3._Oxidative_Stress_and_Protein_Carbonylation.pdf

by

Submission date: 23-May-2023 09:18AM (UTC+0700)
Submission ID: 2099711316
File name: 3._Oxidative_Stress_and_Protein_Carbonylation.pdf (319.75K)
Word count: 3651
Character count: 19293

Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(5); 300-305

ISSN: 0976 822X

Research Article

Oxidative Stress and Protein Carbonylation as a New Tool to Assess Redox Status in Liver Toxicity Induced by Iron

Nia Kania[1], Iskandar Thalib[2], Eko Suhartono[3]

[1]*Research Center for Toxicology, Cancer, and Degenerative Disease, Department of Pathology, Ulin Genral Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia*
[2]*Research Unit of Mutiara Bunda Mother and Child Hospital, Martapura of South Kalimantan, Indonesia*
[3]*Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia*

Available Online: 1st October, 2016

ABSTRACT

The objectives of this study were to determine the effect of iron (Fe) on Oxidative Stress Index (OSI) and Protein Carbonylation (PC), and their correlation in liver homogenate in vitro. In this experiment, a liver sample was taken from male rats (*Rattus novergicus*). Samples then homogenized and divided into four groups with; T0 served as control which contain liver homogenate only; T1 which contains liver homogenate+30 μg/dl of ferrous sulphate (FeSO4); T2 which contains liver homogenate+ 300 μg/dl of FeSO4; and T3 which contains liver homogenate+3000 μg/dl of FeSO4. After treatment, liver Total Oxidant (TO), Total Antioxidant (TA), OSI and PC level were estimated. The results revealed that Fe exposure could significantly increased TO, OSI, and PC level, and decreased TA level in liver cells ($P<0.05$). The result also revealed that OSI that induced by Fe have a strong correlation with PC (r=0,9388). These results indicated that Fe exposure induce OS which can be seen from the increasing of TO and OSI levels, and the decreasing of TA. Also, the results indicated that Fe exposure induced PC which related to Oxidative Stress (OS).

Keywords: Coal Dust, Iron, Oxidative Stress, Protein Carbonylation

INTRODUCTION

Indonesia is blessed with abundant mineral resources[1]. One of the world's most plentiful mineral energy that can be found in Indonesia is coal[2]. In Indonesia the coal resources are plenty, particularly in several area including South Kalimantan[3]. It is well documented that the coal usage raises a number of environmental challenges. One of the important enviromental problem caused by coal mining is air pollution[4].

In each process of coal mining, a great amount of dust is produced[5]. Coal mine dust is a mixture that contains more than 50 substances[6]. According to Setiawan et al[7]. study, the coal dust contained several metal with the highest metal level is iron (Fe). Fe is a necessary chemical element, involved in a wide spectrum of fundamental biological processes. However, Fe have several adverse effects in excessive amounts[8]. Several studies reported that exposure to excess Fe doses may lead to liver cell damage, mutation, and malignant transformations[9].

Fe toxicity is developed through the production of reactive oxygen species (ROS)[10]. Our previous study showed that Fe could induced the formation of ROS, such as hydrogen peroxide[11]. Fe may contribute significantly to the generation of ROS via Fenton and Haber-Weiss reaction. Increase the amount of ROS could promote lipid peroxidation and changes in antioxidant acivity[12-13]. This condition could shift the balance of oxidative status, known as oxidative stress (OS)[14].

Recently, OS not only evaluated by analyze the level of oxidants and antioxidants, but also analyze through a proportion or ratio. This ratio was first proposed by Sharma et al.[15] and known as OS index (OSI)[16]. OSI is a ratio between Total Oxidants Status (TOS) and Total Antioxidant Status (TAS)[17-18]. Generally, TOS and TAS was measured by the method of Erel. This present study used a different appoarch. OSI is calculated by measuring the ratio between H_2O_2 level (Total Oxidant (TO)) and the total acitivity of SOD, CAT, and Pox (Total Antioxidant (TA)). OS could promote several biomolecule modifications. Among a wide range of OS-derived modifications, Protein Carbonylation (PC) is known to be a major hallmark of OS[19-20]. PC is a type of protein oxidation that can be promoted by OS condition. ROS during OS condition yields highly reactive carbonyl derivatives resulting either from oxidation of the side chains of lysine, arginine, proline, and threonine residues – particularly via metal-catalysed oxidation-or from the cleavage of peptide bonds by the α-amidation pathway or by oxidation of glutamyl residues. This chain reaction leading to ageneration of some protein carbonyls[20]. PC is the most widely used biomarker for oxidative damage to proteins, and reflects cellular damage induced by multiple forms of ROS[21]. Considering the Fe is related to OS status and OS related to PC, this present study is aimed to measured the OSI, PC level, and correlation between those two parameters in liver homogenate.

*Author for Correspondence: ekoantioxidant@gmail.com

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 Lambung 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 homogenized in phosphate buffer saline (pH 7.0) and was ready to used for in vitro experimental models.

