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Research Article

ANTI-METALOTOXIC PROPERTIES OF KELAKAI (*STENOCHLAENA PALUSTRIS*) LEAVES EXTRACT AGAINST CADMIUM-INDUCED LIVER TISSUE DAMAGE

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ABSTRACT

Objective: The present study was undertaken to investigate the anti-metalotoxic activity of the leaves extract of *Stenochlaena palustris* (*kelakai; S. palsutris)* on cadmium (Cd)-induced liver tissue damage.

Methods: Liver tissue damage was induced by the administration of cadmium sulfate ($CdSO_4$) at a dose 3 mg/l. Anti-metalotoxic effect of the extracts was determined by assessing the concentration of malondialdehyde (MDA), carbonyl compound (CC), conjugated dienes (CD), and advanced oxidation protein products (AOPPs) induced by Cd with and without the presence of the extract.

Results: The results of this present studies showed that treatment with $CdSO_4$ significantly increase the levels of MDA, CC, CD, and AOPPs. The leaves extract of *S. palustris* significantly decrease the levels of all measured parameter in liver tissue.

Conclusion: The present study demonstrated that Cd could induced the liver tissue damage, and the extract of *S. palsutris* showed the anti-metalotoxic activity to reduce the damage.

Keywords: Cadmium, Liver, Oxidative Stress, *Stenochlaena palustris.*

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INTRODUCTION

In South Kalimantan, river is an integral part of human life. Every people in South Kalimantan uses river for various functions of life such as transportation, fishing, and bathing [1]. This causes the people have a great risk to be exposed to various types of pollutants present in river water, such as Cadmium (Cd). Sofarini *et al*. [2] result study indicated that one of the heavy metals that exceed the maximum threshold in the waters of the Barito River is Cd. The previous study by Rahman [3] showed that the average of Cd level in the waters of Takisung and Batakan beach was about 0.06 ppm and 0.074 ppm, respectively. To the best of our knowledge, Cd levels that are still allowed into the water environment are about 0.00011 ppm and the maximum allowable level is 0.01 ppm [4].

After Cd enters the body, it could be irreversibly accumulates in the human body, in particularly, in kidneys and other vital organs such the lungs or the liver [5,6]. These accumulation then might result in some several adverse effects like oxidative stress. Suhartono *et al*. [7] result study showed that subacute and subchronic Cd exposure could increase the level of malondialdehyde (MDA) and advanced oxidation protein products (AOPPs) in kidney homogenates. Furthermore, our several results study showed that Cd exposure could induced oxidative stress, lipid peroxidation, and depletes the enzymatic antioxidant activity in brain, liver, and kidney of rats [8-11].

Recently, the study of medicinal plants to prevent and inhibit the oxidative stress-related disease is increasing. Many plants have been known to have such effects, and one of them is Kelakai (*Stenochlaena palustris*: *S. palustris*). *S. palustris* is one of the plants that often grow on wetlands in South Kalimantan. Based on empirical studies, *S. palustris* has been used daily to treat several medical conditions such as anemia, fever, and skin diseases by the local people of South Kalimantan [12]. Our previous study indicated that *S. palustris* leaves extract could inhibit the formation of methylglyoxal (MG), AOPPs, and carbonyl compound (CC) induced by Cd *in vitro*. Our another previous study also indicated that *S. palustris* leaves extract contained flavonoid and has antioxidant activity which is might be useful to inhibit the oxidative stress [13].

However, no data are available in the literature of *S. palustris* extract on the inhibition of oxidative stress in liver homogenate induced by Cd. Therefore, we undertook the present investigation to examine the anti-metalotoxic effects of *S. palustris* leaves extract on the Cd-induced liver tissue damage.

METHODS

Collection and identification of plant materials

The fresh leaves of *S. palustris* were collected from Gambut subdistrict, South Kalimantan, Indonesia, in April 2017. The plant was authenticated by the Department of Biology of the Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, South Kalimantan, Indonesia. Before use, it was ensured that the leaves and bark were free from contamination, sand, and no microbial growth. The bark and leaves were shade dried and were made into coarse powder using a commercial blender.

Preparation of extracts

Extraction was done by maceration methods. 50 g of shade-dried leaves of *S. plaustris* was weighed, and 250 ml of 70% ethanol-water was added to it, respectively. Then, the mixture was allowed to concentrated in 3 days. The mixture was then filtered 3 times until the filtrate is clear colored. All the filtrate is made into one and then evaporated until thick. Furthermore, the filtrate re-macerated for 24 h and placed in a test tube in the fridge until it uses for further examination.

