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The Effect of Ion Mn(II) in the Kidney and Liver and Protective Effect of *Terminalia catappa* pericarp Antidote in Rat Liver

Sofian W.[1], Edy F.[2], Djong H. Tjong[3], Ety Y.[4] and Rahmiana Z.[5*]

[1]*Department of Anatomy, Faculty of Medicine, University of Prima Indonesia/Royal Prima Hospital,Medan, North Sumatera, Indonesia*
[2]*Laboratory of Molecular Biology, Faculty of Medicine, University of Prima Indonesia, Medan, North Sumatera, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang,West Sumatera, Indonesia*
[4]*Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, West Sumatera, Indonesia*
[5]*Laboratory of Environmental Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatera, Indonesia*

ABSTRACT

Manganese (Mn) is one of the trace elements that is required for human metabolisms and activity of several enzymes, but it also could induce toxic effect when its ingested in high amount. The present study was carried out to evaluate the protective effect of T.catappa pericarp antidote against $MnCl_2$ induced renal toxicity and hepatotoxicity in experimental rats. Experimental rats were administered orally of T.catappa pericarp antidote 5 mL x b.w/200 g b.w against the renal toxicity and hepatotoxicity induced by administration of $MnCl_2$ 1000 mg/L. Efficacy of T.catappa pericarp antidote against the renal toxicity and hepatotoxicity was evaluated by evaluating the biochemical serum parameters and histopathological changes. The treatment with $MnCl_2$ 1000 mg/L induced apoptotic and necrotic cells in liver. Pre treatment with T.catappapericarp antidote could reduce significantly the levels of malondialdehyde, urea, creatinine, SGOT and SGPT as 30,33%, 66,03%, 48,83%, 50,62% and 49,01% respectively compared to $MnCl_2$ treatment group. Pre treatment with T.catappa pericarp antidote also prevented the deteriorative effect induced by $MnCl_2$ through protective mechanisms of ROS scavenging and metal chelating.

Key words: *Terminalia catappa*, Manganese, oxidative stress, antioxidant

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INTRODUCTION

Metal compounds are found throughout the human environment. Industrial activity contributed greatly to human metal exposure [1]. Some of heavy metals have importance function as trace elements in organisms, but the toxic effect of many of them in human metabolisms need a great concern [2]. Manganese (Mn) is one of trace element that is required for the the activity of several enzymes, but it also could induce chronic toxicity when ingested in high amount, such as inhalation of the miners [3]. Manganese particles exists as airborne particulate matter in ferrous foundries and because of their small size particles, manganese incline to remain and stand in the air for long periods of time. Exposure in high amounts of manganese could cause accumulation in the basal ganglia in the brain that lead to neurological disorder similar to Parkinson's disease [4]. Because manganese in absorbed and excreted by the liver, the liver is also a primary target for its toxicity, through the mechanism of the generation of free radicals in

mitochondria and lead to oxidative stress [5]. Manganese exposure could induce reactive oxygen species (ROS) production and ROS induces the oxidation of membrane polyunsaturated fatty acid, resulted a multitude of lipid peroxidation products [6]. Oxidative stress is defined as an imbalance between production of free radicals and oxidants, and it could eliminate by antioxidant system. The term of antioxidant leads to a broad spectrum of compounds that are able to donate the electrons and neutralize free radicals, thus preventing cell damage [7].Terminalia catappa or ketapang Indonesian language, is a plant that easily found growing on the beach, on the road side, and planted as a shade tree in the garden. Ketapang is plant that has many uses, from roots, stem, and leaves. Ketapang seeds can be used to make various cakes, breads, jams and tempeh, while the shell of the fruit usually discarded [8]. The previous studies reported that *T.catappa* fruit powder was an effective adsorbent for removal of ion Mn(II). The optimum amount of *T.catappa* fruit powder biomass to remove Mn(II) ions was 1 g and able to remove Mn(II) ions up to 75,22%, with optimum sorption capacity 460,18mg/g. The optimum conditions were at pH 6, 15 minutes for contact time and 6900 mg/L for Mn(II) initial concentration [9]. The research on the effectiveness of *T.catappa* pericarp antidote in alleviating oxidative stress and liver and kidney damage caused by Mn(II) toxicity has not been done. Hence it is necessary to investigate the effect of pre treatment using *T.catappa* pericarp antidote against Mn(II) toxicity in liver and kidney of experimental rats through examination some biochemical serum parameters and histology analysis

