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Effect of Phaleria Macrocarpa Flesh Fruits Extract on MDA Level, SGOT and SGPT Activity in Serum of Experimental Rats Contaminated by Cd (II) Ion

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Keywords: Phaleria macrocarpa fruit extract; Cd (II) ion; MDA; SGOT; SGPT

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Abstract

BACKGROUND: Cd (II) ion is a heavy metal that has a toxic ability in the human body. P. macrocarpa has been used as anticancer, Diabetes Mellitus and antimicrobe because it consists of flavonoid, steroid, and tannin.

AIM: The purpose of this study was to investigate the effectiveness of P. macrocarpa fruits extract as an antidote for the toxicity of Cd (II) in the liver of experimental rats.

METHODS: The experimental laboratory was done by using 9 female of Wistar rats (Rattus novergicus) that divided into 3 groups with the age between 2.5-3 months and weight between 133-160 grams. The first group was a control given distilled water and a normal diet. The second group was given antidote 5 mL of P. macrocarpa fruit extract x BW/200 g dosage for 7 days and induced by 1000 mg/L of Cd (II) ion with dosage of 1 mL x BW/200 g. The third group was given 1000 mg/L Cd (II) ion only of 1 mL x BW/200 g. After 5 hours, the blood sample was taken for analysis of MDA, SGOT, and SGPT.

RESULTS: As the result of experimental rats exposed with Cd (II) ion, there are significant decreasing of all the observed parameters including MAD, SGOT and SGPT with percentage 71.5%, 72.1%, and 93.6% respectively.

CONCLUSION: The rats given with the antidote of Phaleria macrocarpa flesh fruit were able to protect the liver from damage due to exposure to Cd (II) as seen from the decrease in liver function enzyme parameters namely SGOT and SGPT.

Introduction

The development of rapid industrialization generated many negative consequences for environmental pollution affecting human health [1]. Many processes in industries including mining, electroplating, dyeing, paper, and crude oil generate wastes that containing heavy metals that toxic to living organisms [2]. Cadmium (Cd), mercury (Hg), Copper (Cu), arsenic (As) and lead (Pb) are dangerous heavy metals that have high toxicity and tendency to accumulate in the food chain even at low concentration. Particularly for Cd (II) ion and its compound, this heavy metal contributes to a large number of serious health problems including heart disease, cancer and diabetes [3]. Cd (II) ion has been known could cause various health problems such as impaired liver function, lung, bone defects, cancer and hypertension in humans [3]. Exposure to heavy metals such as Cd (II) and As (II) could cause metabolic disorders such as 'itai-itai' emphysema and testicular atrophy [1].

Conventional methods of physical-chemical including electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and absorption to remove heavy metals are economically high cost and have disadvantages including requires reagent in quantities and generate secondary wastes. Biosorption is an alternative method for removing heavy metals from aqueous solutions and industrial waste [4], [5], [6]. Biosorption process generally low cost and does not pollute the environment [7]. *Phaleria macrocarpa* is a plant that grows in tropical

areas primarily in Papua, Indonesia, and is used to address a wide range of health problems such as diabetes and hypertension [8].

The previous study reported that the seed and fruit flesh of *P. macrocarpa* effective for adsorption of Cd (II) with maximum biosorption capacities (Q) were 21.4592 mg/g and 24.7629 mg/g respectively [9]. The previous research of Nasution *et al.,* (2015) explained that *P. macrocarpa* contained hydrogen stretching due to inter and intramolecular interaction of alcohol, phenol and carboxylic acid [10]. This functional group will contribute to preventing metal ion from destroying organs.

Based on the Atomic Absorption Spectroscopy (AAS) analysis by Adrian *et al.,* (2015) showed the leaves of *Manihot utilissima* have a potentially used as an absorbent for Cd (II) in aqueous solution. In the year, the researchers continue the research about the effect of Cd (II), mainly in the kidney and liver [11]. The researcher administrates intraperitoneally of cadmium lead to elevated levels of cadmium content in almost organs in rat except in the brain. Administration of cadmium also increases some biochemical parameters and enzymes in serum include malondialdehyde, urea, creatinine, SGOT, and SGPT [10].