Experimental Models

Homogenate samples were divided into 4 groups (1 control group and 3 treatment groups). Control (T0) group: liver homogenate only; Treatment 1 (T1) group: liver homogenate + 30 μg/dl of ferrous sulphate ($FeSO_4$); Treatment 2 (T2) group: liver homogenate + 300 μg/dl of ferrous sulphate ($FeSO_4$); Treatment 3 (T3) group: liver homogenate + 3000 μg/dl of ferrous sulphate ($FeSO_4$). Each solution then incubated at 37°C for 1 hour. After incubation, liver TOS, TAS, OSI and PC level were estimated.

TO analysis

The TO was analyze through the measurement of H_2O_2. H_2O_2 level was calculated by the FOX2 method with slight modification. Solutions measured spectrophotometrically at λ = 505 nm. Standard and test solutions consisted of 1 M H_2O_2 200 µL and 200 µL serum, respectively, with the addition of 160 µL PBS pH 7.4, 160 µL $FeCl_3$ (251.5 mg $FeCl_3$ dissolved in 250 ml distilled water) and 160 µL 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 $FeCl_3$ 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 λ=505 nm, using the supernatant of each solution. H_2O_2 level was expressed as μM[22-23].

TA analysis

TA is simply the sum of the measurable antioxidants. The measurable antioxidants were SOD, CAT, and Pox. SOD was measured according to the method that previously described by Misra and Fridovich[13]. CAT activity was measured by the method of Aebi[24]. Pox activity was measured by the method of Pruitt[25].

Oxidative stress index analysis

The OSI was a ratio between TO and TA. It was calculated following to equation:

$$\text{OSI } (\mu\text{M/unit}) = \frac{\text{TO } (\mu\text{M})}{\text{TA (unit)}}$$

Protein carbonyl level analysis

PC was calculated by measuring the total protein carbonyl content. The total protein carbonyl content was determined by colorimetric method. The liver homogenate (0.5ml) was pipetted into 1.5 ml centrifuge tube and 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine in 2 M HCl was added and allowed to stand at room temperature for 1 hour, with vortexing every 10-15 minutes. Then, 0.5ml of 20% Trichloroacetic acid was added followed by centrifugation. The supernatant was discarded and the pellets was washed 3 times with 1 ml ethanol-ethyl acetate (1:1) to remove free reagent. The obtained precipitated protein was redissolved in 0.6 ml guanidine solution. Carbonyl content was calculated from maximum absorbance (390nm)[22].

Statistical analysis

The results were expressed as mean±SE for three replicates. Significance of mean differences of all TO, TA, OSI, and PC between treatment and control groups were statistically compared using one-way Analysis of Variance (ANOVA) or Kruskal-Wallis test and followed by a post hoc Tukey's Honestly Significant Difference (HSD) or Mann-Whitney test for multiple range test. To determine the relationship between OS and PC, OSI was correlated to PC. The linear correlation regression was used to analyse the relationship between those variables and the correlation coefficient (r) was derived to measured the strength of association between those two parameters. Significance was set at $P<0.05$. The software used for the data analysis were the Statistical Package for the Social Sciences (SPSS) version 16.0 and Microsoft Excell 2010 for Windows Vista.

RESULTS

In this present study, OS status in liver homogenate induced by iron was evaluated by measuring OSI. To obtained the level of OSI, TO and TA must calculated. The results shows in figure 1 and 2. According to figure 1 and 2, Fe exposure seems to increase the level of TO and decrease the level of TA. ANOVA test results shows that Fe exposure cause a significant increase in TO level and significant decrease in TA level ($p<0.05$). Post Hoc Tukey HSD test results show that there are significant differences in TO level between between T0 and T3, and T1 and T3 group, and in TA there are significant differences between group of treatments except between T2 and T3 group (table 1).

After determine the TO and TA level, the level of OSI can be obtained. The results shows in figure 3. After the administration Fe, OSI level seems to be increase compared to the controls (Figure 3). There was a dose dependent increase in the OSI level in the liver tissues. ANOVA test results shows that Fe exposure cause a significant increase in OSI level ($p<0.05$). Post Hoc Tukey HSD test results show that there are significant differences between T0 and T3, and T1 and T3 group (table 1).

The effect of different dose of Fe on PC level is presented in figure 4. It was observed that the PC level increase in all Fe treatment groups as compared to control group. The increase in PC level were in a dose dependent manner. However, statistical analysis test results shows that the decreasing of MPO activity were not statistically significant (Kruskal-Wallis test, $P>0.05$). Figure 5 shows