Experimental protocol

The liver samples were collected from 24 male rats (*Rattus norvegicus*) with 2–3 month old, weighing 200–250 g. Then, liver samples were taken by surgically procedure with ketamine as anesthesia. Then, the liver fixed in phosphate buffer at pH 7.0. The liver was ground to form a liquid. Subsequently, the solution was taken and centrifuged at 3500 rpm for 10 min, and the top layer was taken and stored until it uses.

Furthermore, the liver homogenate was prepared to experimental *in vitro* models. Samples divided into 4 groups (1 control group and 3 treatment groups). Control group (C-group): 1 ml of liver homogenate+1 ml of 3 mg/l of cadmium sulfate ($CdSO_4$)+1 ml of phosphate buffer with 7.4 pH. Treatment 1 group (T1-group): 1 ml of liver homogenate+1 ml of 3 mg/l of $CdSO_4$+1 ml of phosphate buffer with 7.4 pH+1 ml of 5 mg/l of *S. palustris* leaves extract. Treatment 2 group (T2-group): 1 ml of liver homogenate+1 ml of 3 mg/l of $CdSO_4$+1 ml of phosphate buffer with 7.4 pH+1 ml of 10 mg/l of *S. palustris* leaves extract. Treatment 3 group (T3-group): 1 ml of liver homogenate+1 ml of 3 mg/l of $CdSO_4$+1 ml of phosphate buffer with 7.4 pH+1 ml of 15 mg/l of *S. palustris* leaves extract.

Each solution then incubated at 37°C for 1 h. After incubation, liver MDA, CC, conjugated dienes (CD), and AOPPs concentration were estimated. All animals used and care was in compliance of the Ethics Commission of Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia.

Liver MDA inhibition assay

The liver MDA concentration was analyzed according to TBARS method which is previously described by Buege and Aust. 1 ml samples were quickly transferred to assay tubes containing 100 µL of 100% trichloroacetic acid (TCA), 100 µL of 1% Na-Thiobarbiturate, and 250 µL of 1 N hydrochloride acid (HCL). The solution then heated at 100°C for 20 min and followed by cooling using ice at room temperature. Then, the solution was centrifuged at 3500 rpm for 10 min, and the supernatant was taken. The absorbance of the supernatant was determined at 532 nm against a blank solution that contained all reagents, except sample. The MDA concentrations are expressed as µM [14].

Liver CC inhibition assay

The liver CC concentration was estimated using spectrophotometric 2,4-dinitrophenylhydrazine (DNPH) assay. CC was measured using two tubes, a control and sample tube. The sample tube contained 1 ml sample and 1 ml DNPH, while the control tube contained 1 ml hepatic homogenate control and 2.5 ml HCl. Then, both tubes were incubated for 45 min at room temperature and protected from sunlight. Both tubes then centrifuged at 1400 rpm for 5 min.

In each tube, 1 ml of 20% TCA was added and centrifuged again at 1400 rpm for 5 min to remove the supernatant. 1 ml of 10% TCA was added to each tube and followed by centrifugation again at 1400 rpm for 5 min to remove the supernatant. The precipitate then washed 3 times with ethanol/ethyl acetate then dissolved the precipitate in both tubes with 1 ml of 6 M guanidine hydrochloride at 37°C for 15 min with vortex mixing. The absorbance of both tubes was read at 366 nm. The CC level than can be calculated using equation: [15]

$$\text{CC level } (\mu M) = \frac{\text{Sample Absorbance} - \text{Control Absorbance}}{26}$$

Liver CD inhibition assay

Liver CD concentration was assayed as previously described by Recknagel *et al*. with minor modifications. Briefly, the sample extracted with chloroform: methanol (2:1) at a ratio of 1:2.5 homogenate to organic solvent mixture. The resulting mixture was centrifuged at 2000 rpm for 8 min. The organic phase containing the CD was taken, dried under nitrogen, solubilized in hexane, and rinsed with 0.003 N HCl. Samples were analyzed spectrophotometrically at 233 nm, the maximal absorbance for CD, and expressed per nanomole of phosphate. Total phosphates were quantified by incubating a portion of the dried organic phase for 3 h with 70% perchloric acid at 130°C, followed by the addition of ammonium molybdate and Fiske-Subbarow reagent. Samples or standards were then reheated to 110°C for 10 min, cooled, and analyzed spectrophotometrically at 820 nm. Data are expressed as optical density at 233 nm/nmol phosphate [16].

