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Oxidative and Chlorinative Stress Biomarkers in Liver Cells of Rats Exposed to Cyanide *In-Vitro*

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ABSTRACT

Cyanide is one of the rapid acting poisons. Exposure to cyanide can cause several health problems, including liver cells damaged. However, the mechanism of liver cells damage by cyanide still not clear. In this present study, we proposed that cyanide could induced liver cells damaged via oxidative and chlorinative stress pathway. Thus, our study aimed to investigated the effect of cyanide on liver cells via oxidative and chlorinative stress pathway by measuring the levels of hydrogen peroxide (H₂O₂) malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPPs), the myeloperoxidase (MPO) activity, and the chlorinative index (CI). The results revealed that cyanide exposure positively correlated with H₂O₂, MDA, AOPPs level, and CI, and negatively correlated with MPO activity in liver cells homogenate. These results indicated that cyanide exposure induce liver cells damage through the oxidative and chlorinative stress pathway which can be seen from the correlation between cyanide exposure and the levels of H₂O₂, MDA, and AOPPs, the activity of MPO, and the CI.

Keywords: Chlorinative Stress, Cyanide, Oxidative Stress

INTRODUCTION

Cyanide is one of the most deadly rapid-acting poisons. Cyanide toxicity is generally considered to be a rare form of poisoning¹. Although less frequent than another poisoning, cyanide poses significant physical harm, due to its rapid and potentially fatal adverse effects². Recently, this compound was popular especially in Indonesia. It is because the case of Australian student with Indonesia citizenship, named Jesica Kumolo Wongso, who is a murder suspect in the case of cyanide-laced coffee in Indonesia³. Cyanide is one of the most toxic chemical substances on Earth. Cyanide may be produced by toxic gases during the pyrolysis of plastic or nitrile-based polymer fibres, by extracts of plants containing cyanogenic glycosides (e.g. cassava) or from industrial waste (e.g. electroplating)⁴. The extreme toxicity of cyanide arises from its readiness to react with other elements and hence interfere with normal biological processes⁵. Both acute and chronic exposures of cyanide can cause severe poisoning in human and animals⁶. Experimental studies on different animal species have shown that prolonged sublethal cyanide exposure can induce pathologic effects on various organs, including liver^{4,7}. The mechanisms by which cyanide exert its effects to liver have not all been fully elucidated. It is also widely accepted that cyanide itself stimulates product¹ of intracellular reactive oxygen species (ROS). It is thought that cyanide exposure causes rising

of oxidative stress by glutathione (GSH) depletion and inhibition of a series of antioxidant enzymes. Also, it is reported that cyanide exposure increases levels of ROS and reactive nitrogen species (RNS) and the amount of lipid peroxidation⁸. The increasing of ROS by cyanide will increase the concentration of hydrogen peroxide (H₂O₂) to form radical hydroxyl with the presence of transition metal⁹⁻¹¹. These ROS can randomly react with lipids, proteins and nucleic acids causing oxidative stress and damage in these macromolecules. When ROS target lipids, they can initiate the lipid peroxidation process, a chain reaction that produces multiple breakdown molecules¹². Among these multiple breakdown molecules, malondialdehyde (MDA) is the one of the most well studied markers of lipid peroxidation¹³⁻¹⁵. However, the increasing concentration of H₂O₂ also have another effect. H₂O₂ can be used by myeloperoxidase enzyme (MPO) to produce another oxidants, known as hypochlorous acid (HOCl)⁹. HOCl can promote a further reaction resulted in a condition known as chlorinative stress¹⁶⁻¹⁹. According our previous study, chlorinative stress can be measured by calculating both the ratio between H₂O₂ level and MPO activity, known as chlorinative index (CI) and the level of Advance Oxidation Protein Products (AOPPs)¹⁹⁻²¹. Based on the fact that oxidative and chlorinative stress may be involve in cyanide toxicity. Thus, we hypothesized that, cyanide-induced liver cells damage may follow the oxidative and

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chlorinative stress pathway; and we designed the present study to explore this hypothesis by measured several parameters, such as H₂O₂, MDA and AOPPs levels, MPO activity, and CI value in liver cells homogenate.

