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Anti-Lipid Peroxidation Activities of Three Selected Fruits Juices Against Cadmium Induced Liver Damage In Vitro

Eko Suhartono^{1*}, June Astri Nijka², Vini Yulia Anhar², Rizki Amalia Sari², Edyson¹, Dona Marisa³

¹Medical Chemistry and Biochemistry Department, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, Indonesia

²Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, Indonesia

³Physiology Department, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, Indonesia

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ABSTRACT

Cadmium (Cd) is one of the most toxic heavy metal. Numerous tissues both in vitro and in vivo suggest that Cd induced lipid peroxidation. The presence of antioxidant can inhibit this process. This present study was designed to determine the effects of mango, papaya, and banana juices on malondialdehyde levels (MDA) during Cd exposure in vitro. The result of this study showed that there were a significant difference MDA level between three selected fruits juices. The result of this study also showed the significant differences MDA level between the concentration of each fruit juices. The highest correlation was found between banana juices and MDA levels with IC₅₀ 1.12 and followed by papaya and mango juices with IC₅₀ 1.37 and 3.87 respectively. The findings of this study suggest that the three selected fruits juices may exert its protective action against Cd-induced lipid peroxidation in liver homogenate in vitro possibly through its antioxidant mechanism.

Keywords: antioxidant activity, cadmium, fruits juices, lipid peroxidation

INTRODUCTION

Cadmium (Cd) is heavy metal that is produced from many industries, such as a battery, electroplating, pigment, plastic, and fertilizer industries [1-2]. Cd contamination and toxicity have become a matter of concern in recent years. Cd has been recognized as one of the most toxic environmental pollutants [3,4]. Acute or chronic exposure to Cd can damage various organs including lung, liver, kidney, bone, testis and placentas [5,6].

It has been well known that the mechanisms responsible for Cd immunotoxicity involved oxidative stress [7]. Oxidative stress is an imbalance between the oxidant and antioxidant. Oxidative stress can cause damage to macromolecules such as lipids and called lipid peroxidation [8-9].

Lipid peroxidation is characterized by some complex compounds formation including reactive carbonyl compounds, such as MDA [10]. MDA has been found to play a major role in the toxicity of Cd [11]. Previous

studies showed that there were significant differences in kidney MDA levels between control and Cd treatment groups [12]. Other previous studies also revealed that Cd exposure increase the level of MDA. MDA levels were increased with the increasing of time exposure and Cd concentration [13].

South Kalimantan is one of the areas in Indonesia that has a variety of fruits [14-15]. It is well known that the fruits are good for health. It is because the fruits contained compounds that act as an antioxidants [16-18]. Antioxidants can terminate or retard the oxidation process, as well as lipid peroxidation [19-20].

However, effects of these three selected fruits juices (bananas, mango, and papaya), as an inhibitor of lipid peroxidation induced by Cd have not yet been studied. Therefore, the aim of this study was to investigate the anti-lipid peroxidation of *Musa acuminata*, *Carica papaya* and *Mangifera indica* juices against cadmium-induced lipid peroxidation in vitro.

MATERIALS AND METHODS

Chemical and Materials

Phosphate buffer saline, CdSO₄, SDS (sodium dodecyl sulfate), acetic acid, TBA (thiobarbituric acid) solution, N-butanol, pyridine were from Sigma.

*Corresponding author:

Eko Suhartono

Medical Chemistry and Biochemistry,
Faculty of Medicine, University of Lambung Mangkurat,
Banjarasin-Indonesia

E-mail: ekoantioxidant@gmail.com

Fruit Materials

Three types of tropical fruits and used as reference were studied. They were Maui bananas (*Musa acuminata*), Papaya (*Carica papaya*), and Mangga (*Mangifera indica*). The fresh fruits species were procured from the local market for the present study.

Juice Preparation

The fruits were separated carefully from the skin and seeds. Then the juice was made with the help of juicer. The fresh juice was used for all investigations. The juice was extracted from the homogenized flesh of the fruits of each cultivar. The juice was separated from the pulp by pressing it several times and centrifuging it at $1500 \times g$ for 20 min. The supernatant juice was filtered and kept at $4^{\circ}C$ until use.

Experimental Section

Male albino rats (*Rattus novergicus*) weighing 200-259 g, were used in this study. All rats were caged separately for acclimation period for one week. During the acclimation period, the rats were drink and fed with the same waters and food. PDAM water as drinking water and C-05 pellets as foods. The rats were fasted for 1-2 hours to ensure that the rat stomach empty before treatment. Ethical clearance was obtained from the faculty of medicine, Lambung Mangkurat University, South Kalimantan, Indonesia.

