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The Effect of ion Cd(II) in the Kidney of Experimental Rats and Utilization of Cassava Leaves (*Manihot utilisima*) as Antidote.

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ABSTRACT

The present study investigated the protective effect of *Manihot utilissima* leaves against cadmium induced oxidative stress in kidney of experimental rat. The administration intraperitoneally of cadmium lead to elevated levels of cadmium content in almost organs in mice except in brain. Administration of cadmium also increase some biochemical parameters and enzymes in serum include malondialdehyde, urea, creatinine, SGOT and SGPT. Pre oral treatment of *Manihot utilissima* leaves antidote could reduce the level of cadmium in all affected organs and biochemical parameters significantly. pre treatment with antidote could reduce the level of MDA,creatinine, urea, SGPT and SGOT to 52,8%, 30,3%, 15,2%, 64,3%, and 78,8% respectively compared with the cadmium induced rats group.From these result it can be concluded that antidote of *Manihot utilissima* leaves give preventive effect against cadmium toxicity.

Keywords: *Manihot utilissima*, antidote, cadmium, oxidative stress

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INTRODUCTION

Heavy metal toxicity has attracted the interest of many researchers in recent years due to serious impact resulted on human health. The research conducted focused on finding more effective way in the management of heavy metals toxicity, mainly the use of antioxidant vitamins and nature materials that have minimal side effects [1]. Cadmium is a heavy metal widely dispersed in the environment. Cadmium is naturally found in environment especially in ocean and fewer in surface water and ground water [2]. Cadmium accumulates primarily in the kidney and liver, these two organs are critical targets for acute cadmium toxicity. Acute cadmium poisioning causes several symptoms and damages include nausea, vomiting, testicular damage, renal damage and hepatic necrosis [3]. The most direct way to reduce acute cadmium toxicity is using chelation therapy. However, because there are unavoidable side effects that can not be avoided for renal toxicity, chelator like dithiocarbamates and monoisoamyl meso-2,3-dimercaptosuccinate (MiADMS) only effective if administered within short time after cadmium exposure and cannot be used for oxidative stress in the liver and brain [4]. *Manihot utilissima* is a well known local vegetable in Indonesia which have been found to have antioxidant, anti tumour and antimicrobial activity [5,6]. *Manihot utilissima* also known as cassava or tapioca shoots, which originates from South America. This plant can also be found in most of tropical countries. The previous study describes that *Manihot utilissima* leaves have ability in removal of Cd (II) in aqueous solutions with some functional groups involved in adsorption, with optimum conditions at pH 6, contact time 90 minutes, initial concentration 500 mg/L and biosorbent dosage 0,1 g with sorption capacity of *Manihot utilissima* leaves was 22,24 mg/g [7]. Study concerning the effectiveness of *Manihot utilissima* leaves in alleviating oxidative stress and damage of kidney caused by cadmium has not been done. Therefore it was considered of interest to investigate the effects of pre treatment of *Manihot utilissima* antidote in combating Cd toxicity in rat kidney and by examining the level of some biochemical serum parameters.

MATERIALS AND METHODS

Experimental Plant Material

The experimental plant *Manihot utilissima* leaves was collected from local market in Padang, West Sumatera

Preparation of antidote of Manihot utilissima leaves

The leaves of *Manihot utilissima* were washed with running water and wind dried for a week in room temperature. After dried, the leaves were mashed by using a blender to be in powder form. Approximately 2 g of the powder were add with distilled water then transferred into a beaker glass and add more distilled water until total volume 120 mL were reached. The solution then heated to boil, filtered and stored in a closed bottles.

Chemicals

All chemicals including cadmium acetate was obtained from Merck (Darmstadt, Germany). All chemicals were analytical grade and were purchased from standard commercial suppliers.

Test Animals

Adult male white rats weighing 140-160 g were purchased from Andalas University. They were housed in proper cage and maintained on *ad libitum* diet and water. All treatments and procedures were in accordance with the protocol of animal ethic committee of Andalas University.

Experimental design

Adult mices were randomly divided into 3 groups, each group contained 3 animals. The first group served as control and was given only distilled water. The second group was given 1 mL x bw Cd (II) 1000 mg/L intraperitoneally. The third group was treated 5 with 5 mL x bw/200 g bw of Manihot utilissima leaves antidote for a week, followed by administration of 1 mL x bw/200 g bw Cd (II) 1000 mg/L intraperitoneally. After 5 hours, the animals were sacrificed by choloform anesthesia and the blood was collected for biochemical serum

analysis and oxidative stress. The organs include brains, lungs, liver, kidney, testis and spleen from each sacrificed rats were dissected out to measure the content of cadmium in each organ. the kidney then excised and fixed in Bouin's solution for histological studies.

