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Research Article

The Inhibition Effect of Kelakai (*Stenochlaena palustris*) Extract on Cadmium-Induced Glycation and Fructation *In-vitro*

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ABSTRACT

The objectives of this study were to determine the inhibition effect of kelakai (*Stenochlena palustris*) extract against cadmium (Cd)-induced glycation and fructation in vitro. The inhibiton effect of kelakai extract was determined by assessing the concentration of methylglyoxal (MG), Advanced Oxidation Protein Products (AOPPs), and carbonyl compound (CC). In this present study, glycation and fructation reaction were made using bovine serum albumin (BSA) as a protein and glucose or fructose as a reducing sugar and Cd as a catalyst. Each model then divided into 5 groups consisting of: BSA + glucose or fructose as group 1 (T1); BSA + glucose or fructose + Cd as group 2 (T2); BSA + glucose or fructose + Cd + 5 mg/l of kelakai extract as group 3 (T3); BSA + glucose or fructose + Cd + 10 mg/l of kelakai extract as group 4 (T4); and BSA + glucose or fructose + Cd + 15 mg/l of kelakai extract as group 5 (T5). Results of this present study shows that Cd could increased the rate constant of MG, AOPPs, and CC formation both in glycation or fructation. Administration of plant extract could decreased the rate constant of MG, AOPPs, and CC formation induced by Cd both in glycation and fructation. The results also shows that the rate constant of the formation of MG, AOPPs, and CC in fructation were higher than glycation. In conclusions, the resuts of this present study indicated that Cd could increased the rate constant of MG, AOPPs, and CC formation while the administration of kelakai extract could decreased the rate constant. It suggest that kelakai extract have inhibitory effect against Cd-induced glycation and fructation reaction in vitro.

Key Words: Cadmium, Fructation, Glycation, *Stenochlaena palustris*

INTRODUCTION

Industrial and agricultural development have resulted in increased levels of toxic substances in the environment, including heavy metals, such as cadmium (Cd)[1-2]. Cd is widely used in several industries, such as pigment production, electroplating, alloys, batteries, and welding industry[3]. Cd also present in tobacco and as a side product in mining industry[4]. The presence of Cd caused by those industrial and agricultural processed has raised concern because this metal can bioaccmulates and biomagnificates in the upper levels of food chain[5]. As a consequence, human at the top at the food chain can aquire high levels of Cd which can caused several toxicological effects, including kidney injury[6-7]. Cd are mostly enter the human body via food ingestion and air inhalation[8]. Once enter the human body, Cd can accumulates mainly in liver and kidney[9-11]. Several studies suggested that Cd exposure induced kidney injury. The kidney injury by Cd is well characterized by various signs of tubular dysfunction, such aslow molecular weight proteinuria, glucosuria, and aminoaciduria,and pathologically by chronic interstitial nephritis[12].

The main pathomechanism for describing the toxic effect of Cd in kidney is not well understood. Our previous studies suggested that Cd involve in the glycation and fructation with methylglyoxal (MG), advanced oxidation protein products (AOPPs), and carbonyl compound (CC) as a side products. We have recently shown that Cd concentration have a significant correlation with the formation of MG in vitro[13]. Also, in another previous study indicated that 3 mg/l of cadmium sulphate ($CdSO_4$) could increase the formation of MG in vitro[9]. Our previous results also shows that Cd exposure induced the formation of AOPPs, MG, and CC both in vivo and in vitro condition[1,7,14-16]. Side product of glycation and fructation has been known can caused several toxic effects. This toxic effects can be prevented by the use of the medicinal plants. Kelakai (*Stenochlaena palustris*) is one of the plants that often grow on wetlands in South Kalimantan. Based on empirical studies, kelakai has been used daily to treat several medical conditions such as, anemia, fever and skin diseases by the local people of South Kalimantan. Our previous study indicated that kelakai extract has a potential analgesic and antipyretic effects[17]. Kelakai extracts can also decrease the number of Circulating Endhotelial Cells (CEC) and peroxidatif index in liver of mice[18-19]. This is might be caused by the bioactive substances flavonoid which can act as antioxidants[20].

