

# 9. The Inhibition Effect of Kelakai (*Stenochlaena palustris*) extract on Cadmium-Induced Glycation and Fructation In-vitro

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## 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 (*Stenochlaena palustris*) extract against cadmium (Cd)-induced glycation and fructation in vitro. The inhibition 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 increase the rate constant of MG, AOPPs, and CC formation both in glycation or fructation. Administration of plant extract could decrease the rate constant of MG, AOPPs, and CC formation induced by Cd both in glycation and fructation. The results also show that the rate constant of the formation of MG, AOPPs, and CC in fructation were higher than glycation. In conclusion, the results of this present study indicated that Cd could increase the rate constant of MG, AOPPs, and CC formation while the administration of kelakai extract could decrease the rate constant. It suggests that kelakai extract has an 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)<sup>1-2</sup>. Cd is widely used in several industries, such as pigment production, electroplating, alloys, batteries, and welding industry<sup>3</sup>. Cd also present in tobacco and as a side product in mining industry<sup>4</sup>. The presence of Cd caused by those industrial and agricultural processes has raised concern because this metal can bioaccumulate and biomagnify in the upper levels of food chain<sup>5</sup>. As a consequence, human at the top of the food chain can acquire high levels of Cd which can cause several toxicological effects, including kidney injury<sup>6-7</sup>. Cd mostly enters the human body via food ingestion and air inhalation<sup>8</sup>. Once in the human body, Cd accumulates mainly in the liver and kidney<sup>9-11</sup>. Several studies suggested that Cd exposure induced kidney injury. The kidney injury by Cd is well characterized by various signs of tubular dysfunction, such as low molecular weight proteinuria, glucosuria, and aminoaciduria, and pathologically by chronic interstitial nephritis<sup>12</sup>.

The main pathomechanism for describing the toxic effect of Cd in kidney is not well understood. Our previous

studies suggested that Cd involves in the glycation and fructation with methylglyoxal (MG), advanced oxidation protein products (AOPPs), and carbonyl compound (CC) as side products. We have recently shown that Cd concentration has a significant correlation with the formation of MG in vitro<sup>13</sup>. Also, in another previous study indicated that 3 mg/l of cadmium sulphate (CdSO<sub>4</sub>) could increase the formation of MG in vitro<sup>9</sup>. Our previous results also show that Cd exposure induced the formation of AOPPs, MG, and CC both in vivo and in vitro conditions<sup>1,7,14-16</sup>. Side product of glycation and fructation has been known to cause several toxic effects. These toxic effects can be prevented by the use of 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<sup>17</sup>. Kelakai extracts can also decrease the number of Circulating Endothelial Cells (CEC) and peroxidative index in liver of mice<sup>18-19</sup>. This might be caused by the bioactive substances flavonoid which can act as antioxidants<sup>20</sup>.

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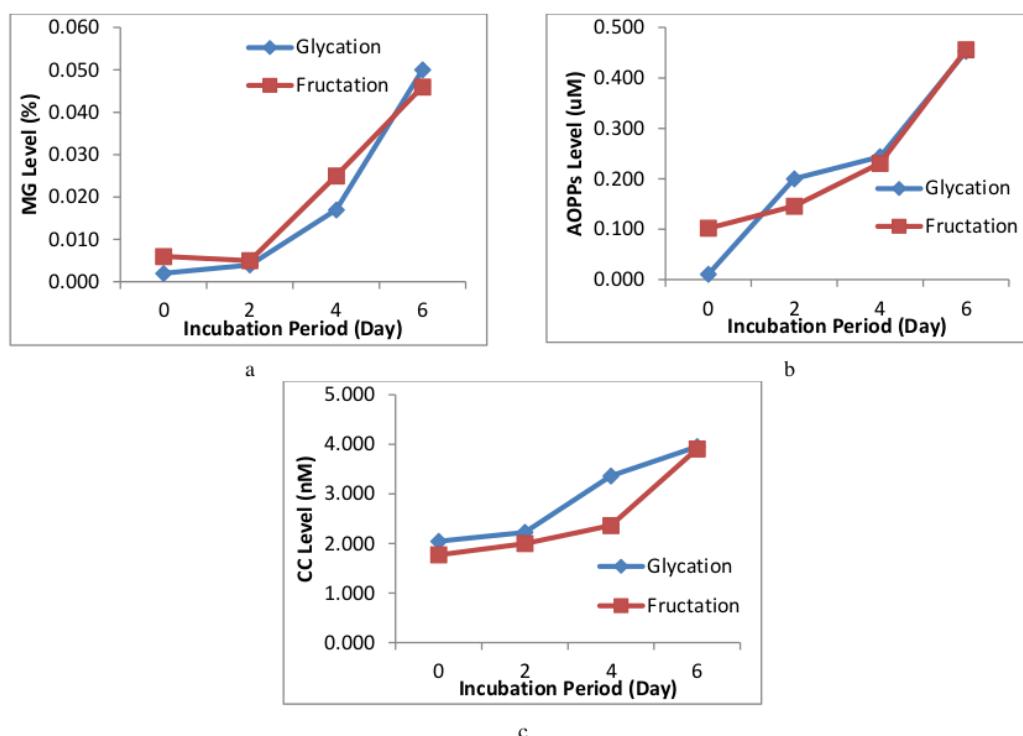