3. MATERIAL AND METHODS

Animals and Homogenate Preparation

Male rats (*Rattus novergicus*) weighing 200–250 gram with 2-3 months old were obtained from the Abadi Jaya farm at Yogyakarta, Indonesia, in healthy condition. The experiment was approved by the Ethical Committee from the Lambu Mangkurat University, South Kalimantan, Indonesia. Animals were fed under standard conditions and acclimatized with a 12 hours light/dark cycle. The animals were sacrificed by surgical procedure and the livers was removed. Then, the organs homogenize in phosphate buffer saline (pH 7.0) and was ready to used for in vitro experimental models.

Experimental Models

The present study was a experimental study design to examine the liver celles damage induced by cyanide through the oxidative and chlorinative stress pathway. Liver cells will exposed to different concentration of cyanide. The cyanide concentrations were 0 mg/l, 0.5 mg/l, 1 mg/l, and 2 mg/l. Each solution then incubated at 37°C for 3 hour. After incubation, liver H₂O₂, MDA, and AOPPs level, MPO activity, and CI were estimated.

H₂O₂ level analysis

H₂O₂ level was calculated by the FOX2 method with slight modification. Solutions measured spectrophotometrically at $\lambda = 505$ nm. Standard and test solutions consisted of 1 M H₂O₂ 200 μ L and 200 μ L serum, respectively, with the addition of 160 μ L PBS pH 7.4, 160 μ L FeCl₃ (251.5 mg FeCl₃ dissolved in 250 ml distilled water) and 160 μ L o-fenantroline (120 mg o-phenantroline dissolved in 100 ml distilled water) for both solutions. The composition of the blank solution was identical to that of the test solution, except for absence of FeCl₃ in the blank. Subsequent to preparation, all solutions were incubated for 30 minutes at room temperature, then centrifuged at 12,000 rpm for 10 minutes, and the absorbance of the standard (As), test (Au) and blank (Ab) solutions measured at $\lambda=505$ nm, using the supernatant of each solution²².

MDA level analysis

MDA level was calculated by thiobarbituric acid reactive substances (TBARS) by the technique already proposed by Buege and Aust. The supernatant were put into a Pyrex tube that contained 10% of trichloroacetic acid and 0.67% of TBARS and incubated at 100°C for 15 min. Then chill the mixture on ice for 5 min and add the 1.5 ml of n-butyl-liquor. Let the mixture stand for 40 s and centrifuged at 1000 rpm for 15 min. The TBARS value was calculated by the spectrophotometer at the absorbance of 532 nm and figured utilizing the coefficient 1.56×10^5 mol/cm. The MDA concentration expressed in μ mol MDA. As a standard solution we used commercially MDA²².

MPO activity analysis

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MPO activity was measured spectrophotometrically using o-dianisidine (Sigma-Aldrich) and H₂O₂. In the presence of H₂O₂ as oxidizing agent, MPO catalyses the oxidation of o-dianisidine yielding a brown coloured product, oxidized o-dianisidine, with a maximum absorbance at 470 nm. One unit (U) of MPO activity was defined as that degrading 1 μ mol of H₂O₂ per minute at 25°C²³.

Chlorinative index analysis

Chlorinative index was a ratio between H₂O₂ level and MPO activity. Chlorinative index was calculated following to equation²⁰.

$$CI = \frac{\text{Hydrogen Peroxide Level}}{\text{MPO Activity}}$$

AOPPs concentration analysis

AOPPs concentration analysis were calculated by spectrophotometric methods which was first performed by Witko-Sarsat *et al*¹⁵. with slight modification. 200 μ l of supernatant from the liver 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. AOPPs concentrations were expressed as μ mol/l of chloramine-T equivalents²⁴.

Statistical analysis

The results were expressed as mean \pm SE for six replicates. The data was analysed between each parameter level and cyanide concentration. For analyzing the data, microsoft excell 2010 was used and was examined by simple correlation regression.

