram BB/day for 14 days, isoniazid is given once daily for oral using gastric sonde); group G1 (rats were given cork fish extract 20 ml/kg BW/day and after two h isoniazid with a dose of 37.8ml/200 gram BB/day for 14 days. Isoniazid and *Canna striata* fish extract are given once daily peroral using gastric sonde); group G2: Rats were given 40 ml/kg B/day cauliflower extract two times using gastric sonde and after two hrs isoniazid with a dose of 37.8ml/200 gram BB/day per oral once daily using gastric sonde for 14 days; and G3 group (rats were given 60 ml/kg B/day extract of cork fish three times using gastric sonde and after two hours isoniazid with the dose of 37.8ml/200 gram BB/day per oral once daily using gastric sonde for 14 days).

On the 22nd day, blood samples were taken for examination of MDA levels in each mouse. Blood collection is done intra-cardinal (cardiac puncture)

RESULTS AND DISCUSSION

Using the Sodium Dodecyl Sulfate Polyakrilamide Gel Electrophoresis (SDS-PAGE) tool and using Coomassie Blue staining, the bands of the same good cinnamon albumin protein steamed at 25˚C (A1) and 40˚C (A2) . By calculating the value of Rf it is found ten protein bands with the size as in figure 3.1 that is, 15,41 KDa, 19,74 KDa 28,6 KDa, 38,87 KDa, 46,08 KDa, 72,45 KDa, 94,42 KDa , 120.82 KDa, 142.80 KDa, and 155.97 KDa.

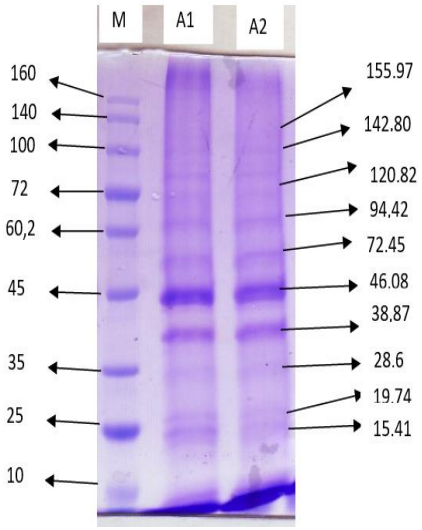

Figure 1. Protein Albumin Profile of *Canna striata*

The following are table which shows levels of Malondyaldehyd on the treatment Rat white

Table 1. MDA Blood Rat Level

The Treatment	MDA					$\bar{X}$
	R_1	R_2	R_3	R_4	R_5	
Control (+)	5.281	5.281	5.281	5.662	4.136	5.128
Control (-)	6.281	6.899	7.899	5.281	7.662	6.804
G1	5.281	5.281	5.662	5.188	5.188	5.320
G2	5.188	6.044	5.281	5.281	5.281	5.415
G3	5.662	4.188	4.518	5.281	5.662	5.062

Table 1 shows that the highest average MDA value is in positive control group that is equal to 6,804 and the lowest MDA value is in group of treatment of three (G3) that is equal to 5,062. To further clarify Table 1, a bar chart shown in Figure 2 below shows that *Canna striata* fish extract can decrease MDA levels of white rats induced by isoniazid toxic doses.

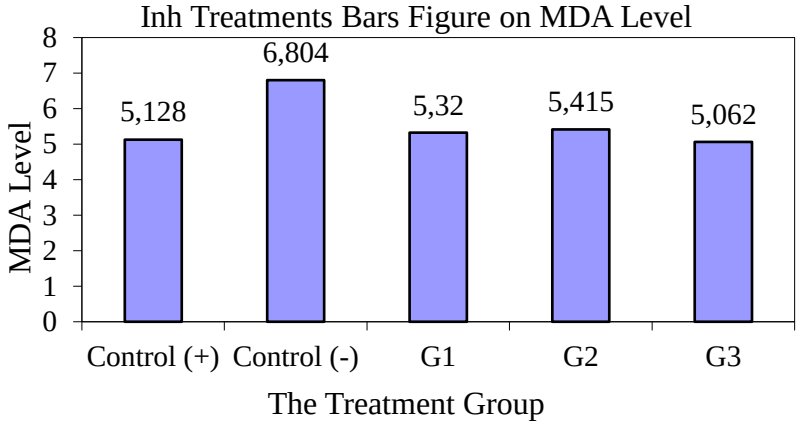

Figure 2. INH Treatment Bars Table on MDA Levels

Before testing the effect of the extract of *Canna striata* on the levels of Malondialdehyde (MDA) in white rats (*Rattus novergicus*) induced by Isoniazid (INH), it is necessary to test the ANOVA and Post Hoc Tukey test in the discussion.