Biochemical serum analysis and oxidative stress

Oxidative stress product was estimated by MDA (malondialdehyde) content in serum. Liver function parameters include SGOT and SGPT and kidney function parameters include urea and creatinine were measure using two reagents method (Substrate Start)

Histopathology analysis

The kidney were washed with sterile saline and fixed in Bouin's solution. The kidney were excised by using rotary microtome and stained with hematoxylin and eosin dye for histopathology analysis.

Statistical analysis

The data was statistically analyzed using Statistical Package for Socal Science Program ver. 16 (SPSS ver. 16). The analysis eas conducted using analysis of variance (ANOVA) followed by Tukey Test and Bonferroni test

RESULT AND DISCUSSION

Cadmium content in liver and kidney

Cadmium levels in liver and kidney and the effect of pre treatment with Manihot utilissima leaves antidote were shown in Table. 1. Table 1 indicates that there are decreased level of cadmium in liver and kidney significantly in group 3 compared to group 2. Lakshmi *et al* [8] in their study reported that ethanol extract of *Tribulus terrestris* administered to rats exposed to cadmium could reduce the accumulatin of cadmium in liver and kidnet by 39% and 60% respectively. Embugishiki *et al* [9] reported that mice pre treatment wih carrot juice could reduce the accumulation of cadmium in the liver and kidney as 38% and 40% respectively. Besides liver, kidney is one of the primary target of cadmium exposure. The decreasing concentration of cadmium in kidneys possibility due to the spreading of cadmium to other organs or the complexes formation with functional groups in *Manihot utilissima* leaves.

Table 1: Cadmium levels in liver and kidney in experimental rats

No	Organs	Group 1 (control)	Group 2 (Cd treatment; (mg/g))	Group 3 (antidote pre treatment (mg/g))
1	Liver	ND	0,0456	0,0026*
2	Kidney	ND	0,0117	0,0011*

*P<0,05 compare to group 2

Biochemical serum analysis and oxidative stress

Table 2: The level of malondialdehyde, urea, creatinine, SGOT and SGPT in serum

No	Parameters	Group 1 (Control)	Group 2 (Cd treatment)	Group 3 (pre treatment with antidote)
1	Malondialdehyde (MDA) (mg/dl)	3,61	14,9	7,19*
2	Urea (mg/dl)	38,36	33,4	28,33*
3	creatinine (mg/dl)	0,21	0,79	0,55
4	SGOT (U/L)	111,987	236,97	84,73*
5	SGPT (U/L)	25,88	203,33	43,14*

*P<0,05 compare to group 2

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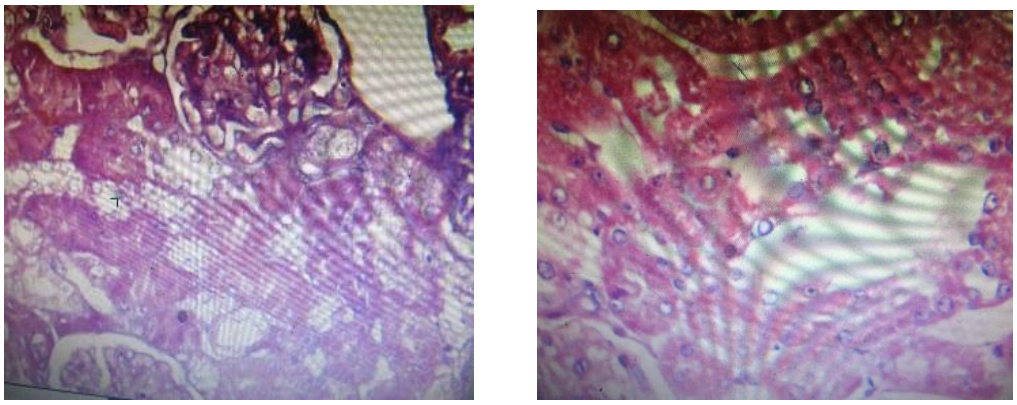
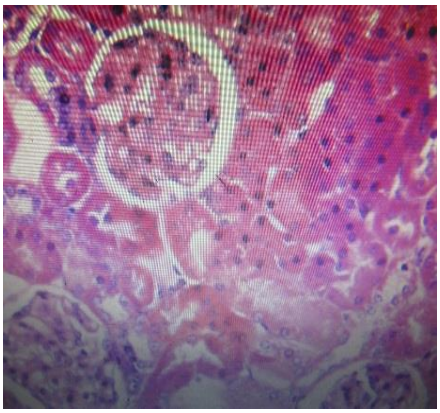
The rat blood from control group, group 2 and group 3 was taken then centrifuged and the biochemical parameters from serum were analyzed. The result of biochemical serum analysis and oxidative stress are shown in Table 2

Administration of Cd (II) solution 1000 ppm resulted in elevated levels of MDA in mice by 76% compared to control, while in group 3 the MDA levels increase by 50% compared to control. Pre treatment with antidote also able to reduce the levels of urea and creatinine which is parametes of kidney function. The similar pattern of result also occurred in liver function enzyme, SGPT and SGOT, where the antidotes of cassava leaves were capable to reduce the elevated leaves of the enzymes.

