

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## The Effect of Pb(II) in the Kidney of Experimental Rats and the Effectiveness of Papaya (*Carica papaya*) Leaves Powder as an Antidote.

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### ABSTRACT

The present study investigated the protective effect of papaya (*Carica papaya*) leaves powder antidote against Pb(II) induced nephrotoxicity in experimental rats. The administration of Pb(II) ions 1.000 mg/L intraperitoneally in experimental rats lead to increased levels of serum biochemical parameters and oxidative stress including SGPT, SGOT, urea, creatinine and malondialdehyde significantly. Pre treatment with papaya leaves powder antidote could significantly reduced the level of SGPT, SGOT, urea, creatinine and malondialdehyde up to 69,15% , 59,70%, 51,42%, 5,26%, and 32,55% respectively, compared to Pb(II) treated group only. Histopathologically, Pb(II) exposure could lead severe necrosis of kidney cells and swelling of tubular cells and pre treatment with *C.papaya* leaves powder antidote could reduce the damage effect induced by Pb(II). From this result, it can be concluded that pre treatment with *C.papaya* leaves powder antidote could give preventive effect against Pb(II) induced nephrotoxicity in experimental rats

**Keywords:** *Carica papaya*, antidote, Pb(II), nephrotoxicity, antioxidant

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## INTRODUCTION

Lead (Pb) is a toxic heavy metal the environment, especially the water bodies through variety of industries including metal production industries, fertilizer industries, batteries, air conditioning industries and electroplating [1]. Pb is a dangerous heavy metal even in a very small amount. However, human are exposed by Pb through the environment that contaminate by industrial waste, and by the diet, so more than 75% exposure of Pb comes from ingestion [2]. Kidney is one of a primary organ target for toxicity of Pb. Excessive exposure of Pb could lead to nephrotoxic effects of both chronic and acute [3]. Lead nephropathy is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis [4]. At present, chelating agents such as disodium edentate calcium (CaNa<sub>2</sub>EDTA) and sodium dimercaptosuccinate (NaDMS) is commonly used to treat Pb poisoning. The chelating agent will form an insoluble complex with Pb to remove Pb in tissue, but these agents are not able to remove metal until in intracellular and could lead to redistribution of toxic metal, loss of essential metals and dysfunction of liver and kidney [3]. In order to solve this problem, natural therapies to promote chelation, detoxification and protection are gaining popularity because of minimal effects. Medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability [5]. Papaya (*Carica papaya* Linn) is commonly called as paw-paw and it belongs to the family Caricaceae, and its commonly known for its food and nutritional values throughout the world [6]. Previous study reported that papaya leaves can be used as adsorbent of Pb(II) in optimum conditions at pH 4,0, initial concentration 5400 mg/L, optimum contact time at 15 min, biosorbent mass 0,1g with biosorption capacity (Q) 284,35 mg/g [7]. This study aim to investigate the effect of *C.papaya* leaves powder antidote against nephrotoxicity induced by Pb(II) in experimental rats.

## MATERIALS AND METHODS

### Plant Material

*C.papaya* leaves that used in this study was collected from local garden in Padang, West Sumatera, Indonesia

### Preparation of antidote of papaya leaves

The *C.papaya* leave were air dried for 2 weeks at room temperature and then grinded into a powder form using a blender. 2 g of *C.papaya* leaves powder then mashed with distilled water and then transferred into a bealer glass, then distilled water was added to achieve 120 mL total volume. The solution was heated to boil, then filtered and stored in sealed bottles.

### Experimental Design

9 experimental rats were divided into 3 groups with 3 rats in each group. Group 1 as control, only given distilled water with a normal diet. In group 2, the rats were given Pb(II) 1.000 mg/L only. In group 3, the rats were given pre-treatment with antidote orally for 8 days, and followed by administration of Pb(II) 1.000mg/L intraperitoneally. After 5 hours of exposure to Pb(II), rats in group 2 and 3 anaesthetized using chloroform, and sacrificed to drawn the blood and the kidney. The blood was collected for biochemical serum analysis and oxidative stress parameter. The kidney then fixed in Bouin's solution for histopathology analysis

### Biochemical Serum Analysis and Oxidative Stress Parameters

Lipid peroxidation were determined based on the levels of malondialdehyde in serum. Serum biochemical parameters determined include liver function enzyme, SGPT and SGOT, and kidney function parameters which include urea and creatinine. Liver function enzyme and renal function parameters were determined using 2 reagents method.

### Histopathology Analysis

The kidneys were washed with sterile saline and the fixed in Bouin’s solution. The kidney then excised using microtome and stained with hematoxylin and eosin. The kidney damage then analyzed histopathology using light microscope.

### Statistical Analysis

The result of biochemical serum analysis was statistically analyzed using Statistical Package for Social Science Program ver.16 (SPSS ver.16). The analysis was conducted using analysis of variance (ANOVA) followed by Tukey test.

