In Vitro Study of DNA Adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) Formation Based on Fenton-like Reaction Using Chromium (III) and Benzo[a]pyrene

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Abstract: In this research, study of 8-hydroxy-2'-deoxyguanosine (8-OHdG) caused by exposure of Chromium (III) and Benzo[a]pyrene was conducted. This study was done by reacting 2'-deoxyguanosine as DNA base with xenobiotic like Benzo[a]pyrene with variation of pH (7.4 and 8.4), incubation time (7 and 12 hours), and incubation temperature (37°C and 60°C). On this mixture, another observation was conducted with addition of Chromium (III) and H_2O_2 as the Fenton-like reaction reagent. 8-OHdG DNA Adduct was then analyzed with High Performance Liquid Chromatography (HPLC) reversed phase with UV detector on 245 nm wavelength. The mixture of pH 6.7 Phospate Buffer 10mM and LC-grade methanol with ratio of 85:15 and 1 mL/minute flow rate were used in the measurement of 8-OHdG. On every mixture in all pH, time, and temperature variation, 8-OHdG was detected with the concentration below the Limit of Quantification, thus the concentration cannot be quantified. Addition of the Fenton-like reaction reagent also impacted on higher 8-OHdG concentration in result. Longer incubation time and higher incubation temperature were proved to generate more 8-OHdG, meanwhile the variation of pH did not significantly affect the concentration of generated 8-OHdG in the mixture.

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1 INTRODUCTION

Benzo[a]pyrene (BaP) is a Polycyclic Aromatic Hydrocarbon (PAH) compound. PAH is an aromatic molecule composed of carbon and hydrogen atoms, and consists of two or more aromatic ring molecules. Some PAH compounds are known to have quite high carcinogenic properties, especially those with 4 to 6 aromatic rings (Luch & Baird, 2005). BaP is a PAH compound that has the highest carcinogenic potential and is used as an indicator of PAH contamination in the environment. BaP can be formed through pyrolysis and pyro synthesis. Pyrolysis is the reaction of breaking organic matter into simple fragments, while pyro synthesis is the reaction of the formation of aromatic compounds from pyrolysis fragments. Pyrolysis occurs at sufficiently high temperatures in a dry environment or without water. BaP is formed when the pyrolysis temperature is above 425°C (Guillén *et al*., 2000).

Food processing such as smoking, grilling, or roasting may lead to the increase of BaP concentration in food, since BaP in the smoke that originated from the woods can be easily absorbed by the food during the process. Stolyhwo in 2005 (Stołyhwo & Sikorski, 2005) reported that on the outside skin of the smoked fish contained 50 µg/kg BaP. Similar research is also conducted by Kafeelah A. in 2015 (Kafeelah *et al*., 2015), which states that in smoked fish, contained Polycyclic Aromatic Hydrocarbons (PAHs) with a very significant amount compared to non-smoked fish.

The exposure of a xenobiotics in the human body can cause many variety of risks, depending on the xenobiotic toxicity. BaP is a carcinogenic compound, since BaP can trigger free radical formation in the human body through Fenton / Fenton-like reaction. Radical compound such as radical hydroxyl can react with the forming base of Deoxyribonucleic Acid (DNA), and result in a conformational change in the basic structure of the DNA. This occurence produces a new compound called DNA adduct. This is a reversible event, in which the DNA in the body has its regeneration

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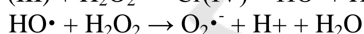
mechanism to recover its structure. However, if this event occurs repeatedly or exposure to xenobiotics persists, it can be resulted in permanent damage to the structure of DNA and increasing the risk of DNA mutation on the cell (carcinogenesis) in the body of the living organism (Briedé *et al.,* 2004).

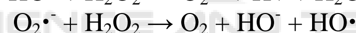
DNA that have mutated by exposure to xenobiotics through a series of reactions that produce radical compounds can form one of the DNA adduct compounds 8-hydroxy-2'-deoxyguanosine (8-OHdG). These compounds can come out of the cell nucleus and contained in human blood or urine. 8-OHdG may act as a biomarker of DNA structure modification; if the biological sample analysis detects the presence of 8-OHdG, then it is can be confirmed that the DNA structure has been damaged.