MATERIALS AND METHODS

Experimental Plant

The experimental plant *T.catappa* pericarp was collected from local market in Padang, West Sumatera

Preparation of antidote of *Terminalia catappa* pericarp

The pericarp of *T.catappa* were washed with running water and wind dried for 2 weeks at room temperature. After dry, the pericarp were mashed using a blender until it becomes a powder. 2 g of the of pericarp powder were added with distilled water until total volume 120 mL were reached. The solution was heated to boil and then stored in a sterile sealed bottle.

Chemicals

All chemicals including $MnCl_2$ was obtained from Merck (Darmstadt, Germany). All chemicals were analytical grade and were purchased from standard commercial suppliers

Experimental Rats

The adult male white rats weighing 140-160 g provided by Andalas University. The experimental rats were put into caged and ad libitum diet and water were given during the experimental period. All treatments and maintenance procedures has been approved by the animal ethics committee of Andalas University

Mn (II) Toxicity Experimental Design

The experimental rats were randomly divided into 3 groups which is consists of 3 rats in each group, where the first group was the the control which given distilled water only. The second group was administered with 1 mL x b.w Mn(II) 1000 mg/L intraperitoneally. The third group was administered with 5 mL x b.w/200 g b.w of *T.catappa* pericarp antidote for 7 days by oral intubation, and followed by administration of 1mL x b.w/200 g b.w Mn(II) 1000 mg/L intraperitoneally.

Collection of Experimental Samples

After 5 hours, the animals were sacrificed by chloroform anesthesia. The blood was collected from the animals by cardiac puncture. The aliquots of blood samples were preserved for biochemical serum analysis and oxidative stress. Organs of rats which include liver and kidney were dissected and preserved for determination of Mn(II) level which accumulates in each organ. The liver and kidney were fixed in Bouin solution for histological analysis.

Analytical Procedures

Mn(II) concentration in each organ was determined using atomic absorption spectroscopy (Varian Spectra AA 240 Spectrometer). The liver and kidney function parameters and oxidative stress product were determined using 2 reagents method (Start Substrate).

Histopathological Studies
Slices of liver and kidney from each group were routinely stained with haematoxylin and eosin (H&E) and assessed in a light microscope.

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

RESULTS AND DISCUSSION

Accumulation of Mn (II) in Liver and Kidney
The accumulation of Mn(II) in liver and kidney and the effect of pre treatment by oral intubation with *T.catappa* pericarp were shown in Table.1.

Table 1 : Mn content in liver and kidney in experimental rats

No	Organs	Group 1 (control)	Group 2 (Mn (II) treatment; (mg/g))	Group 3 (antidote pre treatment (mg/g))
1	Liver	ND	0,00648	0,00439*
2	Kidney	ND	0,00455	0,0016*

**P<0,05 compare to group 2*

Table 1 shown there are significantly decreased of Mn(II) levels in liver and kidney after pre treatment with antidote. The result shown that the rats were exposed to Mn(II) will lead to accumulation of Mn(II) in liver than the kidney. It might be due to manganese in absorbed and excreted by the liver, so the the liver is a primary target for its toxicity, through the mechanism of the generation of free radicals in mitochondria and lead to oxidative stress. The primary targets of Mn toxicity are central nervous system and brain. Mn has been shown to be deposited in certain regions of brains mainly in basal ganglia region and could lead to a progressive, permanent and neurodegenerative damage [10,11]. Based on epidemiological and experimental studies, in addition to the brain, the liver also a major target organ for accumulation and toxicity of Mn [12]. The pre treatment with *T.catappa*pericarp antidote could reduce the level of Mn(II) in liver and brain as 32,25% and 84,73% respectively. This result may be due to metal chelating activity of *T.catappa*. The result of in vitro antioxidant studies assessed that *T.catappa* show higher metal chelating activity (390,00±08,66 for leaf and 480,00 ±08,66 for stem) [13].