## RESULT AND DISCUSSION

### Biochemical Serum Analysis and Oxidative Stress

The result of serum analysis and oxidative stress was showed in Table.1

**Table 1. The level of malondialdehyde (MDA), urea, creatinine, SGOT and SGPT in serum**

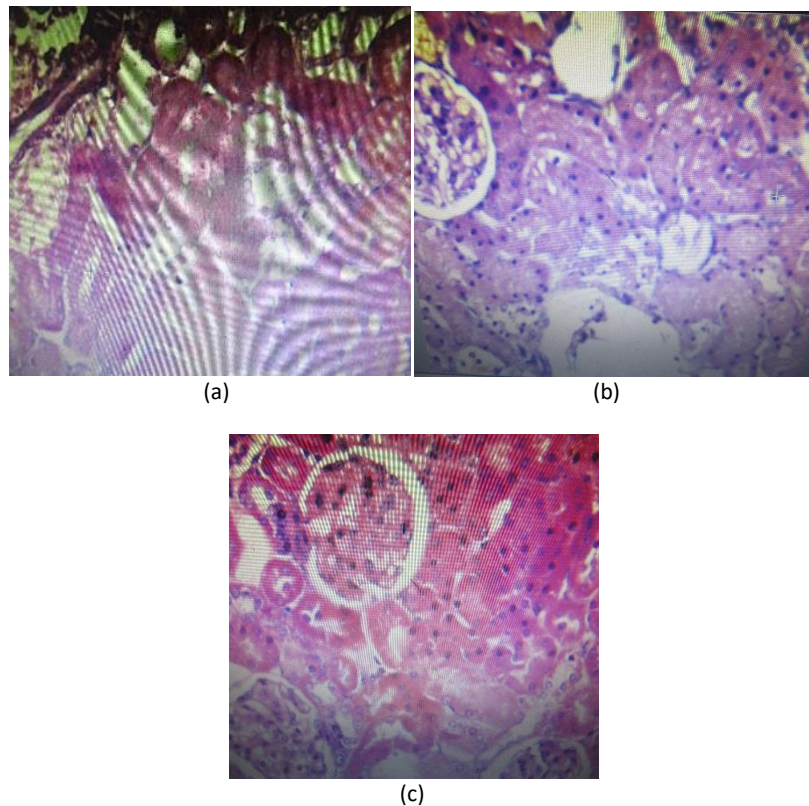
No	Parameters	Group 1 (Control)	Group 2 (Pb(II) treatment)	Group 3 (pre treatment with antidote)
1	Malondialdehyde (MDA) (mg/dl)	3,61	6,88	4,64*
2	Urea (mg/dl)	38,36	43,4	21,08*
3	creatinine (mg/dl)	0,21	0,57	0,54
4	SGOT ( U/L)	111,987	182,22	73,42*
5	SGPT (U/L)	25,88	59,43	18,33*

\*P<0,05 compare to group 2

Table 1. showed that in the group 2 there were elevated levels of serum biochemical paramaters after the rats were exposed to Pb(II). In group 3, in which mice were pre-treated with the *C.papaya* leaves powder antidote, decreased levels of serum parameters were observed. Decreased levels of MDA, urea, creatinine, SGOT and SGPT in rats in group 3 compared to group 2 were 32,55%, 51,42%, 5,25%, 59,70% and 69,15% respectively. Hussein et al [8] reported that the rats that were exposed with Pb acetate 30 mg/kg by orally once daily for 20 weeks, showed a significant increase levels of malondialdehyde, creatinine, urea, SGOT and SGPT compared with the control group. Administration of Pb(II) in rats associated with the elevated production of ROS and NO inactivation. High levels of ROS after exposure to Pb(II) could increase the occurrence of superoxide anion, raising the possibility of interaction between NO and ROS to produce nitrite peroxy compound, a molecule that is highly destructive. A significant increase in ALT and AST levels in rats that were exposed to Pb might be due to oxidative damage to liver tissue resulting lipid peroxidation in membrane and lead to chaotic on some biochemical pathway of liver cells and energy metabolism. SGOT and SGPT are known to be marker for liver activity [9]. The increased of SGOT and SGPT in serum is used as indicator of the damage of plasma membrane permeability, cellular damage and metabolic disorders for toxicity due to Pb [10]. The elevated levels of urea and creatinine was also observed in rats exposed to Pb when compared to control group. The increase in creatinine and urea due to an imbalance in renal function because intrinsic lesions of the kidneys, decreased of kidney perfusion obstruction of the lower urinary tract [8]. Measurement of malondialdehyde is widely used as an indicator of lipid peroxidation. The increased in malondialdehyde in the group treated with the metal could be used as indicators of cell membrane damage. In addition, the end product of lipid peroxidation could be mutagenic and carcinogenic [11]. Pre-treatment with *C.papaya* leaves powder antidote, capable of lowering the levels of MDA, urea, creatinine, SGOT and SGPT compared to those that exposed with Pb(II) only. It indicates that *C.papaya* leaves powder antidote capable of providing the protective effect against liver and kidney function as a result of exposure to Pb(II). The protective effect is likely due to the antioxidant content of papaya leaves. *C.papaya* leaves are known to have phenol and phenolic compounds which is a secondary metabolites and play an important role in the antioxidant. Phenolic compound responsible for antioxidant properties of *C.papaya* leaves, since phenolic compounds are able to act as free radicals scavenging. In addition, *C.papaya* leaves also contained polyphenols and flavonoids that could have positive effect on free radical scavenging [12].