Liver AOPPs inhibition assay

AOPPs measurement was made by spectrophotometric methods as describe by WitkoSarsat *et al*., with slight modification. Briefly, AOPPs were measured by spectrophotometry on a microplate reader and were calibrated with chloramine-T solutions that in the presence of potassium iodide at 340 nm. In test wells, 200 ml of plasma diluted 1/5 in phosphate buffer solution was placed on a 96-well microtiter plate, and 20 ml of acetic acid was added. In standard wells, 10 ml of 1.16 mol potassium iodide was added to 200 ml of chloramine-T solution (0–100 mmol/l) followed by 20 ml of acetic acid. The absorbance of the reaction mixture is immediately read at 340 nm on the microplate reader against a blank containing 200 ml of phosphate buffer solution, 10 ml of potassium iodide, and 20 ml of acetic acid. The chloramine-T absorbance at 340 nm being linear within the range of 0–100 mmol/l. AOPP concentrations were expressed as µmol/l of chloramine-T equivalents [17].

Statistical analysis

Data were presented as the mean±standard error (SE) values. One-way analysis of variance or Kruskal–Wallis test and followed by a *post hoc* Tukey's Honestly significant difference or Mann–Whitney test for multiple range test among the group of treatments were performed using a statistical package program (SPSS version 16) for Windows 10. $p<0.05$ was considered as statistically significant.

RESULTS

Figure 1a depicts the effect of *S. palustris* over the actions of MDA. In case of liver damage, the lipid peroxidation levels are meant to get highly elevated, and it was clearly observed in C-group when compared to another group, and in the groups treated with the smallest concentration of *S.* palustris (T1-group), the lipid peroxidation levels were significantly reduced when compared to T1-group. In T2- and T3-groups also exhibited increased levels of reduced MDA in comparison with C- and T1-groups.

Figure 1b demonstrates that Cd exposure (C-group) is significantly increased the level of liver CC. These figure further demonstrates that *S. palustris* extract is significantly decrease the level of liver CC, in a concentration-dependent manner.

Figure 2a represented the mean values±(Mean±SE) of liver CD concentration. All groups of treatment (T1-, T2-, and T3-groups) show a lower CD concentration compare to C-group. Statistical analysis test results show treatment with *S. palustris* extract led to a significant decrease of their levels in comparison with the C-group.

Figure 2b represented the mean values±(Mean±SE) of liver AOPPs concentration. All groups of treatment (T1-, T2-, and T3-groups) show a lower AOPPs concentration compare to C-group. Statistical analysis test results show treatment with *S. palustris* extract led to a significant decrease of their levels in comparison with the C-group.

DISCUSSION

The present study reflects the anti-metalotoxic activity of *S. palustris* extract against Cd-induced oxidative stress in liver tissue of rats. In our experiments, Cd exposure had increased peroxidation of membrane lipids of liver tissue. It was indicated by the increasing of liver MDA and CD concentration. Lipid peroxidation is primarily an outcome of oxidative stress by reactive oxygen species (ROS) [18]. The mechanism of induction of lipid peroxidation by Cd is still poorly understood. In general, Cd is unable to directly cause the formation of ROS under

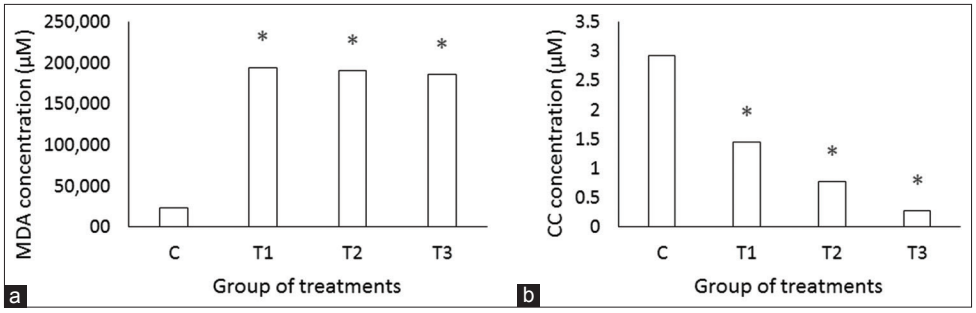

Fig. 1: Effect of *Stenochlaena palustris* on liver (a) malondyaldehyde and (b) carbonyl compound concentration over cadmium sulfate administration. Values are mean±standard error of the mean of four replicates in each groups of treatment. Statistical significance *p<0.05 in comparison with control group