Tarasub *et al* [10] in a study has been reported the protective effect of curcumin against nephrotoxicity in rats exposed to Cd (II). Administration of 150 mg/kg bw curcumin 1 hour before Cd (II) exposure showed the decreased levels of MDA compared to control group (administration Cd (II) only). Bu *et al* [11] reported a protective effect of quercetin against testicular germ toxicity in rats induced by cadmium. the administration of Cd 4 mg/kg and quercetin concurrently could reduce the levels of MDA in rats compared with control. Cadmium is a highly toxic environmental contaminants that cause the production of *reactive oxygen species* (ROS) such as hydroxyl radicals, superoxide anions, nitrogen oxides and hydrogen peroxide. ROS would enhance lipid peroxidation. Peroxidation of lipids known to play important role in Cd induced renal damage and MDA is one of the products so the measurements of MDA could be used to examine the levels of lipid peroxidation [10]. Exposure to cadmium reported could cause damage to the kidneys. It could be seen from the elevated levels of urea and creatinine. In the liver, the protein molecules is metabolized to form final product ammonia and carbon dioxide and then converted to urea in the liver. From the liver, urea diffuse in the blood to the kidneys and then excreted into the urine. In cadmium exposed rats, cadmium accumulates in the kidneys, caused a defect in glomerular filtration, lead to increased levels of creatinine and urea in the urine [12].



(a) (b)



(c)

Figure 1 : (a). photomicrographs of rats kidney in group 2 (Cd (II) 1000 ppm administration); degeneration and necrosis occurred in most of epithelium lining the tubules. Fig 1 (b). photomicrographs of rats kidney in group 3 (pre treatment with Manihot utilissima antidote) occurred cloudy swelling and less necrotic cells. Fig 1 (c) photomicrograph of control kidney

Tsumbu *et al* [13] has reported the antioxidant activity of *Manihot utilissima* leaves. The study reported that cassava leaves contain polyphenols and flavonoids. Aqueous extract of cassava leaves significantly reduce the formation of lipid peroxidation markers including lipid hydroperoxide and ethylene. Further the extract showed the ability to inhibit the formation of free radicals in the early stages of lipid peroxidation.

Histopathology Analysis

The protective effect of *Manihot utilissima* leaves powder antidote against nephrotoxicity histopthologically described in Figure.1. Cadmium mainly accumulates in the kidney and liver. These organs is an important target organs for acute toxicity of cadmium [3]. The similar result was reported by Aslam *et al* [14] which reported a protective effect of cadmium toxicity by using seeds of *P.grande.*The experimental rats initially were administered the seed extract of P.grande at dose 60 mg/kg bw and 120 mg.kg bw for 4 days. After 4 days the rats were injected with $CdCl_2$ at a dose of 20 mg/kg bw. The protective effecr of low-dose *P.grande* extract (60 mg.kg bw) on the kidneys showed focal necrosis of proximal tubular epithelial cells and the occurrence of desquamation of the cells in tubular lumina. The protective effect of *Manihot utilissima* leaves powder against cadmium induced kidney damage, due to high content polyphenol in *Manihot utilissima* leaves. Polyphenols are reportedly able to reduce oxidative stress in humans. In addition to polyphenols, flavonoids contained in *Manihot utilissima* leaves also reported could inhibit oxidative stress by scavenging free radicals, act as reducing agents, hydrogen molecules donors and chelating agent. The carbonyl groups contained in flavonoids and phenolics compounds responsible for antioxidant activity [15].

CONCLUSION

Cadmium exposure to experimental rats leads to changes in the levels of serum biochemical parameters and oxidative stress. The administration of *Manihot utilissima* leaves powder antidote could reduce the changes of biochemical serum levels. Exposure to Cd (II) could leads to abnormal changes on kidney histology in the form degeneration and necrosis cells. Pre treatment with *Manihot utilissima* antidote could reduce the damage of the kidney so that the antidote of *Manihot utilissima* is expected to have a protective effect against cadmium induced nephrotoxicity.

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