The Fenton-like reaction is a formation of a radical compound, using a metal compound beside iron as the catalyst. In this study, Chromium (III) were used in the in vitro reaction. Chromium is one of the most abundant metals in nature and can be exposed into living processes through its use in industries such as wood preserving, and plating (Metze, 2005). The presence of Chromium (III) metal becomes the catalyst for H2O2 to form a hydroxyl radical. This can be understood by the following series of reactions (Nixon, 2005):

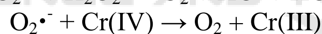
$$Cr(III) + H_2O_2 \rightarrow Cr(IV) + HO^- + HO\bullet$$



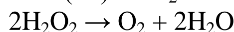
$$HO\bullet + H_2O_2 \rightarrow O_2\bullet^- + H+ + H_2O$$



$$O_2\bullet^- + H_2O_2 \rightarrow O_2 + HO^- + HO\bullet$$



$$O_2\bullet^- + Cr(IV) \rightarrow O_2 + Cr(III)$$



$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

In this in vitro research, the damage of the DNA structure as the result in the xenobiotic BaP exposure to one of the DNA base 2'-deoxyguanosine-5'monohydrate (2'-dG) and the formation of 8-OHdG as a DNA Adduct were studied. This reaction were involving the radical hydroxyl pathway from the Fenton-Like reaction using Chromium (III)..

2 MATERIAL AND METHODS

2.1 Material

The materials that used in the in vitro study are 2'deoxyguanosine -5'-monohydrate (Sigma Aldrich), Hydrochloric Acid, Sodium Hydroxide, Phospate Buffer, Acetic Buffer, Chromium (III) Oxide (Merck), LC-Gradient Grade Methanol (Merck), Dimethyl Sulfoxide (Merck), Benzo[a]pyrene, 8-hydroxy-2'-deoxyguanosine standard (Sigma Aldrich), H_2O_2 (Merck). All materials were used without further purification. HPLC data were acquired using Hitachi Primaide HPLC with YMC-TriartC18/S-5um/12nm, 250 x 4.6 mml.D reversed phase column and 254 nm UV Detector.

2.2 Methods

2.2.1 Characterization and Method Validation of 8-OHdG Measurement

20 µL of 8-OHdG standard with the concentration between 10-100 ppb were injected into HPLC column using sodium phosphate pH 6.7 buffer 10 mmol/L and methanol with the ratio of 85:15 as the mobile phase and 1mL/minute flow rate. The results are then plotted on the calibration curve of 8-OHdG. 8-OHdG quantification in the sample determined from the linearity equation from the calibration curve. Repeatability test was done by injecting 30 ppb and 80 ppb concentration of 8-OHdG standard to the HPLC column 6 times continuously. The repeatability then acquired by comparing the standard peak in every measurements.

2.2.2 In Vitro Study

The DNA base used was 2'-deoxyguanosine (6 ppm) incubated with BaP (60 ppm) under variation of condition in pH (7.4 and 8.4), temperature (37°C and 60°C), and incubation time (7 hours and 12 hours), then formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) is observed. Incubation with Chromium (III) metal (120 ppm) and H_2O_2 (120 ppm), and with the combination was also performed. Before analyzed, mixture was first centrifuged for 15 minutes and then decanted. A total of 20 µL sample were injected into a reversed-phase HPLC column under the same condition as in the characterization and method validation. The results of the sample measurements were then compared with the 8-OHdG standard calibration curve. The quantification of 8-OHdG in the sample was done by measuring the peak area of the sample, then calculated using the equation from 8-OHdG standard calibration curve at various concentrations.

3 RESULTS AND DISCUSSION

In this study, High Performance Liquid Chromatography were used to determine the concentration of DNA Adduct formed. Linearity test

was done to determine the detector response in the analyte concentration alteration. This test was done with injecting 8-OHdG standard in a series of concentration (10, 30, 50, 80, and 100 ppb) to the instrument, and then the peak area plotted in the calibration curve. Coefficient of correlation 0.9975 and the regression equation of y = 81.169x – 321.52 were obtained, and the limit of detection of 5.19 ppb and limit of quantification 17.29 ppb also obtained by a statistical equation. Limit of detection defined as the concentration in which the analyte can be confirmed as 8-OHdG, but cannot be quantified. As for the limit of quantification, any concentration of analyte in the sample that fall upper the limit can be quantified statistically (Riyanto , 2014).