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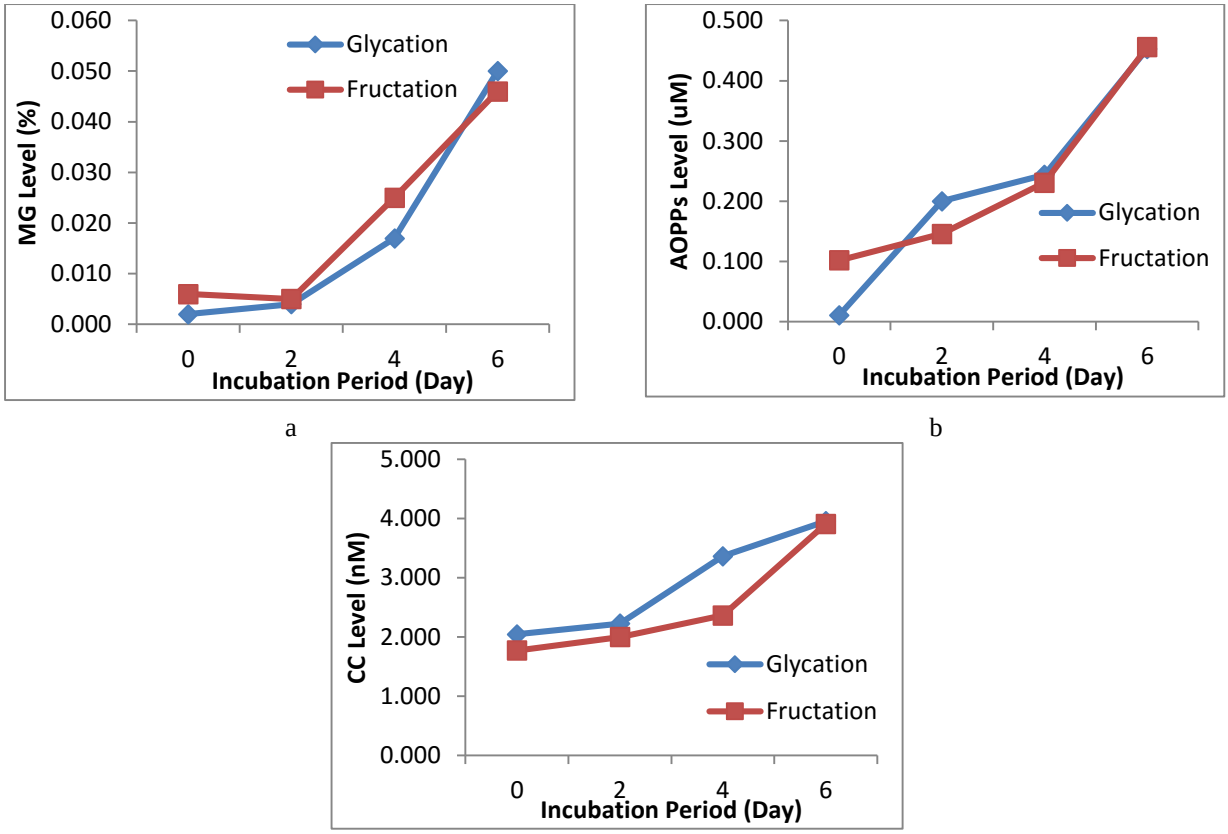

Figure 1: The formation of (a) MG; (b) AOPPs; and (c) CC in glycation and fructati

Table 1: The rate constant of MG, AOPPs, and CC formation in glycation and fructation reaction

Parameters	Glycation		Fructation	
	k (s^{-1})	r	k (s^{-1})	R
MG	0.007	0.914	0.008	0.935
AOPPs	0.069	0.975	0.076	0.903
CC	0.343	0.969	0.489	0.869

Based on several facts as mentioned above, this present study is designed to to study the inbition effect of kelakai extract on glycation and fructation reaction model with Cd was used as a catalyst in vitro.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The fresh leaves of kelakai was collected from Gambut subdistrict, South Kalimantan, Indonesia in January 2015. The plant was authenticated by Biology Department, Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University. Before use, it was ensured that the leaves were free from contamination, sand and no microbial growth. The leaves were shade dried and was made into coarse powder using a commercial blender.