RESULTS AND DISCUSSION

In this present study, to investigate the involvement of oxidative stress in cyanide- induce liver cells damage, the level of liver H₂O₂ and MDA was investigated. The results shows in figure 1 and 2. Cyanide exposure caused the increasing of liver H₂O₂ and MDA levels. From figure 1 and 2, we can see that the increasing of cyanide concentration can increase the level of both H₂O₂ and MDA. Cyanide concentration was strong positively correlated with liver H₂O₂ and MDA (rH₂O₂= 0,906; rMDA= 0,992). The results of this present study shows that cyanide have a strong positive correlation with liver H₂O₂ and MDA level. It means the increasing level of cyanide in liver will increase both H₂O₂ and MDA level. The results in this study confirm that cyanide exposure increase oxidative stress and lipid peroxidation in the brain and kidney of mice and rats^{25,26}. It is been widely accepted that cyanide-induced cellular oxidative stress, i.e., increase of superoxide anions, lipid peroxide, hydroxide radicals, hydrogen peroxide and others, appears to arise through multiple pathways. At the cellular level, cyanide produces chemical hypoxia by

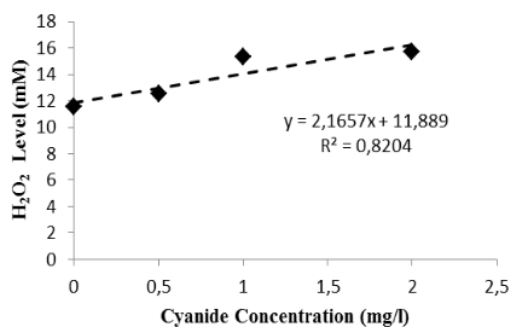


Figure 1: Correlation between cyanide concentrations and liver H₂O₂ level

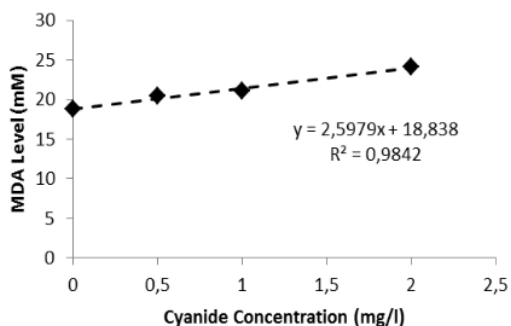


Figure 2: Correlation between cyanide concentrations and liver MDA level

inhibiting cytochrome c oxidase in complex IV of the mitochondrial oxidative phosphorylation chain to markedly reduce ATP²⁷. Furthermore, it can block the flow of electrons through complex IV to prevent oxidative metabolism and enhance ROS generation at complex III²⁶. Another mechanism that cyanide-induced cellular oxidative stress is inhibits antioxidant enzymes like GPx and depletes non-enzymatic antioxidants like intracellular GSH²⁸. It was also reported that cyanide causes an increase in lipid peroxidation and a decrease in GSH levels, and GPx, SOD, and CAT activities in liver of rats²⁹. Okolie and Iroanya³⁰ reported inhibition of SOD and CAT in liver, kidney and lung tissues of the cyanide treated rabbits. Such enzymes containing metal porphyrins as peroxidase, CAT and xanthine oxidase are inhibited strongly by cyanide⁸. MPO activity in the liver that received different doses of cyanide are presented in the Figures 3. After the administration cyanide, MPO activity seems to be decreased (Figure 2). There was a dose dependent decrease in MPO activity in the liver tissues. Cyanide concentration was strong negatively correlated with liver MPO activity. MPO is a member of peroxidase. MPO is a heterodimeric, cationic and glycosylated haem enzyme³¹. The basic reaction of MPO is decompose H₂O₂ into water and oxygen³². In the other hand, in several conditions, MPO also decompose the oxidation of chloride to HOCl³³. The results of this present study shows that