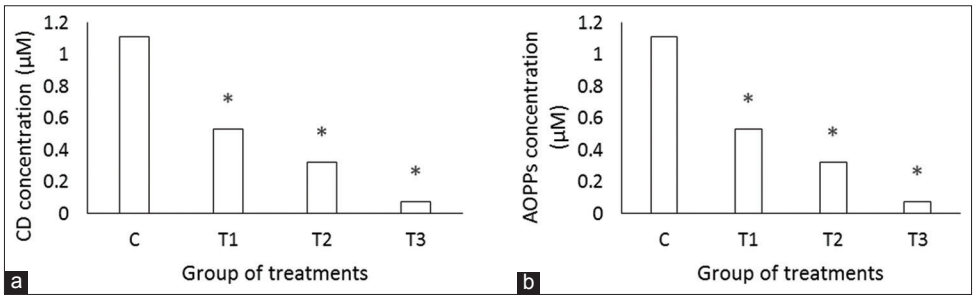

Fig. 2: Effect of *Stenochlaena palustris* on liver (a) conjugated dienes and (b) advanced oxidation protein products concentration over cadmium sulfate administration. Values are mean±standard error of the mean of four replicates in each group of treatment. Statistical significance *p<0.05 in comparison with the control group

physiological conditions [19]. Several previous authors suggested that Cd could induced the formation of ROS through severar pathway: (1) Replacing Fe in Fenton reaction; (2) affect the enzymatic antioxidant; (3) impaired glutathione which is known as ROS scavenger; and (4) inhibited complex II and III in electron transport chain reaction [20]. Those four pathways will trigger the formation of ROS which will promote a further reaction resulted in lipid peroxidation that is characterized by the formation of MDA and CD [21,22].

Besides lipid, protein also a main target for oxidative stress condition. ROS could directly oxidized protein to form several protein carbonyl compound. This in line with the result of this present study that Cd exposure which is increase the formation of ROS result in increase the level of liver CC. ROS could oxidize arginine and proline to produce glutamic semialdehyde, and lysine to produce aminoadipic semialdehyde. Both protein oxidation products by ROS known as CC which is the most widely used biomarker for oxidative damage [23].

Recently, another biomarker for protein damage in oxidative stress condition was proposed, known as AOPPs. It was first described in 1996 by Witko-sarsat *et al*. who found this new biomarker in chronic uremic patients [24]. AOPPs are described as dityrosine containing cross-linked protein products which are result from the direct reaction between ROS and protein [25]. The result of our present experiments indicated that Cd increases the formation of AOPPs. The main reason might why Cd could increase AOPPs might because the ability of Cd generate ROS as mentioned before.

The result of this present study also indicated that the *S. palustris* extract is found to be effective in decreasing the lipid and protein damage by Cd in liver tissue. The result of this study is also accordance with our previous experiments which reported decrease MG, MDA, and AOPPs by *S. palustris* extract in glycation and fructation reaction model with Cd as a catalyst [26].

The inhibition effect of *S. palustris* extract might because of the phytochemical constituents such as flavonoid, phenolic compound, and alkaloid. Those all phytochemical constituents were responsible for inhibiting the Cd toxicity. All phytochemical constituents in *S. palustris* extractable to bind particularly to metals, including Cd. Thus, the extract will slow down the rate formation of all measured parameters [13].

CONCLUSION

It can be concluded from the presented results that Cd-induced oxidative damage in liver tissues by enhanced lipid peroxidation as well as protein damage. Moreover, the extract of *S. palustris* expressed anti-metalotoxic activity against Cd. *S. palustris* extract may exert its anti-metalotoxic actions against Cd-induced oxidative stress in liver tissues possibly through its chelating metal mechanism. The results raise the possibility of *S. palustris* extract being considered as one of the promising medicinal plants of the people in the areas, where they may have chances of exposure to Cd occupationally or environmentally. Further studies are undergoing to clarify their molecular mechanisms.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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