The repeatability test was performed to determine the accuracy of the HPLC method used in the measurement. This test is done by measuring the 8-OHdG standard at concentrations of 30 ppb and 80 ppb repeatedly as much as six times. One of the values that can precipitate precision is the value of the coefficient variation. In this research, the value of coefficient variation for 8-OHdG standard with concentration of 30 ppb is 1,627% and for 8-OHdG standard with concentration 50 ppb is 0,996%. The incubation of mixture was done under various condition of pH (7.4 and 8.4), temperature (37°C and 60°C) and incubation time (7 and 12 hours). pH 7.4 and 37°C was used to be the analogue of the physiological pH and human body temperature, a higher pH, temperature, and the variation of incubation time were used to characterized the DNA Adduct formation profile. Also, pH 8.4 can be used as an analogue of the human bile condition (Sutor & Wilkie, 1976).

The stationary phase used in this study is column C18. In this condition the peak was obtained at a retention time of 6,007 min for standard 2'-dG pH 7.4, 6,007 min for standard 2'-dG pH 8.4, and 7,473 min for 8-OHdG. Standard chromatogram profiles for standard 2'-dG pH 7.4, 2'-dG pH 8.4, and 8-OHdG can be seen in Fig. 1.

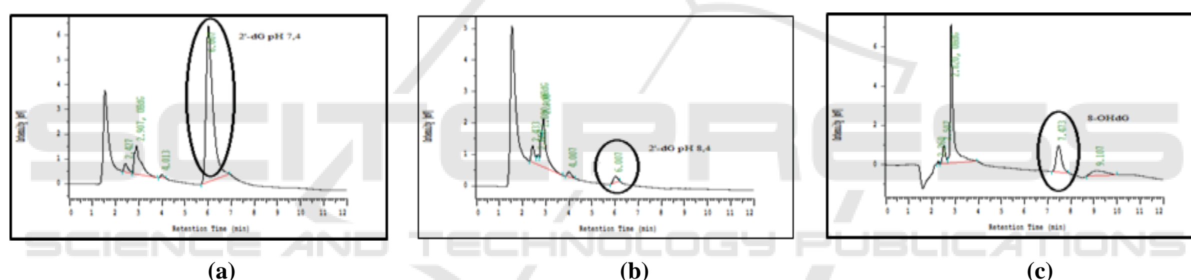
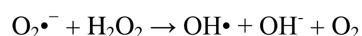


Figure 1: (a) Standard Chromatogram dG pH 7.4 6 ppm, (b) Standard Chromatogram dG pH 8.4 6 ppm, (c) Chromatogram Standard 8-OHdG 500 ppb

The results obtained from the mixture chromatograms show that 8-OHdG is formed at all time, temperature, and pH variations. This suggests that 8-OHdG can be formed at human physiological temperatures of 37°C, and at physiological pH of 7.4, so that 8-OHdG is shown to act as a biomarker of DNA structure damage to the human body. 8-OHdG is considered detectable because all values are above LOD, but the value cannot be quantified because it is below the LOQ.

To observe the effect of metal addition in the DNA Adduct formation on 2'-dG, whether Cr (III) react independently in the formation of 8-OHdG or synergistic with the xenobiotic (in this case BaP), incubation of the 2'-dG and metal mixture Cr (III) was done. Although the pathway and its formation mechanisms are not yet known, on the measurement results, it is concluded that Cr (III) and 2'-dG can form DNA Adduct. This indicates that the effect in addition of Cr (III) metal in the BaP and 2'-dG mixture can increase the concentration of the 8-OHdG. This statement corresponds to the concentrations obtained from the 2'-dG and BaP mixtures, the result of most of the mixtures indicating that the obtained 8-OHdG concentrations were greater in the 2-dG, BaP, and Cr (III) mixtures. This can be due to the addition of Cr (III) to give a synergistic effect with BaP because Cr (III) can also damage the DNA structure and produce DNA Adduct (Fang *et. al*, 2014).

The increase in 8-OHdG concentration in H_2O_2 addition can be correlated by the fact that H_2O_2 is a strong oxidant which can then be reduced to a hydroxyl radical (•OH) (Nixon, 2005) with the reaction:



$$O_2\bullet^{-} + H_2O_2 \rightarrow OH\bullet + OH^{-} + O_2$$

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$$Cr^{3+} + H_2O_2 \rightarrow Cr^{6+} + OH\bullet + OH^-$$

To analyze the effect of H_2O_2 addition in 8-OHdG formation, it can be done by comparing the results of 8-OHdG concentrations obtained on mixtures using H_2O_2 and mixtures not using H_2O_2. For the effect on the addition of metal and H_2O_2 as oxidizing agent can be seen in Figure 2.