Preparation of Extracts

Extraction was done by maceration methods.[21] Powdered material of kelakai leaves is taken for maceration with 150 ml of distilled water for 1 hr on rotary shaker. The extract then filtered using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath.

Experimental Protocol

In this present study, Bovine Serum Albumin (BSA) was used as a protein, and glucose and fructose were used as reducing sugar for maillard reaction model. There are two maillard reaction model of which glucose as a first model, and fructose as a second model. Each model then divided into 5 groups as follow: group 1 (T1): BSA + glucose or fructose; group 2 (T2): BSA + glucose or fructose + Cd; group 3 (T3): BSA + glucose or fructose + Cd + 5 mg/l of kelakai extract; group 4 (T4): BSA + glucose or fructose + Cd + 10 mg/l of kelakai extract; and group 5 (T5): BSA + glucose or fructose + Cd + 15 mg/l of kelakai extract. Each solution then incubated at 37°C for 1 hour. After incubation, MG, AOPPs and CC levels were estimated.

MG concentration analysis MG compounds are measured using modified Dinitro-Phenyl hydrazine (DNPH) method[13]. From each test solution, 0.5 ml solution was taken, and then each solution was divided to 2 tubes with 0.25 ml volume in each tube. The first tube was the sample (A) and the second tube was blank (B) solution. Then 1 ml DNPH were added into each A tube and 1 ml HCl 2.5 mol/l into each B tube. The tubes were incubated for 45 min in room temperature and protected from light, and then tubes were shaken with a vortex for 15 min. The next step is added 1 ml of TCA 20% into each tube (A and B),