Biochemical serum parameters and oxidative stress product
The blood of rats from each group were taken and centrifuged to obtain serum. The result of analysis include liver function enzyme, markers of renal function and malondialdehyde (MDA) are shown in Table.2

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	5,77	4,02*
2	Urea (mg/dL)	38,36	83,4	28,33*
3	creatinine (mg/dL)	0,21	0,43	0,22*
4	SGOT (U/L)	111,987	189,00	93,32*
5	SGPT (U/L)	25,88	80,37	40,98*

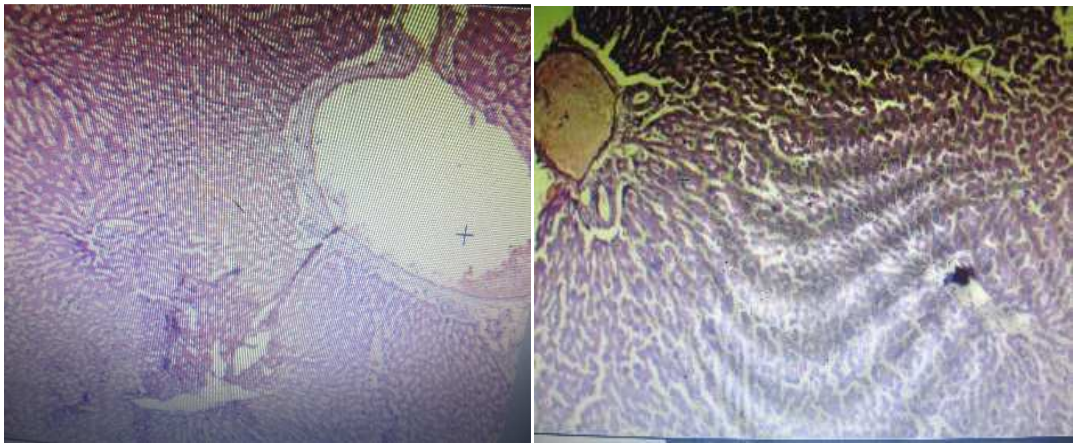
**P<0,05 compare to group 2*

Table 2 showed that in group 2, all parameters measured were increased. The levels of malondialdehyde (MDA), urea, creatinine, SGOT dan SGPT were elevated in Mn treated animals (Group 2) compared with the control groups. The level of SGOT and SGPT were elevated in rats treated with Mn(II) as , it might be due to increased cell membrane permeability or damage of hepatocytes. The increasing levels of SGOT and SGPT in serum of treated rats is mainly due to the leakage of these enzyme from the liver cytosol into the blood stream [14].. Razavian and Rabiee [15] reported that Mn exposure could lead to hepatotoxicity as indicated by the elevated level of SGPT (ALT) and SGOT (AST). In their research, the miners had higher AST and ALT than others. AST and ALT increased significantly in miners with 300µg/L serum Mn concentration. Higher AST and ALT in miners indicate more hepatotoxic and less cholestasis. The parameters then decreased with statistically differences ($p<0,05$) in Group 3 when compared with Group 2. It is indicated that *T.catappa* pericarp antidote has hepatoprotective effect