The aim of the study was to investigate the effectiveness of *P. macrocarpa* fruits extract as an antidote for the toxicity of Cd (II) in the liver of experimental rats.

Materials and Method

Preparation for experimental rats

Experimental rats used in this study were 9 animals which were divided into 3 groups. The treatment group was as follows group I administration with distilled water and normal diet, group II administration with antidote and Cd (II) 1000 mg/L and group III administration with Cd (II) 1000 mg/L only.

Analysis of Biosorbent Function Groups with FTIR

FTIR (Fourier Transform Infrared AA240) analysis of bio sorbents was carried out before and after the absorption of metal ions. The flesh and seeds of the crown of the deity are mashed with mortar added with KBr, then printed into thin discs or pellets. Samples were analyzed by FTIR to determine the functional groups of the flesh of the crown god.

Preparation antidote of P. macrocarpa

The antidote was prepared by weighing 2 grams of the fruit of *P. macrocarpa* and then mashed with distilled water, transferred into a beaker and then add distilled water approximately 120 mL, heated to boiling and then stored in vials tube. The rats induced with the antidote every day for one week with a dose of 5 mL x bw/200 g bw orally.

Histopatology analysis

After one week, the rats were injected with a solution of Cd 1000 mg/L with a dose of 1 mL x bw/200 g bw intraperitoneally. After 5 hours, the blood samples and liver were taken for analysis of MDA, SGOT, and SGPT, and histology, respectively. The histopathology analysis was observed using an Olympus microscope. In the third group, the rats only induced using Cd with a dose of 1 ml x bw/200 g bw intraperitoneally, and after 5 hours, the blood samples were taken for analysis of MDA, SGOT, and SGPT.

Measurement of MDA

Provided 3 test tubes containing blanks (distilled water), standard MDA solution, serum (sample) 0.5 mL. Add 2.5 ml of 5% TCA each. Mix using a vortex mixer, centrifuge for 10 minutes at 2000 rpm. Each pipette 1.5 mL filtrate, put into a tube in accordance with the label. Add 1.5 mL Na each. Thio Barbituric Acid mix with a vortex mixer, heat it in a water bath for 30 minutes. Cool, read the adsorbent with a spectrophotometer at a wavelength of 530 nm.

Measurement of SGPT Levels in Serum

Tubes are arranged on a shelf that has been provided, then labeled, starting sample 1 (S1), sample 2 (S2), sample 3 (S3) and so on. Then 100 µl of serum 1 was taken into the S1 tube, 1000 µl of reagent 1 was added (Tris pH 7.15 100 mmol/L, L-alanine 500 mmol/L, LDH (lactate dehydrogenase ≥ 1700 U/L)), mixed, incubated at room temperature for 5 minutes, 250 µl reagents 2 were added (2-oxo-glutaric 15 mmol/L, NADH 0.18 mmol/L, pyridoxal-5-phosphate 0.09 mmol/L, buffer pH 9.6 0 , 7 mmol/L), mixed and read the absorbance after 1, 2 and 3 minutes.

Measurement of SGOT Levels in Serum

The tubes are arranged in a rack that has been provided, then labeled, starting sample 1 (S1), sample 2 (S2), sample 3 (S3) and so on. Then, 100 µl of serum 1 input was taken into the S1 tube, 1000 µl of reagent was added (Tris pH 7.65 80 mmol/L, L-Aspartate 240 mm0l/L, MDH (malate dehydrogenase) ≥ 600 U/L, LDH ≥ 900 U/L) mixed, Incubation at room temperature for 5 minutes, added Reagent 2 (2-oxo

glutaric 12 mmol/L, NADH 0.18 mmol/L pyridoxal-5-phosphate 0.09 mmol/L, buffer pH 9.6 0.7 mmol/L), as much as 250 µl, mixed and read the absorbance after 1, 2 and 3 minutes.

Result

Analysis of Adsorbent Function Groups with FTIR

FTIR (Fourier Transform Infrared) spectroscopy is used to identify functional groups in bio sorbents that might be involved in the process of biosorption of Cd(II) ions.