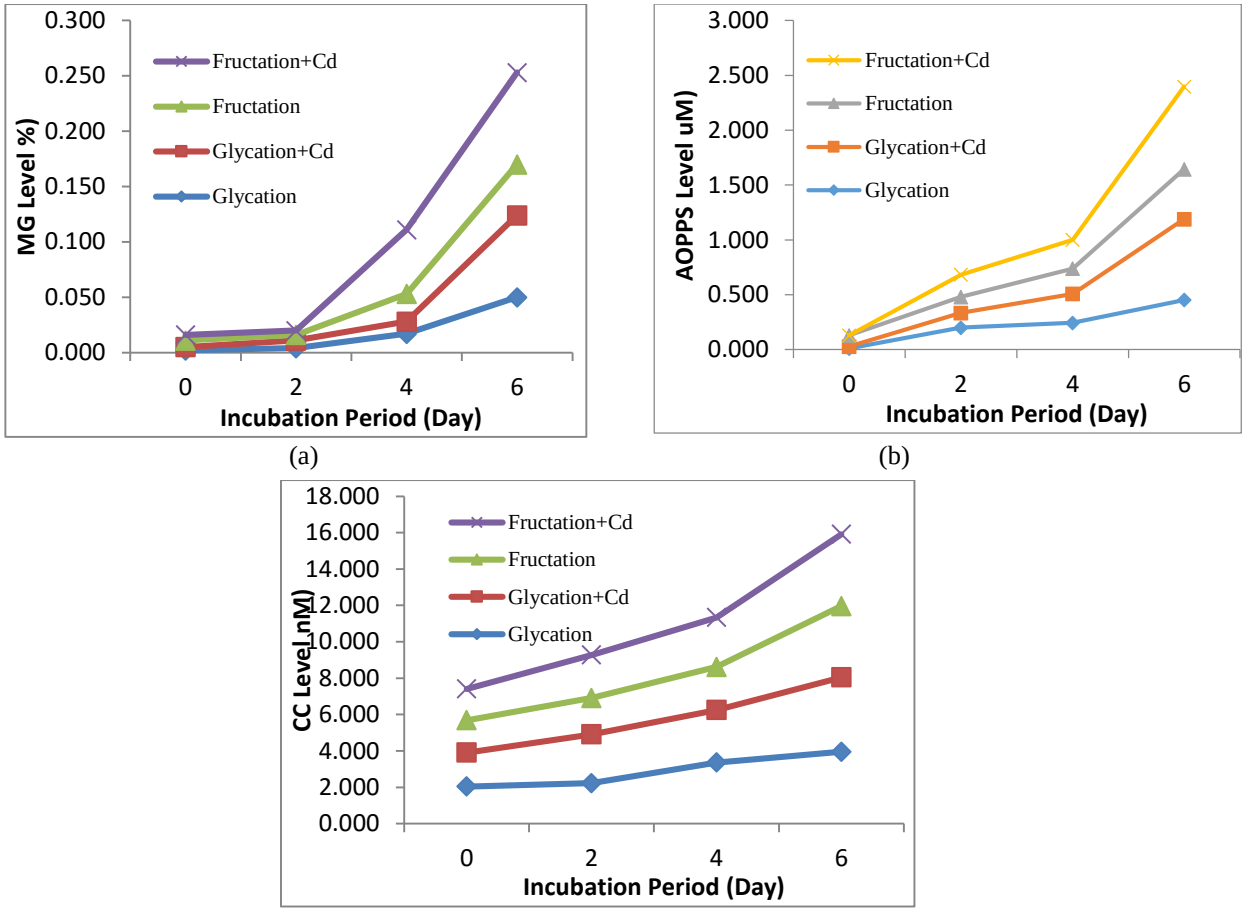

Figure 2: The formation of (a) MG; (b) AOPPs; and (c) CC by glycation and fructation reaction with the presence of Cd

Table 2: The rate constant of MG, AOPPs, and CC formation in glycation and fructation reaction with the presence of Cd

Parameters	Glycation		Fructation	
	k (s^{-1})	r	k (s^{-1})	R
MG	0.011	0.832	0.014	0.942
AOPPs	0.114	0.936	0.131	0.942
CC	0.344	0.966	0.552	0.971

then the tubes were incubated for 5 min. Tubes were centrifuged for 5 min with 1400 rpm of speed to separate the supernatant. The pellets are centrifuged and washed three times with the addition of 1 ml ethanol-ethyl acetate. The last step was added 1 ml of urea 9 mol/l and incubates the solution for 10 min in 37oC while it was shaken. The solution was centrifuged again for 5 min in 1400 rpm of speed. Then the absorbance of tube A and B were measured at λ = 390 nm (ΔA). Furthermore, a total of 25 µl of the homogenate was added to 350 µl DNPH (0.1% DNPH in 2 mol/l HCl) and then 2.125 ml distilled water was added. It is incubated for 15 min at 37°C, then 1.5 ml NaOH 10% was added. Absorbance was measured at λ = 576 nm (A1). MG level was calculated following to equation:

MG level (%) = (A1÷ΔA) × 100%

AOPPs concentration analysis

AOPP concentration analysis were calculated by spectrophotometric methods which was first performed by Witko-Sarsat *et al.*, with slight modification. 200 µl of supernatant from the kidney homogenate were diluted with phosphate buffer solution. Then, placed on 96-test wells. Add 20 ml of acetic acid in each test well. For the standard, add 10 ml of 1.16 mol potassium iodide, 200 ml of chloramine-T solution (0–100 mmol/l), and 20 ml of acetic acid. Placed the standard mixture into standard wells. Then, read the absorbance of the mixture at 340 nm. The absorbance was read against a blank solution. A blank solution is a mixture between 200 ml of phosphate buffer solution, 10 ml of potassium iodide, and 20 ml of acetic acid. AOPP concentrations were expressed as µmol/l of chloramine-T equivalents[14].