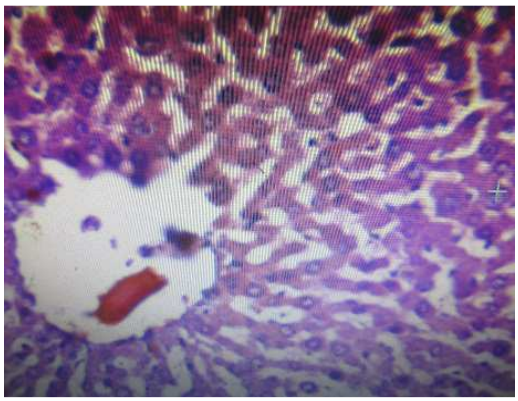
[16]. Administration of Mn (II) 1000 ppm lead to increased levels of malondialdehyde significantly in rats. Malondialdehyde (MDA) is one of lipid peroxidation product. The measurement of MDA can be used to assess lipid peroxidation [17]. Pre treatment with antidote could reduce the elevated levels of malondialdehyde until 30,32%. It might be due by the act of antioxidants in *T.catappa* as free radical scavengers by preventing and repairing damages caused by reactive oxygen species (ROS). Pre treatment with antidote also able to reduce the levels of urea and creatinine which is parameters of kidney function. Chtourou et al [18] reported administration of silymarin (SIL), a natural flavonoid, reduced the alterations in the renal and urine markers in manganese induced nephrotoxicity and oxidative stress in rat. Administration of SIL significantly prevented Mn induced nephrotoxicity, indicated by both diagnostic indicator of kidney injury like plasma urea, uric acid and creatinine. The protective effect of *T.catappa*pericarp in kidney might be due by increasing the antioxidant cascade and decreasing lipid peroxidation markers[18].

Histopathology Study

The protective effect of *T.catappa*pericarp antidote against Mn induced hepatotoxicity and nephrotoxicity showed in Figure.1 and Figure.2 respectively