Then the animals were sacrificed by a surgical procedure with ether as an anesthesia. The abdomen was opened; the liver was removed and then homogenized in phosphate buffer saline (pH 7.0). Then 1 ml liver homogenate (10% w/v) was added to test juices of different concentrations.

Anti-Lipid Peroxidation Assay

The lipid peroxidation was initiated by adding the $CdSO_4$ solution to the liver homogenate, and then the fruits juices in different concentrations were added. Then the MDA levels were measured by the modified method of Ohkawa et al. and Masao et al. [21]. The percentage of MDA formation inhibition was calculated using the formula:

$$\text{Percentage Inhibition} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}}$$

Abs control: Blank solution absorbance that contained liver homogenate and $CdSO_4$ solution.

Abs sample: Solution absorbance that contained liver homogenate and $CdSO_4$ solution with the fruits juices.

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Statistical Analysis

The results are expressed as the mean \pm SD for three triplicates. Linear regression analysis as used to calculate IC50 value.

Kruskal-Wallis test evaluated the difference of MDA formation inhibition between three selected fruits and between concentration. All data were entered into and processed by SPSS 17,0 for Windows.

RESULTS AND DISCUSSION

Several concentrations of the three selected juices fruits were tested for their anti-lipid peroxidation activity induced by $CdSO_4$ in the liver homogenate. The result showed in figure 1 A, B, and C.

Figure 1 A, B, and C shows the inhibition effect of three selected fruits on MDA formation. Kruskal-Wallis test result that there are significance differences in MDA formation among the three selected fruits and the three concentration ($p < 0,05$). It means the three selected, and the concentration of fruits juices affect the MDA formation during Cd exposure.

The Cd is a toxic metal that may induce oxidative damage by disturbing the balance of prooxidant-antioxidant in the tissues [1]. One of oxidative damage indicator by Cd is the accumulation of MDA, which is a breakdown product of lipid peroxidation [22].

The Cd is unable to generate free radicals itself, but somehow Cd could generate the free radicals indirectly. The Cd could generate free radicals via the Fenton chemistry. Cadmium could replace other metals such as, iron and copper, which in turn to release and increase the concentration of unbound iron or copper ions. These free ions participate in causing oxidative stress via the Fenton reactions. Recently, Watjen and Beyersmann showed evidence in support of the proposed mechanism [23].

Also, cadmium was shown to inhibit complex III of the mitochondrial respiratory chain. The inhibition of complex III would lead to the accumulation of semi ubiquinones and the formation of radical superoxide [24].

Another mechanism how is Cd could generate the formation of free radicals were disturbing the cellular antioxidant and induced the formation of pro-inflammatory cytokines [24-25].

These excessive of ROS production by a several mechanism, as mentioned above can promote the lipid peroxidation process. The first step of lipid peroxidation process called initiation. First, a hydrogen atom from a methylene group in the lipid was abstracted. Then, the presence of a double bond adjacent from the