CC concentration analysis

Sample derivatization. Two 1-µl aliquots are needed for each sample to be assayed. Samples are extracted in a final concentration of 10% (w/v) TCA. The precipitates are treated with 500 µl of 0.2% DNPH or 500 µl of 2 M HCl. Samples are incubated at room temperature for 1 h with vortexing at 5-min intervals. The proteins are then

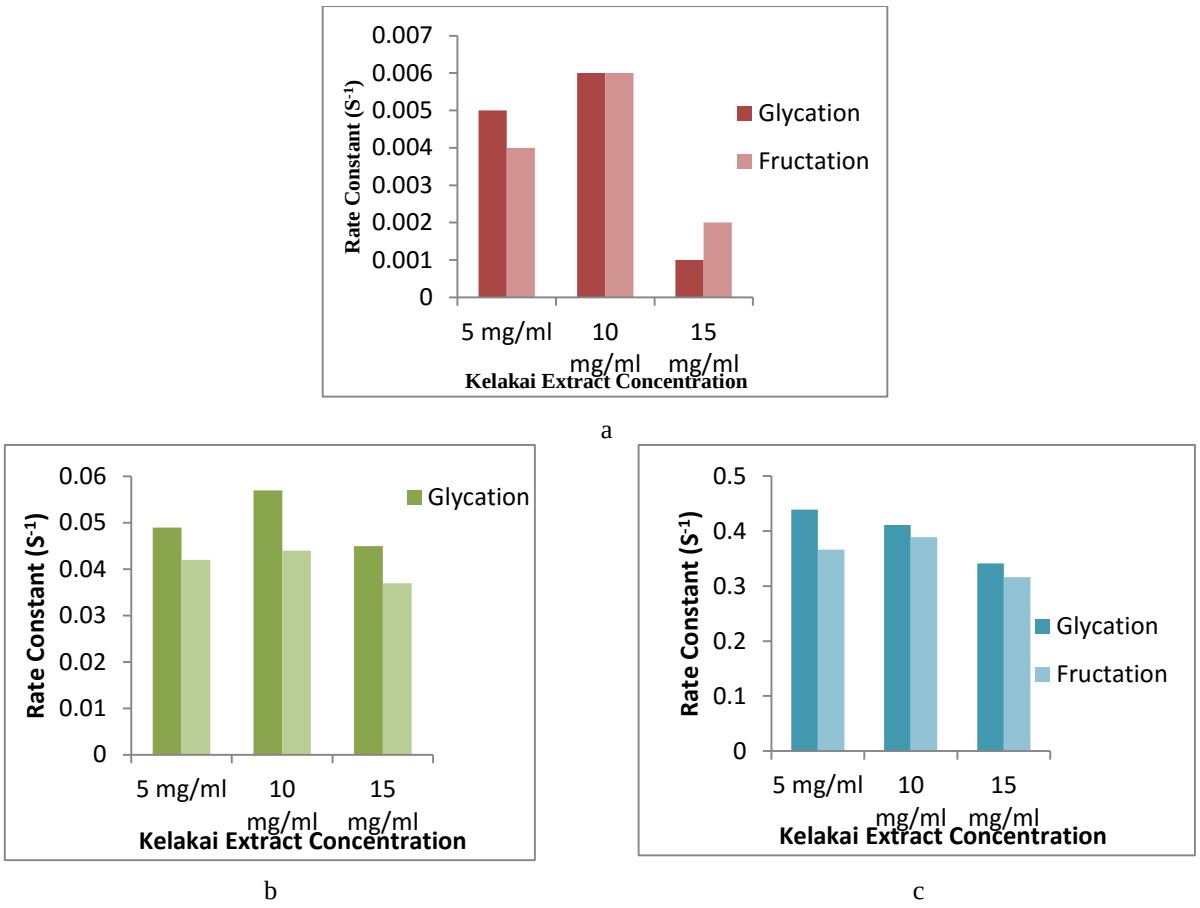

Table 2: The inhibition effect of kelakai extract on the rate constant of (a) MG; (b) AOPPs; and (c) CC formation in glycation and fructation reaction with the presence of Cd

precipitated by adding 55 µl of 100% TCA. The pellets are centrifuged and washed three times with 500 µl of the ethanol: ethyl acetate mixture. The pellet is then dissolved in 600 µl of 6 mol guanidine hydrochloride. The CC level was determined by reading the absorbance at the optimum wavelength 390 nm[22].