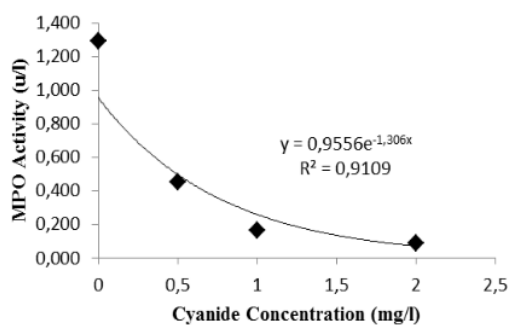


Figure 3: Correlation between cyanide concentrations and liver MPO activity

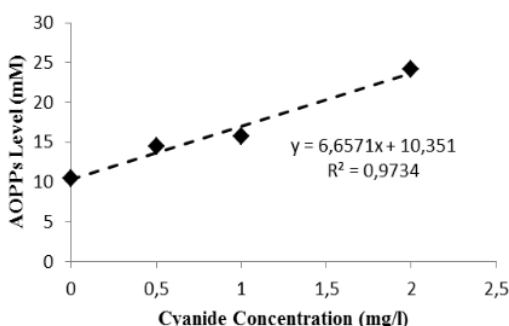


Figure 4: Correlation between cyanide concentrations and liver AOPPs level

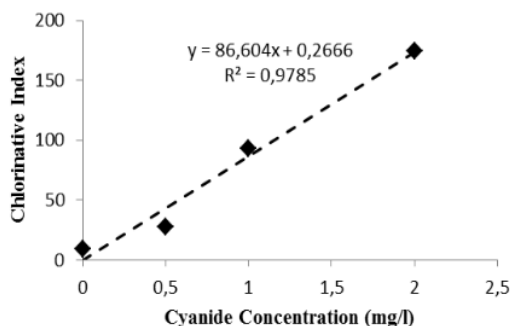


Figure 5: Correlation between cyanide concentrations and liver CI

cyanide interrupt the MPO activity. The interruption of MPO activity by cyanide may be cause by the effect of cyanide to induce a chlorinative stress condition. It is based on our several results study. Based on our several results, the presence of H₂O₂ which in this study induced by cyanide, can activated MPO to promote a further reaction to form HOCl and induce a condition, known as chlorinative stress^{17,19-20}. To prove it, in this present study we measured another two parameters that indicate chlorinative stress, such as AOPPs and CI (figure 4 and 5). From figure 4 and 5, we can see that cyanide exposure in liver is strong positively correlated with AOPPs level and CI. This two parameters indicated that cyanide