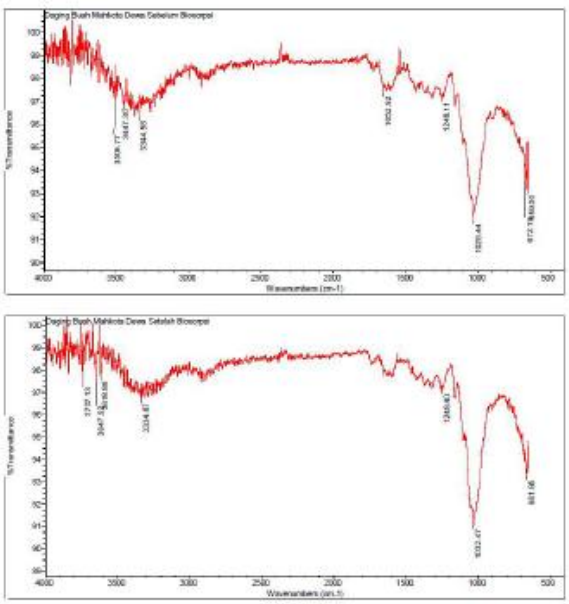

Figure 1: FTIR spectrum of P. macrocarpa; A) before biosorption; B) after biosorption

Histological Analysis

The administration of Cd (II) ions and the antidote of the crown god flesh powder to Cd (II) in the liver is shown in Figure 2.

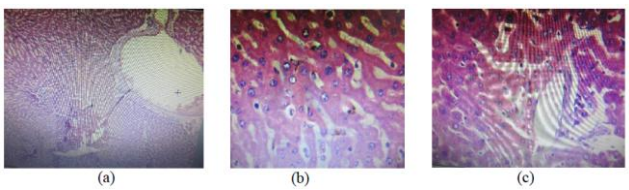

Figure 2: Histopatology of liver on experimental rats; A) control; B) P. macrocarpa flesh fruit extract with Cd (II) induction; C) Cd (II) ion induction without antidote, magnification 40 X

Examination of MDA, SGOT, and SGPT levels in Serum

Blood of rats was taken from each group. Then centrifuged to get serum. Biochemical results of serum blood are shown in Table 1.

Table 1: The levels of serum biochemical parameters and oxidative stress including MDA, SGOT and SGPT

No	Parameters	Group 1	Group 2	Group 3
1	MDA (mg/dl)	3.61	4.24	14.9
2	SGOT (U/L)	111.987	66.08	236.97
3	SGPT (U/L)	25.88	12.94	203.33

Discussion

Analysis of Adsorbent Function Groups with FTIR

Figure 1 indicated the functional group that existed in *P. macrocarpa* flesh fruit. The spectrum presented the hydroxyl group at 3504.77 cm^{-1} that shifted to 3737.13 cm^{-1}. This phenomenon exhibited the interaction between *P. macrocarpa* flesh fruit with Cd (II) ion. The functional group in the *P. macrocarpa* flesh fruit formed a stable compound with metal ions so that metal ions could not react further to harm the other organs.

Strong and dense bands at wavelengths of 3500 – 3200 cm^{-1} indicate the presence of O-H stretch groups resulting from intra-molecular and intermolecular bonds of hydrogen from alcohols, phenols and carboxylic acids [12]. The band at a wavelength of 3000-2850 cm^{-1} is estimated to be a C-H stretch group while a carbonyl C = O stretch group is shown at a wavelength of 1760-1690 cm^{-1}. Bands at wavelengths from 1500 to 1400 cm^{-1} indicate C-C stretch or C-H groups. C-H and C-O functional groups are shown at wavelengths of 1320-1000 cm^{-1} [5]. Figure 1 shows the functional groups found in the flesh of the crown of the gods. The wavenumber at 3344.58 cm^{-1} is an OH group which then experiences a shift to 3334.87 cm^{-1} after adsorption occurs.

Histological Analysis

Figure 2 presented liver histopathology on experimental rate after Cd (II) ion and antidote exposure. Figure 2B indicated the surface of the normal liver with light fatty. Meanwhile, Figure 2C indicated that hepatic cells of the liver consisted of lobules with dilatation of Venne centrally. Some of the hepatic cells were cloudy swelling and necrosis.