### Histopathology Analysis

The protective effect of pre treatment with *C. papaya* leaves powder antidote described on kidney of rats in group II and group III histopathologically, described in Figure 1.



**Figure 1. Photomicrograph of kidney (a). kidney histopathology with Pb(II) exposure, there are swelling of tubular, the epithelial cells undergo necrosis partially (b). histopathology of kidney with *C.papaya* leaves powder antidote pre-treatment, there are necrosis but with smaller amounts (c). photomicrograph of control kidney**

Missoun et al [13] reported that Pb exposure to acute doses or high doses could lead an imbalance of proximal tubular function that could lead to aminociduria, glycosuria and hyperphosphaturia. The effects might be reversible. Repeated exposure might lead to toxic stress and chronic damage to the kidney or nephropathy. Missoun et al [13] reported that rats exposed to 1000 mg/L Pb acetate in drinking water for 8 weeks led to an increase in the fraction of the excretion of calcium and phosphorus, increased urea and creatinine and decreased creatinuria and glycemia which is clinically indicates kidney damage. The protective effect against nephrotoxicity of *C.papaya* leaves powder is might be due to removal of Pb through chelating process. Phenolic compound and flavonoids contained in *C.papaya* leaves plays important role in chelating process. Antioxidants could act as chleating agents by reducing the redox potential, thereby stabilizing the oxidized form of the metal ion. Moreover, the protective effect of of *C.papaya* leaves powder estimatedly come from phenolic compounds or polyphenols contained in *C.papaya* leaves which are the secondary metabolites and have a high antioxidant activity. In general, mechanism for antioxidant activity of phenolic compounds is to neutralize free radicals and prevent the decomposition of hydroperoxide into free radicals [14].

### CONCLUSION

Exposure of Pb(II) in experimental rats could increase the level of biochemical serum parameters and oxidative stress which include malondialdehyde (MDA), urea, creatinine, SGOT and SGPT. Increased level of urea and creatinine indicates nephrotoxicity. Histologically, Pb(II) exposure in rats lead to swelling of tubules and necrotic in epithelial cells. Pre-treatment with *C.papaya* leaves antidote could reduce the level of biochemical serum parameters and mitigate the effects of damage to the kidney caused by exposure to Pb(II), so it concluded *C.papaya* leaves has potential to reduce the toxicity effects of Pb(II).



## REFERENCES

- [1] Jadav JN, Maind SD, Bhalerao SA., Oct. J. Env. Res., 2015; 3(1) : 067-079
- [2] Patrick L., Altern. Med. Rev., 2006; 11 : 2-22
- [3] Li L, Zhang Y, Ma J, Dong W, Song Q, Zhang J, Chu L., Scientific World J., 2014; 1-9
- [4] Goyer RA., Toxicol.Lett., 1989; 46 : 153-162
- [5] Janmeda P, Sharma V, Singh L, Paliwal R, Sharma S, Yadav S., Asian Pacific J. Cancer Prev., 2011; 12:677-683
- [6] Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B., Int. J. Herb. Med., 2014; 2(5) : 01-08
- [7] Suyono T, Yuser MA, Munaf E, Aziz H, Tjong DH, Zein R., J.Chem.Pharm.Res 2015; 7(9):100-106
- [8] Hussein SA, Hassanenin MRR, Ali AH., Benha Veterinary Medical Journal 2014; 27(2) : 382-395
- [9] Alagammal M, Sakhtidevi G, Mohan VR. Journal of Advanced Pharmaceutical Sciences 2013; 3(1) : 385-393
- [10] Metwally El Sayed AM, Negm FA, El-din RAS, Nabil EM. J. Biomed. Mater. Res., 2015; 3(4) :34-45
- [11] Bharali MK. Journal of Global Biosciences 2013; 2(5) : 121-125
- [12] De Mandal S, Lalmawizuala R, Vabeiryureilai M, Kumar NS, Lalnunmawii E. Research & Reviews : Journal of Botanical Sciences, 2015; 4(3) : 42-45
- [13] Missoun F, Slimani M and Aoues A., Afr. J.Biochem.Res., 2010; 4(2) : 21-27
- [14] Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R and Mulatsih W., Int. Food Res. J., 2010; 17 :97-106