exposure induce chlorinative stress in liver. AOPPs is a novel markers of oxidant-mediated protein damage by the reaction of plasma proteins with chlorinated oxidants³⁴. Since AOPPs formed by chlorinated oxidants, it can also used as chlorinative stress condition²¹. Besides AOPPs, chlorinative stress can also be seen by CI. It is based on previous research by Hartoyo¹⁹ which investigated the involvement of chlorinative stress in dengue hemorrhagic fever patients. Also, another previous study by Marisa¹⁷ and Kania²⁰ in anti-tuberculosis medicine and iron induced liver toxixicty through the chlorinative stress pathway. In conclusion, the present study demonstrated that cyanide induced oxidative and chlorinative stress as can be seen from the level of H₂O₂, MDA, and AOPPs, the activity of MPO, and the CI in liver cells.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Bel Waer I, Masri W, Chaouali N, Khelifi F, Gana I, Nouioui A, Salah DB, Amira D, Ghorbel H, Hedhili A. Acute cyanide poisoning from jewelry cleaning solutions. Arab Journal of Forensic Science and Forensic Medicine 2015; 1 (1): 134-137.
2. Huzar TF, George T, Cross JM. Carbon monoxide and cyanide toxicity: etiology, pathophysiology and treatment in inhalation injury. Expert Review of Respiratory Medicine 2013; 7 (2): 159-170.
3. Margan M, Pearl H. Australian student charged with murdering her friend with cyanide-laced coffee in Indonesia 'won't face death penalty in found guilty'. Mail Online 2016; Available from: www.Dailymail.co.uk.
4. Sadati F, Shahsavani D, Baghshani H. Biochemical alterations induced by sublethal cyanide exposure in Common Carp (*Cyprinus carpio*). Journal of Biological Environmental Science 2013; 7 (20): 65-69.
5. Manjunatha B, Tirado JO, Selvanagayam M. Sub-lethal toxicity of potassium cyanide on Nile Tilapia (*Oreochromis niloticus*): biochemical response. International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7(3): 379-382.
6. Tulsawani R, Bhattacharya R. Effect of α -Ketoglutarate on cyanide-induced biochemical alterations in rat brain and liver. Biomedical and Environmental Sciences 2006; 19: 61-66.
7. Okafor PN, Njoku AKC. The potentials of cassava as animal feed and it's toxicity implications. Canadian Journal of Pure and Applied Sciences 2008; 2 (3): 521-525.
8. Kavasoglu M, Saroglu Y, Uysal K, Donmez M, Altikat S, Yetek I, Kuru HI. Effect of sodium cyanide on antioxidant enzyme activities and lipid peroxidation in some tissues of mirror carp (*Cyprinus carpio*). Pakistan Journal of Zoology 2015; 47 (6): 1777-1782.
9. Kistic B, Miric D, Dragojevic I, Rasic J, Popovic L. Role of myeloperoxidase in patients with chronic kidney disease. Oxidative Medicine and Cellular Longevity 2016. DOI: http://dx.doi.org/10.1155/2016/1069743.
10. Tremel J, Smejkal K. Flavonoids as potent scavengers of hydroxyl radicals. Comprehensive Reviews in Food Science and Food Safety 2016; 15: 720-738.
11. Santosa PB, Thalib I, Suhartono E, Turjaman M. Antioxidant and anti-lipid peroxidation activities of leaves and seed extracts of Gemor (*Nothaphoebe coriacea*). International Journal of Pharmacognosy and Phytochemical Research 2016; 8 (7): 1149-1153.
12. Pizzimenti S, Toaldo C, Pettazzoni P, Dianzani MU, Barrera G. The "Two-Faced" effects of reactive oxygen species and the lipid peroxidation product 4-Hydroxynonenal in the hallmarks of cancer. Cancers 2010; 2: 338-363.
13. Ho E, Galougahi KK, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. Redox Biology 2013; 1: 483-491.
14. Suhartono E, Triawanti, Yunanto A, Firdaus RT, Iskandar. Chronic cadmium hepatooxidative in rats: treatment with haruan fish (*Channa striata*) extract. APCBEE Proc 2013;5:441-5.
15. Suhartono E, Iskandar, Hamidah S, Arifin YF. Phytochemical Constituents Analysis and Neuroprotective Effect of Leaves of Gemor (*Nothaphoebe Coriacea*) on Cadmium-Induced Neurotoxicity in Rats: An In-Vitro Study. International Journal of Toxicological and Pharmacological Research 2015; 7(6): 297-302.
16. Bartosz G, Bartosz IS. Oxidative, nitrosative, and chlorinative stress: biomarkers. Dietrich-Muszalska et al. (eds.), *Studies on Psychiatric Disorders*, Oxidative Stress in Applied Basic Research and Clinical Practice. DOI: 10.1007/978-1-4939-0440-2_1.
17. Marisa D, Rudito M, Zagita MG, Biworo A, Suhartono E. Hepatotoxicity effect of rifampicin and isoniazid via chlorinative stress pathway mechanism *In-vitro*. International Journal of Toxicological and Phammacological Research 2016; 8 (1): 18-22.
18. Yunanto A, Iskandar, Suhartono E. In-vitro effects of some antibiotic drugs on saliva thiocyanate and oxidation protein products levels on newborn at risk of sepsis. International Journal of Pharmaceutical and Clinical Research 2016; 8 (1): 86-89.
19. Hartoyo E, Thalib I, Suhartono E, Yunanto A. Oxidative and chlorinative stress in children with dengue hemorrhagic fever. International Journal of Pharmaceutical and Clinical Research 2016; 8 (8): 1186-1191.
20. Kania N, Thalib I, Suhartono E. Chlorinative index in liver toxicity induced by iron. International Journal of Pharmaceutical and Clinical Research 2016; 8 (9): 1300-1304.
21. Suhartono E, Triawanti, Leksono AS, Djati MS. Effects of cadmium exposure on lipid peroxidation and chlorinative stress of rat kidney. Journal of Experimental Life Science 2015; 5 (1): 1-5.