Figure 1: The formation of (a) MG; (b) AOPPs; and (c) CC in glycation and fructati

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

Parameters	Glycation		Fructation	
	k (s <sup>-1</sup> )	r	k (s <sup>-1</sup> )	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 study the inhibition 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.<sup>21</sup> 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<sup>13</sup>. 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),

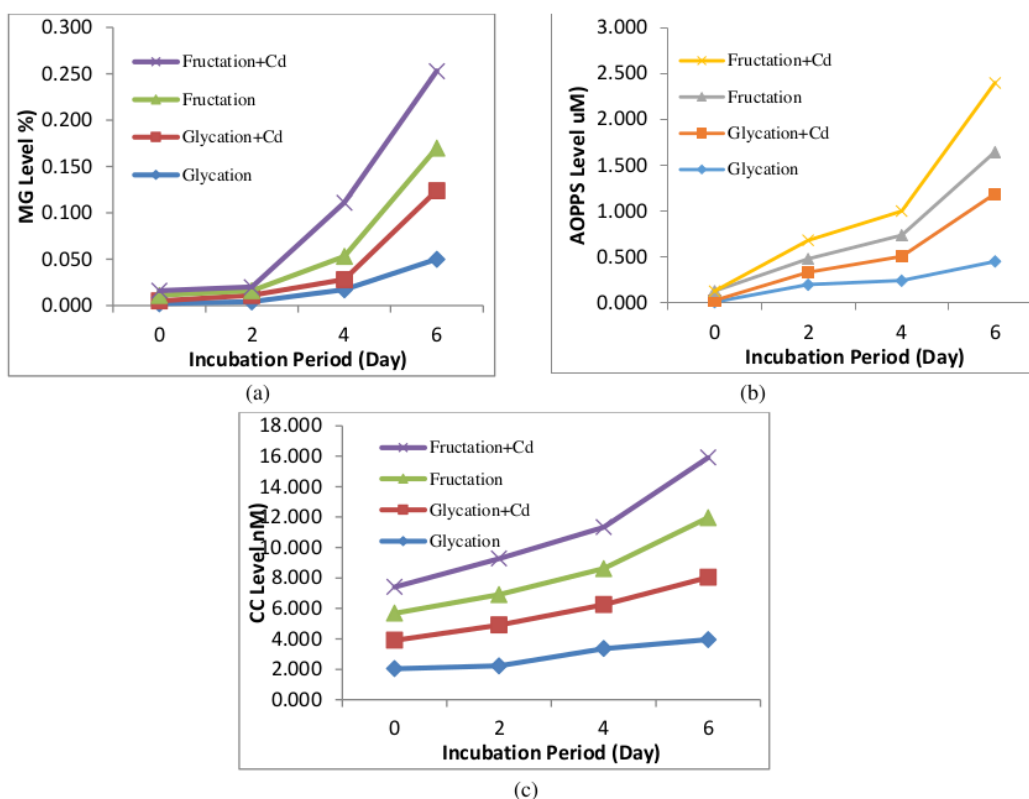


Figure 2: The formation of (a) MG; (b) AOPPs; and (c) CC by glycation and fructation reaction with the presence of Cd

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 <sup>-1</sup> )	r	k (s <sup>-1</sup> )	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 37°C 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  $\lambda = 390 \text{ nm}$  ( $\Delta A$ ). Furthermore, a total of 25  $\mu\text{l}$  of the homogenate was added to 350  $\mu\text{l}$  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  $\lambda = 576 \text{ nm}$  (A1). MG level was calculated following to equation:

$$\text{MG level (\%)} = (A1 \div \Delta A) \times 100\%$$

*AOPPs concentration analysis*

AOPP concentration analysis were calculated by spectrophotometric methods which was first performed by Witko-Sarsat *et al.*, with slight modification. 200  $\mu\text{l}$  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  $\mu\text{mol/l}$  of chloramine-T equivalents<sup>14</sup>.