Based on the results of the study in Figure 3.1, there were ten protein profiles of the same albumin extract of cork fish steamed at 25˚C (A1) and 40˚C (A2). This shows that in the manufacture of cork fish extract the effect of temperature does not give effect on albumin protein content because Albumin protein profile type on A1 and A2 tends to be same so that its Rf price is also identical. By calculating the rate of the Rf price, there were ten protein bands with the size of 15,41 kDa, 19,74 kDa, 28,6 kDa 38,87 kDa, 46,08 kDa, 72,45 kDa, 94,42 kDa, 120, 82 kDa, 142.80 kDa, and 155.97 kDa. And for the next extract, the used cork fish is steamed at 40 of by considering the same protein albumin profile at 25 ºC and 40 ºC.

To see whether there was any effect of the extract of *Channa striata* on the level of Malondialdehyde (MDA) on white rats (*Rattus novergicus*) induced by Isoniazid (INH) then performed by One Way ANOVA test. The test was performed using SPSS version 20.0 with the significance level (α) = 0,05.[10] Test results could see in the table 2.

Table 2. Analysis of One Way Variance (One Way ANOVA)

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.302	4	2.576	6.161	.002
Within Groups	8.360	20	.418		
Total	18.663	24			

From table 2 shows the significance of p-value = 0.002 that is <α (0.05) hence there is influence between giving of *Canna striata* extract to decrease of serum MDA (malondialdehyde) level in Isoniazid induced Rattus novergicus (INH).

Furthermore, the Post Hoc Tukey test was conducted to find out more details about the pair of different groups of samples that differed significantly and the couple of samples did not differ, it will be known which treatment has the most influence on the decrease in serum mileage (malondialdehyde) in Rats (*Rattus novergicus*) induced Isoniazid (INH).

Groups treated with *Canna striata* fish extract showed significant differences (<α (0.05) to group K2, i.e. negative control group (given Isoniazid 37.8 mg / 200g BB).This difference is seen because p <0.05 is normal.

The results showed that there was an influence between the administration of *Canna striata* fish extract on the decrease in serum MDA (malondialdehyde) level in white rats (*Rattus novergicus*) induced Isoniazid (INH). This is evident with p-value = 0.002 ie <α (0.05).

Malondialdehyde as the primary outcome of lipid peroxidation due to MDA-stress oxidative is the end product of lipid peroxidation and is usually used as biological biomarkers to assess oxidative stress.[9]

In the lipid peroxidation process, MDA also forms other free radicals, but the free radical has a short half-life that is difficult to examine in the laboratory. Measurement of serum MDA level could be done with thiobarbituric acid reactive substances (TBARS) test based on spectrophotometric reaction examination.[9]

The results of this study provide answers to the notion that the extract of *Canna striata* is a source of minerals (including zinc, copper, and iron) supporting the process of tissue synthesis, so it is very instrumental in the process of wound healing. Zinc, copper, and iron minerals are indispensable in various metabolic processes of the body.

Putri and Agustina , 2016 in research earlier stated that their that be extract albumin *Canna striata* with a dose 100 mg , 200 mg in the least and though 400 mg influence significantly to the acceleration of a contraction of oral premalignant lesions were incision is on *Rattus novergicus*. The ability of fish extract *Canna striata* catch free radicals allegedly associated with albumin components and minerals contained in it. Albumin is a protein that can bind free radicals in plasma. Molecule albumin has 17 disulfide bonds linking sulfur-containing amino acids. The existence of thiol group bond in albumin extract of cork fish is possible to bind to DPPH and cause high antioxidant capacity of *Canna striata* fish extract.[11]

Albumin and Zn play an important role in healing wounds because albumin had the capacity to bind Zn and take it up in the blood plasma.[12] Another factor that is likely to be the cause of high antioxidant capacity of

Canna striata fish extract is minerals. Minerals Zn, Cu, and Fe are positively charged metals which readily react with atoms or other compounds including Diphenylpicryl Hydrazyl (DPPH). The results of this DPPH analysis show that *Canna striata* fish extract has potential as an antioxidant. Considering figure 2 the extract dose of 20 ml/kg, 40 ml/kg and 60 ml/kg, was found to be effective at high doses of 60 ml/kg in the G3 group as evidenced by the decrease in MDA levels compared to G1 groups and G2.

CONCLUSIONS

Based on the result of characterization of albumin profiles in Canna Striata fish, it was found that albumin protein profile was 15,41 kDa, 19,74 kDa, 28,6 kDa 38,87 kDa, 46,08 kDa, 72,45 kDa, 94,42 kDa, 120.82 kDa, 142.80 kDa, and 155.97 kDa. Giving albumin extract to the levels of Malondialdehyde there are significant effects and differences with the value of p-value 0.002. And the most effective dose can decrease the levels of Malondialdehyde is a dose of 60 ml/kg *Canna striata* extract

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