22. Suhartono E, Triawanti, Leksono AS, Djati MS. Oxidative stress and kidney glycation in rats exposed cadmium. *International Journal of Chemical and Engineering and Applications* 2014; 5: 497-501.
23. Rao AM, Anand U, Anand CV. Myeloperoxidase in chronic kidney disease. *Indian Journal of Clinical Biochemistry* 2011; 26 (1): 28-31.
24. Yunanto A, Firdaus RT, Triawanti, Suhartono E. Advanced Oxidation Protein Products (AOPPs) of newborn at risk of sepsis as novel parameter for early-onset neonatal sepsis. *International Journal of Bioscience, Biochemistry, and Bioinformatics* 2014; 4 (2): 90-93.
25. Mills EM, Gunasekar PG, Pavlakovic G, Isom GE. Cyanide-induced apoptosis and oxidative stress in differentiated PC12 cells. *Journal of Neurochemistry* 1996; 67: 1039-1046.
26. Ola-Mudathir KF, Maduagwu EN. Antioxidant effect of methanol extract of *Allium cepa linn* on cyanide-induced renal toxicity in male wistar rats. *Nigerian Journal of Physiological Sciences* 2014; 29: 147-151.
27. Elsaid FG. Modification by aqueous extracts of *Allium kurrat L.* and *Ricinus communis L.* of Cyanide nephrotoxicity on Balb/C mice. *Oxidative Stress-Environmental Induction and Dietary Antioxidants*, Dr. Volodymyr Lushchak (Ed.), ISBN: 978-953-51-0553-4, InTech 2012. Available from: <http://www.intechopen.com/books/oxidative-stress-environmental-induction-and-dietaryantioxidants/modification-by-aqueous-extracts-of-allium-kurrat-l-and-ricinus-communis-l-on-cyanidenephrotoxic>.
28. Abdel-Zaher AO, Abdel-Hady RH, Abdel-Moneim WM, Salim SY. Alphalipoic acid protects against potassium cyanide-induced seizures and mortality. *Exp. Toxicol. Pathol* 2011; 63: 161- 165.
29. Mathangi D, Shymala R, Vijayashree R, Rao K, Ruckmani A, Vijayaraghavan R, Bhattacharya R. Effect of alphaketoglutarate on neurobehavioral, neurochemical and oxidative changes caused by sub-chronic cyanide poisoning in rats. *Neurochemistry Research* 2011; 36: 540-548. DOI:10.1007/s11064-010-0376-z.
30. Okolie NP, Iroanya CU. Some histologic and biochemical evidence for mitigation of cyanideinduced tissue lesions by antioxidant vitamin administration in rabbits. *Fd. Chemical Toxicology* 2003; 41: 463- 469. DOI:10.1016/S0278-6915(02)00224-7.
31. Olorunniyi FJ, Iniaghe MO, Adebayo JO, Malomo SO, Adediran SA. Mechanism-based inhibition of myeloperoxidase by hydrogen peroxide: enhancement of inactivation rate by organic donor substrates. *The Open Enzyme Inhibition Journal* 2009; 2: 28-35.
32. Kareska S. Factors affecting hydrogen peroxidase activity. *ESSAI* 2009; 7 (27): 82-85.
33. Allen RC, Stephens Jr. JT. Role of lactic acid bacteria-myeloperoxidase synergy in establishing and maintaining the normal flora in man. *Food and Nutrition Sciences* 2013; 4: 67-72.
34. Liu SX, Hou FF, Guo ZJ, Nagai R, Zhang WR, Liu ZQ, Zhou ZM, Zhou M, Xie D, Wang GB, Zhang X. Advanced oxidation protein products accelerate atherosclerosis through promoting oxidative stress and inflammation. *Arteriosclerosis Thrombosis Vascular Biology* 2006; 26: 1156-1162.

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