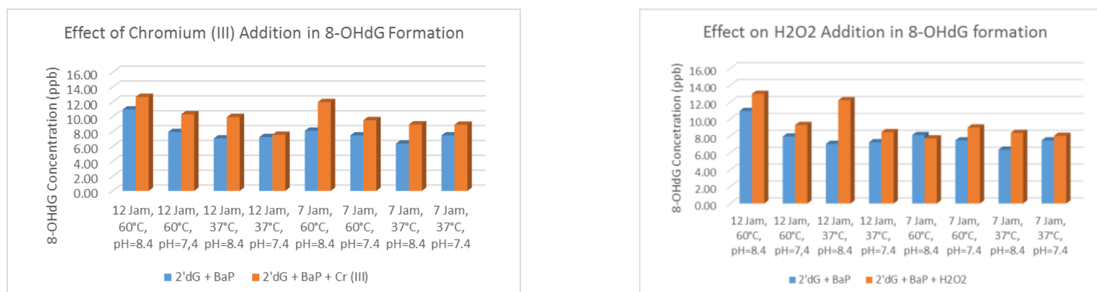

Figure 2: (a). The Effect of Metal Addition (b). The Effect on H_2O_2 Addition

Theoretically, the occurrence of Fenton-like reactions can trigger the formation of more radical compounds so that more DNA adducts are formed. From the result, it can be seen that the addition of Fenton-like reaction reagent increase 8-OHdG formation results on average as much as 40.9%. The highest concentration of 8-OHdG was formed in this mixture, compared to the addition of metal (average concentration increase of 21.4%), or addition of H_2O_2 (average concentration increase 27.5%). The mixture between 2'-dG, BaP, Cr (III), and H_2O_2 becomes a mixture which form 8-OHdG with the highest concentration in all variations. This correlation can be seen in figure 3.

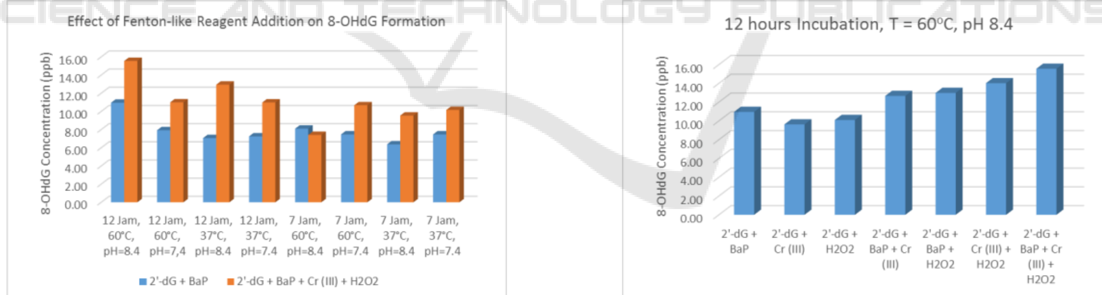

Figure 3: (a) The Effect of Fenton Reagent Addition (b) 8-OHdG Formation under variation of condition

Incubation of mixture at higher temperatures and longer incubation time yields 8-OHdG with higher concentrations. This is because increasing temperature will increase the kinetic reaction, and longer incubation time will increase the duration of collisions between reagents molecule. But changes in pH were observed to have little effect on the formation of 8-OHdG. The correlation can be seen on figure 4.