Data analysis

The data was analysed with the kinetic graphics between the concentration of maillard reaction product and time. Based on preliminary studies, the kinetics in this study is followed the zero-order reaction, with the equation [A] = [A]o - kt.

RESULTS AND DISCUSSION

The rate constant comparison of MG, AOPPs, and CC formation in glycation and fructation

Glycation reaction was first described by Louis Maillard in 1912[23]. The reaction occurs when the amino group of amino acid reacts with a carbonyl group of reducing sugar[24]. However, there are several reducing sugar are known to promote maillard reaction, such as glucose and fructose[25]. Glucose-induced maillard reaction known as glycation while fructose induced-maillard reaction known as fructosylation or fructation[26]. Both glycation and fructation can promote the formation of several toxic compounds. Those several compounds are MG, AOPPs, and CC. It can be seen from our results study that shown in figure 1a, b, and c. Figure 1a, b, and c shows that the rate of MG, AOPPs, and CC formation in glycation and fructation reaction are followed a zero-order reaction. It means both glycation and fructation are affect by the time changing. Also, from the figure 1a, b, and c, the rate constant of MG, AOPPs, and CC formation in glycation and fructation reaction can be known. The results are presented in table 1. Results from table 1 revealed that the rate constant of MG, AOPPs, and CC formation is faster in fructation reaction than glycation. The difference of the rate constant may be due to differences in the balance of open and cyclic form of the reducing sugar molecules. Glucose is more stable than fructose, so the glycation reaction will run slower than fructation[25]. Results from figure 1a, b, and c, and table 1 indicated that both glycation and fructation reaction produce toxic several toxic compounds. There are three toxic compounds we investigated in this present study. The three toxic compounds are MG, AOPPs, and CC. MG and CC are compounds that can be formed by glycooxidation. Glycooxidation can cause the termination of 6 carbon atoms of glucose to 3 carbon atoms which produce MG and CC[27]. In contrast to MG and CC, AOPPs can be formed by the modification of protein structure that is characterized by the formation of cross-linking of the protein that produces AOPPs[28].

The role of Cd in glycation and fructation reaction

Heavy metal such as Cd have been suggested to involve in the glycation and fructation reaction. This in line with the result of this present study. Figure 2a, b, and c shows that the formation of MG, AOPPs, and CC both in glycation and fructation seems to be faster with the presence of Cd. From figure 2a, b, and c, we can find the rate constant of MG, AOPPs, and CC formation both in glycation and fructation reaction with the presence of Cd. The rate constant were presented in table 2. The data from table 2 demonstrated that all the rate constant of the MG, AOPPs, and CC formation were larger than the data from table 1. This results indicated that the addition of Cd can accelerate the formation of MG, AOPPs, and CC both in glycation or fructation. The possible mechanism how Cd can accelerate the MG, AOPPs, and CC formation is Cd can act as the catalyst of the reaction. It is well known that the first step of glycation or fructation reaction is the condensation of the aldehyde group of glucose with an amino group of protein to form Schiff base. The next step is the reversible rearrangement of Schiff base results in Amadori intermediate. The Amadori intermediate then enolizes to form 2,3-enediol by phosphate. The 2,3-enediol can auto oxidized to form dicarbonyl product and MG with the presence of transition metal which act as an catalyst. Also, during the auto oxidation of 2,3-enediol, reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radical can be formed. The ROS production will lead to protein oxidation results in AOPPs formation[7,13,29].