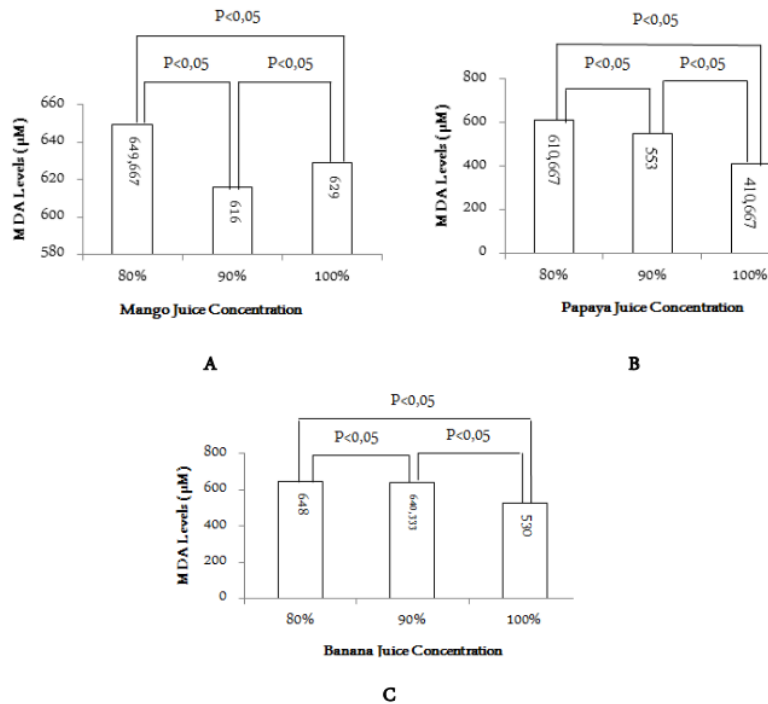


Figure 1. Inhibition effects of (A)Mango; (B)Papaya; (C)Banana juices on MDA formation. Value are expressed as mean±SD. Statistical significance of difference among groups of treatment at $p < 0,05$ using Kruskal-Wallis test.

methylene group weakens the bond between carbon and hydrogen. Further, the hydrogen removed from the fatty acid molecule [25-26].

The initiation process resulted in a fatty acid having one unpaired electron. When oxygen is present in the tissues, the fatty acid from the initiation process can react with it and lead to the formation of lipo-peroxyl radicals ($ROO\bullet$). $ROO\bullet$ can be rearranged via a cyclization reaction to endoperoxides with the final product of peroxidation process being MDA [27].

The presence of fruits juices can inhibit the MDA formation chain reaction. To analyze the correlation between the presence of fruits juices and MDA levels, linear correlation regression analysis was used. The highest correlation was given by banana (0,97) juices, followed by papaya (0,89) and mango (0,67) juices (Table 1).

To compare which of these three selected juices are most effective to inhibit the MDA formation during the exposure of Cd, IC_{50} between these selected fruits juices was evaluated. The IC_{50} value for

each fruit juices, defined as the concentration of extract causing 50 percent inhibition of MDA formation. The lower IC_{50} value indicates higher anti-lipid peroxidation inhibition activity. Table 1 showed that the most effective is banana juices, followed by papaya and mango.

According to the results, all three selected fruits juices have anti-lipid peroxidation activity. The anti-lipid peroxidation activities of three selected fruits juices have been attributed to the presence of antioxidants, such as flavonoids, and vitamins like ascorbic acid (vitamin C), tocopherol (vitamin E) and β -carotene [28].

Previous studies showed that these three selected fruits contain phenolic compounds [29]. Anti-lipid peroxidation action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind iron and copper partially. Also, the phenolic compounds may inactivate iron ions by chelating and additionally suppressing the superoxide-driven Fenton reaction [30]. Another mechanism how phenolic compounds could

Table 1. Percentage of MDA formation inhibition activity of three selected fruits

Concentration	MDA Formation Inhibition (%)		
	Mango	Banana	Papaya
80%	0,00±0,00	0,00±0,00	0,00±0,00
90%	5,18±0,21	1,18±0,07	9,44±0,29
100%	3,18±0,14	18,21±0,03	0,00±0,00
R ²	0,37	0,94	0,80
r	0,61	0,97	0,89
IC50 (%)	3,87	1,12	1,37

reduced the lipid peroxidation becomes hydrogen donors and singlet oxygen quenchers. From this mechanism, phenolic can play a significant role in absorbing and neutralizing free radicals [31].

Another phytochemical constituents in these, three selected fruits juices are ascorbic acid. According to Rekha et al. [33], ascorbic acid acts as a particular antioxidant by reducing ferric ions, this could reduced lipid peroxidation. Also, ascorbic acid could transfer the hydrogen atoms to oxygen. From this mechanism, ascorbic acid make the levels of oxygen are reduced, so the reactive oxygen chain reaction also reduced [32,33].

Tropical fruits such as these three selected fruits in the experimental model also appear to be good sources of carotenoid [34]. Carotenoids principally scavenge two types of ROS: singlet molecular oxygen and peroxy radicals. They deactivate effectively the electronically excited sensitizer molecules, which are involved in the generation of radicals and singlet oxygen [35]. Carotenoid also can bind with lipid peroxy radical resulting in the formation of a carbon-centered radical, it would interfere the propagating step in lipid peroxidation [36].

CONCLUSIONS

The present in vitro studies found that banana juices demonstrated the highest anti-lipid peroxidation activity during Cd exposure and followed by papaya and mango juices. According to biological in vitro assays, certain unknown compounds in these three selected juices may play significant roles as an inhibitor of lipid peroxidation. Overall, the three selected juices could be regarded as a potential anti-lipid peroxidation agent, especially in Cd intoxication. Further investigation of the health benefits using in vivo and human study are required.

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