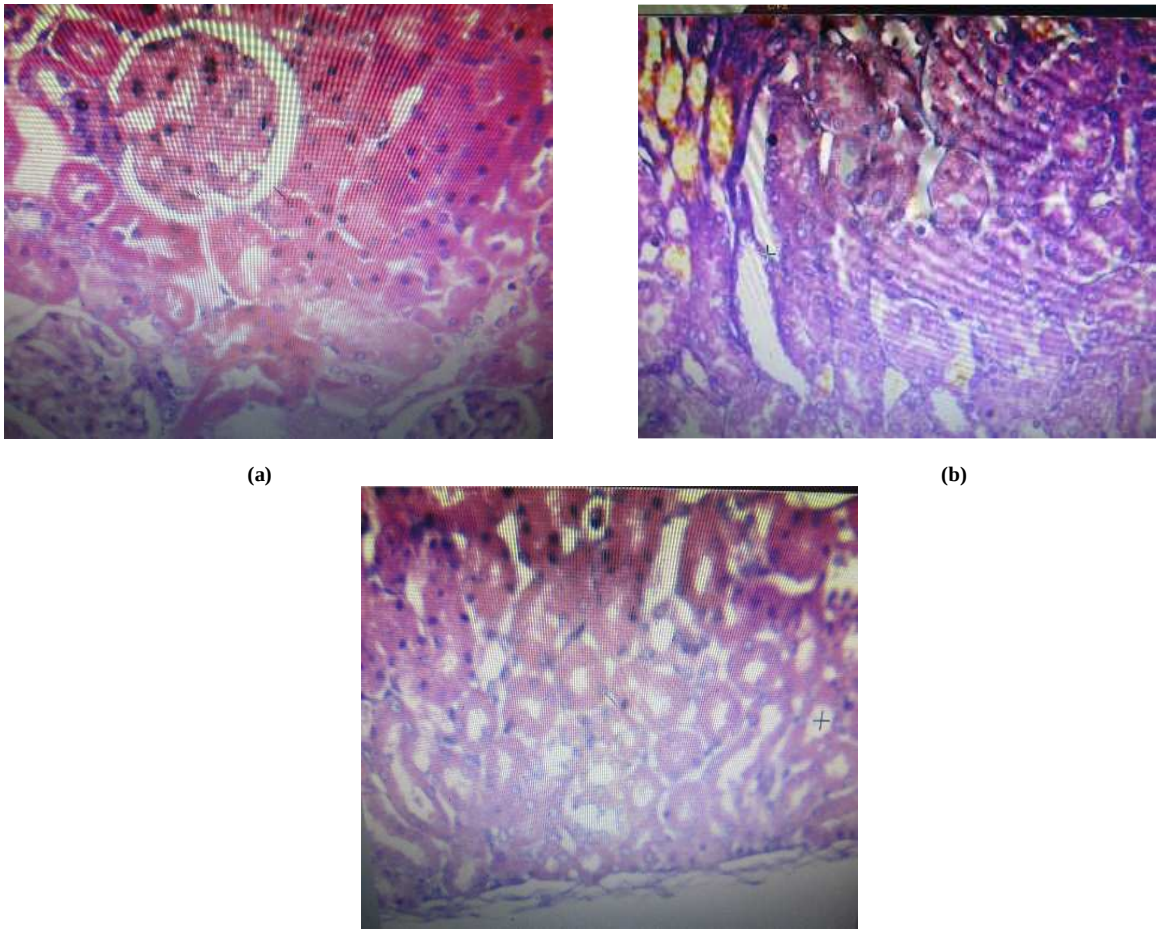


(a) **(b)**



(c)

Figure 1 : (a). photomicrograph of rats liver in control group, (b) photomicrograph of rats liver in group 2 (injection with Mn(II) 1000 ppm) ; slices of liver tissue is composed of cells that arranged to form sinusoid hepatocytes with central venous dilated, apoptotic and necrotic cells (c) photomicrograph of rats liver in group 3 (pre treatment with *T.catappa*pericarp antidote) ; lobules and central venous appeared normal



(a) **(b)**

(c)

Figure 2 : (a). photomicrograph of rats kidney in control group; (b) Photomicrograph of rats kidney in Group 2 (Injection with Mn(II) 1000 ppm) ; kidney tissue composed of cortex and medulla containing glomeruli and tubular, degeneration and necrosis occurs partially in epithelium; (c) photomicrograph of rats kidney in Group 3 (pre treatment with T.catappa pericarp antidote) : occurred cloudy swelling and less necrotic cells

This study was in accordance with Roy et al [19] which reported that manganese could induced hepatotoxicity in poultry birds. Exposure with Mn(II) 50 and 100 mg/kg b.w showed decrease of total protein, an indicative liver damage. The liver tissues accumulated Mn(II) in dose dependent manner and the histopathological study of liver showed apoptotic and necrotic changed. In our study, the kidney showed severe tubular necrosis. The kidney tubular necrosis may be due to the oxidative damage induced by Mn(II). Nuha and Al-Harbi [20] in their research has been investigated histological alteration induced by manganese chloride in the kidney of the albino rats. The rats randomly divided into several groups and exposed with manganese chloride (60,80,100 ppm) by oral administration via drinking water. Histopathological, the most striking lesion were observed in the kidney. The high dose Mn(II) (100 ppm) caused exacerbation in tubular necrosis. the protective effect of *T.catappa*pericarp may be due to metal chelating ability. Prabha [21] reported that pericarp of *T.catappa* which is contain anthocyanin have chelating activity, reducing power and hydrogen peroxide scavenging assay hence it can reduce the toxicity of Mn(II) in liver and the kidney.

CONCLUSION

The exposure with Mn(II) in rats could lead to the elevated levels of serum biochemical parameters including SGPT, SGOT, urea and creatinin and also lipid peroxidation product, malondialdehyde (MDA). The administration of antidote *T.catappa* pericarp could reduce the observed parameters. Exposure to Mn(II) could lead to structural changes in hepatocyte and kidney include hepatocyte necrosis and tubular necrosis. *T.catappa* pericarp antidote

expressed protective role against toxic influence of $MnCl_2$ on all analyzed parameters and its protective activity may be due to anti oxidant mechanisms and metal chelating ability.

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