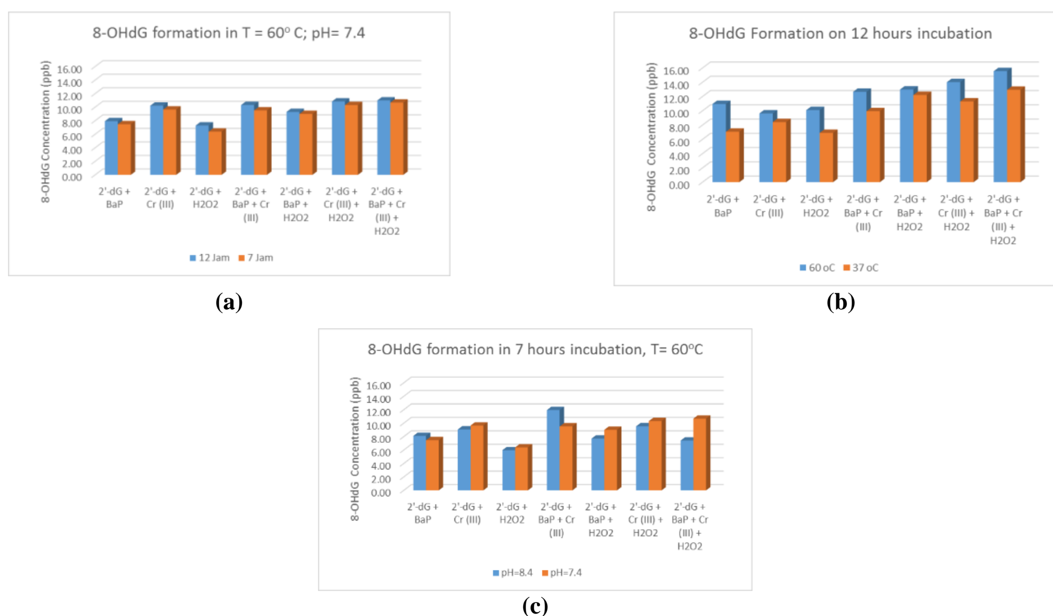

Figure 4: (a) Effect on Incubation time variation (b) Effect on Temperature variation (c) Effect on pH variation

4 CONCLUSIONS

8-OHdG DNA Adduct formed in the mixture was detected on 6.5-7.1 minute retention time in the instrument. Reaction between 2'-deoxyguanosine and BaP under various condition and addition of the Fenton-like reagent resulted in 8-OHdG formation with the concentration above the LOD, but below the LOQ, so the value cannot be quantified. Addition of metal (Chromium (III)) and strong oxidation agent (H2O2) increase the concentration of 8-OHdG in the sample mixture. The mixture between 2'-dG, BaP, and the Fenton-like reagent yield the highest concentration of DNA Adduct detected than other mixture. Reaction on 60oC and 12 hours incubation time increasing the 8-OHdG concentration.

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REFERENCES

Briedé, J., Godschalk, R., Emans, M., de Kok, T., van Agen, E., & van Maanen, J. et al. (2004). In Vitro and In Vivo Studies on Oxygen Free Radical and DNA Adduct Formation in Rat Lung and Liver during Benzo[a]pyrene Metabolism. Free Radical Research, 38(9), 995-1002.

Fang, Z., Zhao, M., Zhen, H., Chen, L., Shi, P., & Huang, Z. (2014). Genotoxicity of Tri- and Hexavalent Chromium Compounds In Vivo and Their Modes of Action on DNA Damage In Vitro. Plos ONE, 9(8), e103194. Moore, R., Lopes, J., 1999. Paper templates. In *TEMPLATE'06, 1st International Conference on Template Production*. SCITEPRESS.

Guillén, M., Sopelana, P., & Partearroyo, M. (2000). Polycyclic Aromatic Hydrocarbons in Liquid Smoke Flavorings Obtained from Different Types of Wood. Effect of Storage in Polyethylene Flasks on Their Concentrations. Journal Of Agricultural And Food Chemistry, 48(10),

Kafeelah, A., Lucy, N., Kafayat, A., Shehu, L., Julius, I., & Titus, O. (2015). Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health. African Journal Of Food Science, 9(3), 126-135.

Luch, A., & Baird, W. (2005). Metabolic Activation and Detoxification of Polycyclic Aromatic Hydrocarbons. The Carcinogenic Effects Of Polycyclic Aromatic Hydrocarbons, 19-96.

Metze, D., Jakubowski, N., Klockow, D., (2005). Speciation of chromium. In: Cornelis, R., Crews, H., Caruso, J., Heumann, K.G. (Eds.), Handbook of Elemental Speciation II: Speciation in the Environment, Food, Medicine & Occupational Health. John Wiley & Sons, Ltd.

Nixon Ogendi Mwebi, (2005). Fenton & Fenton-Like Reactions: Nature of Oxidizing Intermediates. University of Maryland, College Park

Riyanto. (2014). Validasi & Verifikasi Metode Uji: Sesuai dengan ISO/IEC 17025 Laboratorium Pengujian dan Kalibrasi. Yogyakarta: Deepublish, Ed.1, Cet. 1:66

Smith, J., 1998. *The book*, The publishing company. London, 2nd edition.

Stołyhwo, A., & Sikorski, Z. (2005). Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Food Chemistry, 91(2), 303-311.

Sutor, D. J., & Wilkie, L. I. (1976). Diurnal variations in the pH of pathological gallbladder bile. Gut, 17(12), 971–4.s.

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