*CC concentration analysis*

Sample derivatization. Two 1- $\mu\text{l}$  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  $\mu\text{l}$  of 0.2% DNPH or 500  $\mu\text{l}$  of 2 M HCl. Samples are incubated at room temperature for 1 h with vortexing at 5-min intervals. The proteins are then

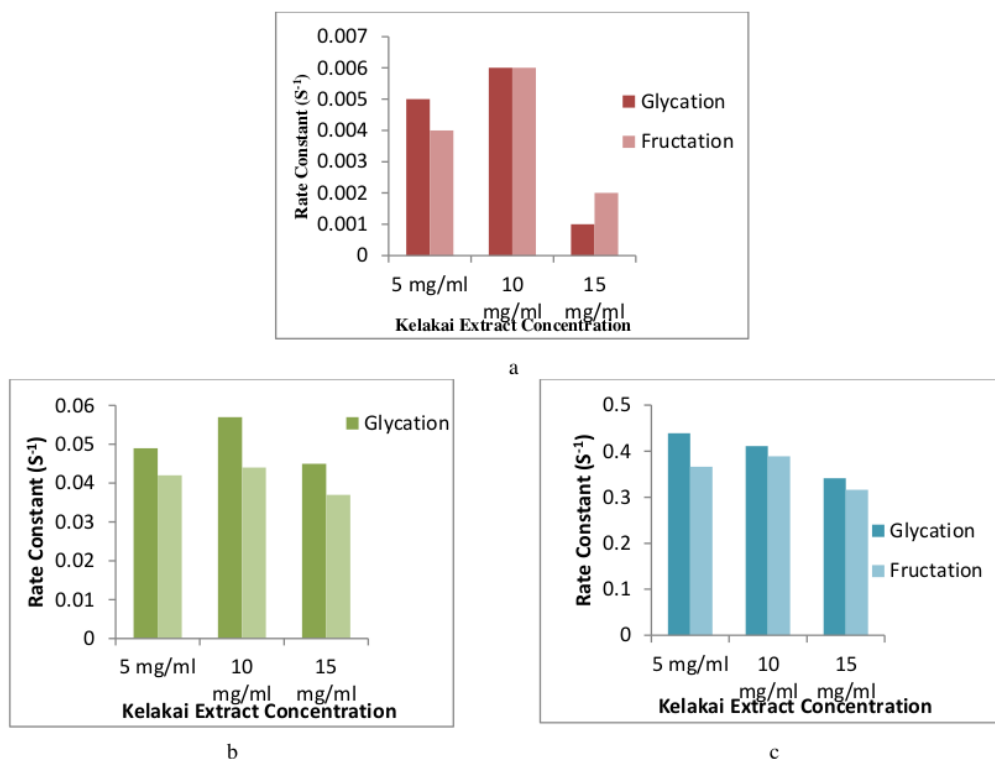


Table 2: The inhibition effect of kelakai extract on the rate constant of (a) MG; (b) AOPPs; and (c) CC formation in glycation and fructation reaction with the presence of Cd

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<sup>22</sup>.

**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  $[A] = [A]_0 - kt$ .

**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<sup>23</sup>. The reaction occurs when the amino group of amino acid reacts with a carbonyl group of reducing sugar<sup>24</sup>. However, there are several reducing sugar are known to promote maillard reaction, such as glucose and fructose<sup>25</sup>. Glucose-induced maillard reaction known as glycation while fructose induced-maillard reaction known as fructosylation or fructation<sup>26</sup>. 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<sup>25</sup>. 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<sup>27</sup>. 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<sup>28</sup>.

*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<sup>7,13,29</sup>.

*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<sup>30-31</sup>. 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<sup>32</sup>.

Phenolic compounds can competitively bind to proteins, so the glycation and fructation reactions will run slower<sup>31</sup>.

Phenolic compounds can bind to glucose, so the glycation and fructation reaction can not occur or the reaction takes place very slowly<sup>31</sup>. 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.

#### ACKNOWLEDGEMENT

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