The inhibition activity of kelakai extract in the formation of MG, AOPPs, and CC with the addition of Cd

The inhibition activities of kelakai extract were shows in figure 3a, b, and c. Results from figure 3a, b, and c clearly indicated that the extract of kelakai can inhibit the formation of MG, AOPPs, and CC induced by Cd both in glycation and fructation. It can be seen from the figure, the kelakai extract seems to decrease the rate constant of MG, AOPPs, and CC formation in both conditions. This inhibition effect may be due to phytochemical constituents with phenolic compound. Phenolic compounds of plants are also very important because their hydroxyl groups confer several protecting ability[30-31]. The protecting ability of phenolic compounds based on several mechanisms, as follow:

Phenolic compounds have a high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind particularly to metals. From this point of view, the phenolic can bind particularly to Cd. This binding then will reduce the formation of MG, AOPPs, and CC induced by Cd[32].

Phenolic compounds can competitively bind to proteins, so the glycation and fructation reactions will run slower[31].

Phenolic compounds can bind to glucose, so the glycation and fructation reaction can not occure or the reaction takes place very slowly[31]. In conclusion, the present study demonstrated that Cd-induced the formation of MG, AOPPs, and CC both in glycation and fructation in vitro. Also, the present study demonstrated that the administration of leaves extract of kelakai can slow down the formation of MG, AOPPs, and CC formation in glycation and fructation in vitro. This indicated that the extract may have an inhibition effect against Cd-induced the formation of MG, AOPPs, and CC both in glycation and fructation.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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REFERENCES

1. Suhartono E, Iskandar, Hamidah S, Arifin YF. Phytochemical constituents analysis and neuroprotective effect of leaves of gemor (*Nothaphoebe coriacea*) on cadmium-induced neurotoxicity in rats: an in-vitro study. International Journal of Toxicological and Pharmacological Research 2015; 7 (6): 297-302.
2. Suhartono E, Nijka JA, Anhar VY, Sari RA, Edyson, Marisa D. Anti-lipid peroxidation activities of three selected fruits juices against cadmium induced liver damage in vitro. Journal of Tropical and Life Sciences 2015; 5 (2): 75-79.
3. Lakhsmi GD, Kumar PR, Bharavi K, Annapurna P, Rajendar B, Patel PT, Kumar CSVS, Rao GS. Protective effect of *Tribulus terrestris* linn on liver and kidney in cadmium intoxicated rats. Indian Journal of Experimental Biology 2012; 50: 141-146.
4. Martine S, Griswold W. Human health effects of heavy metals. Center for Hazardous and Substance Research 2009; 15: 1-6.
5. David GI, Matache ML, Tudorache A, Chisamera G, Rozylowicz L, Radu GL. Food chain biomagnification of heavy metals in samples from the lower prut flood plain natural park. Environmental Engineering and Management Journal 2012; 11 (1): 69-73.
6. Heydarnejad MS, Hemamai MK, Nematollahi A. Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Irish Veterianry Journal 2013; 66 (11): 1-7.
7. Suhartono E, Triawanti, Leksono AS, Djati MS. Oxidative stress and kidney glycation in rats exposed cadmium. International Journal of Chemical Engineering and Applications 2014; 5 (6): 497–501.
8. Wibowo A, Rahaju FA, Iskandar, and Suhartono E. The role of urinary cadmium on liver function and erythrocytes cell count in pregnancy. International Journal of Bioscience Biochemistry and Bioinformatics 2014; 4(4): 224-228.
9. Suhartono E, Santosa PB, Iskandar. Ameliorative effects of different parts of gemor (*Nothaphoebe coriacea*) on cadmium induced glucose metabolism alteration *in vitro*. International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7 (11): 1-4.
10. Suhartono E, Triawanti, Yunanto A, Firdaus RT, Iskandar. Chronic Cadmium Hepatooxidative in Rats:

Treatment with Haruan Fish (*Channa striata*) Extract. APCBEE Procedia 2013; 5: 441-445.
11. Salinska A, Wlostowski T, Olenska E. Differential susceptibility to cadmium induced liver and kidney injury in wild and laboratory-bred bank voles myodes glareolus. Archives of Environmental Contamination and Toxicology 2013; 65 (2): 324-331.
12. Aktoz T, Kanter M, Uz YH, Aktas C, Erboga M, Atakan IH. Protective effect of quercetin against renal toxicity induced by cadmium in rats. Balkan Medical Journal 2012; 29: 56-61.
13. Suhartono E, Triawanti, Leksono AS, Djati MS. The role of cadmium in protein glycation by glucose: formation of methylyglyoxal and hydrogen peroxide in vitro. Journal of Medical and Bioengineering 2014; 3 (1): 59-62.
14. Husna AH, Ramadhani EA, Eva DT, Yulita AF, Suhartono E. The role formation of methylglyoxal, carbonyl compound, hydrogen peroxide and advance oxidation protein product induced cadmium in ovarian rat. International Journal of Chemical Engineeering and Applications 2014: 5 (4): 319-323.
15. Tribowo JA, Arizal MH, Nashrullah M, Aditama AR, Utama DG. Oxidative stress of cadmium-induced ovarian rat toxicity. International Journal of Chemical Engineering and Applications 2014; 5 (3): 254-258.
16. Aflanie I, Muhyi R, Suhartono E. Effect of heavy metal on malondialdehyde and advanced oxidation protein products concentration: A focus on arsenic, cadmium, and mercury. Journal of Medical and Bioengineering 2015; 4 (4): 332-337.
17. Suhartono E, Fachir H, Setiawan B. Capita selecta of biochemistry: Oxidative stress and basic of disease. Pustaka Banua, Banjarmasin, 2007.
18. Suhartono E, Bakhriansyah M, Handayani R. Effect of *Stenochlaena palustris* extract on circulating endothelial cells *Marmota caligata* induced fever. Indonesian Pharmacy Magazine 2010; 21(3): 166 – 170.
19. Suhartono E, Rohman T, Setiawan B. The formation model of Advanced Glycation End Products (AGEs) and protein modification due to glycosylation reaction. Indonesian Medicine Magazine 2005; 55(11): 681-5.
20. Suhartono E. Potention of aquaeus extract of kelakai as antiinflammation by oxidative mechanism. Prsented in Congress International Korean Medicine, Sangji University Korea, 2008.
21. Suhartono E, Viani E, Rahmadhan MA, Gultom IS, Rakhman MF, Indrawardhana D. Screening of medicinal plant for total flavonoid and antioxidant activity in South Kalimantan of Indonesian. International Journal of Chemical Engineering and Applications 2012; 3 (4): 297-299.
22. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Archives of Disease in Chilhood, Fetal, and Neonatal 2006; 91: F208-F212.
23. Nagai R, Jinno M, Ichihashi M, Koyama H, Yamamoto Y, Yonei Y. Advanced glycation end products and their receptors as risk factors for aging. Anti-Aging Medicine 2012; 9 (4): 108-113.
24. Tamanna N, Mahmood N. Food processing and maillard reaction products: Effect of human health and nutrition. International Journal of Food Science 2015; 2015: 1-6.
25. Pena JE, Gil HD. Effect of buffer phosphate in the reaction of hemoglobin with fructose. Avances en Qumica 2014; 9 (2): 49-53.
26. Bose T, Chakraborti AS. Fructose-induced structural and functional modifications of hemoglobin: Implication for oxidative stress in diabetes mellitus. Biochimica et Biophysica Acta 2008.
27. Grillo MA, Colombatto S. Advanced glycation end-products (AGEs): involvement in aging and in neurodegenerative diseases. Amino Acids 2008; 35: 29-36.
28. Sebekova K, Klenoviscova K, Ferenczov J, et al. Advanced oxidation protein products and advanced glycation end products in children and adolescents wtih chronic renal insufficiency. Journal of Renal Nutrition 2012; 22: 143-148.
29. Voziyan PA, Khalifah RG, Thibaudeau C, Yildiz A. Modification of proteins in vitro by physiological levels of glucose. The Journal of Biological Chemistry 2003; 278 (47): 46616–46624.
30. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative Medicine 2012; 12 (221): 1-12.
31. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Study 2006; 15 (4): 523-530.
32. Kumar D. Antioxidant action of phenols under heavy metal stress. The Journal of Phytopharmacology 2012; 1 (3): 4-6