The liver is the primary target for Cd (II) exposure. About half of Cd (II) is absorbed systemically very rapidly in the liver, which then results in reduced Cd (II) availability in other organs such as the kidneys and testicles, which are more sensitive to their toxicity. El-Refaiy and Eissa (2012) in their study reported that giving Cd (II) with chronic doses to rats would produce necrosis of hepatocytes,

fatty, signs of degeneration and infiltration of inflammatory cells. Subchronic exposure to Cd (II) results in day damage in the form of swelling and necrosis. Necrosis generally occurs in centrilobular and spreads throughout the liver lobules. Histopathological changes in the liver exposed to Cd(II) may be caused by the formation of free radicals that are very reactive and cause lipid peroxidation to damage the cell membrane [13].

According to Tarasub *et al.,* (2008), Cd (II) metal generally accumulates in hepatocytes and if the concentration of Cd (II) exceeds the capacity of metallothionein (MT) to bind Cd(II) it will cause liver damage which is generally characterized by increased SGPT enzymes and SGOT. The increased levels of SGPT and SGOT are due to permeability of hepatocyte membranes damaged by exposure to Cd (II) so that these enzymes leak into the bloodstream. In addition, GSH plays an important role in the integrity of lipids and proteins in the liver. Consumption of free radical scavenging produced by Cd (II) causes a significant decrease in GSH levels [14]. Tarasub *et al.,* (2008) reported that in mice exposed to Cd (II), an increase in cytoplasmic hypereosinophilia, cell damage such as pyknosis and necrosis. Damage to liver cells is likely caused by a disruption in thiol hemostasis and ROS production [14].

Examination of MDA, SGOT, and SGPT levels in Serum

The levels of serum biochemical parameters and oxidative stress including MDA, SGOT, and SGPT are shown in Table 1. After the experimental rats exposed with Cd (II), there are significant decreasing of all the observed parameters including MDA, SGOT, and SGPT with percentage 71.5%, 72.1%, and 93.6% respectively, It could be known that the chemical compounds contained in P. macrocarpa fruit extract have the ability to repair the hepatic organ of rats after contaminated by Cd (II). All parts of P. macrocarpa which including pericarp, mesocarp, and seeds contain different kinds of total phenolic and flavonoid compounds. Part of pericarp and mesocarp showed a high content of antioxidant activity using DPPH (71.97% and 62.41%) and free radical scavenging activity (65.68%) [15]. A similar result reported by Embugishiki *et al.,* (2013), which reported the protective effect of pre-treatment carrot juice against oxidative damage induced by Cd (II). Pre-treatment with carrot juice could prevent lipid peroxidation induced by Cd (II), prevent decreasing in non-enzymatic antioxidants and reduce the accumulation of cadmium in the liver and kidneys [16].

Poontawee *et al.,* (2016) was reported a protective effect of *Cleistocaly xnervosum* var paniala fruit extract against oxidative damage due to exposure of Cd (II). Renal oxidative damage occurs after exposure to Cd (II) characterized by increasing of creatinine, blood urea nitrogen (BUN), reduction in glomerular filtration, renal structural damage was accompanied by in increasing of nitric oxide and MDA [17]. It is estimated that the protective effect was due to the antioxidant and free radical scavenging activity, as well as the interaction of bioactive compounds contained in an extract with enzymatic and non-enzymatic antioxidants. Liver damage induced as a result of Cd(II) exposure lead to elevated level in liver function parameters including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP),lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT) total serum bilirubin (TB) and it is also accompanied by an increase of thiobarbituric acid reactive substances (TBARS) [18].

Cd (II) exposure in experimental rats will lead to changes level of serum biochemical parameters such as MDA, SGPT, and SGOT. Pre-treatment with *P. macrocarpa* flesh fruit antidote could significantly reduce the elevated levels of serum biochemical parameters. Pre-treatment with *P. macrocarpa* flesh fruit antidote could reduce the hepatotoxicity effect induced by Cd (II) that might be due to the high content of antioxidant and free radical scavenging activity. On the other hand, the lower of MDA in this research show the effect of the extract which prevents organ damage caused by ROS and Oxidative stress.

This study concluded that the rats given with the antidote of *Phaleria macrocarpa* fruit flesh were able to protect the liver from damage due to exposure to Cd(II) as seen from the decrease in liver function enzyme parameters namely SGOT and SGPT.

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