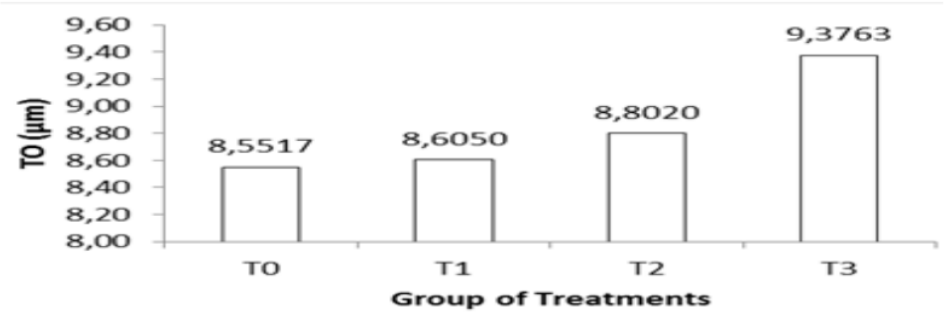

Figure 1: Comparison of TO level between group of treatments

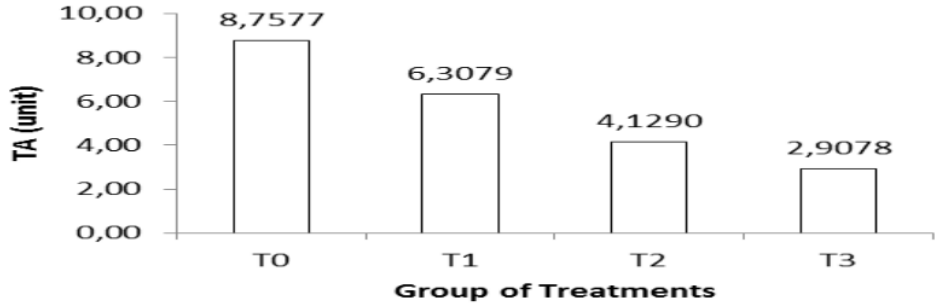

Figure 2: Comparison of TA between group of treatments

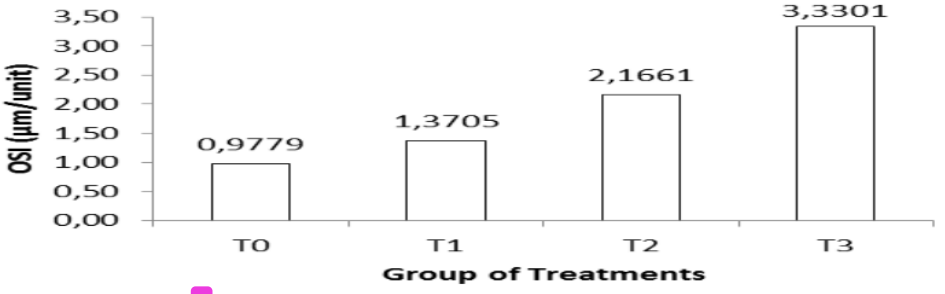

Figure 3: Comparison of OSI level between group of treatments

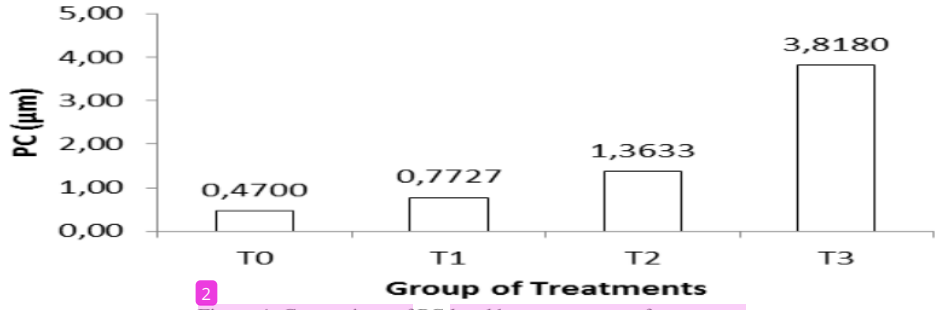

Figure 4: Comparison of PC level between group of treatments

the correlation between the level of OSI and the level of PC in liver homogenate induced by Fe with different concentrations. OSI level was strong positively correlated with PC level (r = 0,938).

Table 1: Post Hoc Tukey HSD test results of TO, TA, and OSI level in the different group of treatments

Group Comparison	TO		TA		OSI	
	p-value	Interpretation	p-value	Interpretation	p-value	Interpretation
T0-T1	0.727	NS	0.001	S	0.285	NS
T0-T2	0.128	NS	0.000	S	0.008	NS
T0-T3	0.001	S	0.000	S	0.000	S
T1-T2	0.218	NS	0.001	S	0.049	NS
T1-T3	0.001	S	0.000	S	0.000	S
T2-T3	0.005	NS	0.029	NS	0.009	NS

TO: Total Oxidant; TA: Total Antioxidant; OSI: Oxidative Stress Index S: Significant; NS: Non-Significant; T0: control group; T1: treatment with 30 μg/dl; T2: treatment with 300 μg/dl; T3: treatment with 3000 μg/dl. Results presented as mean±SD. p-Values were calculated using the One Way ANOVA followed by Post Hoc Tukey HSD or Kruskal-Wallis test and followed by Mann-Whitney test; $p < 0.05$ was considered statistically significant.

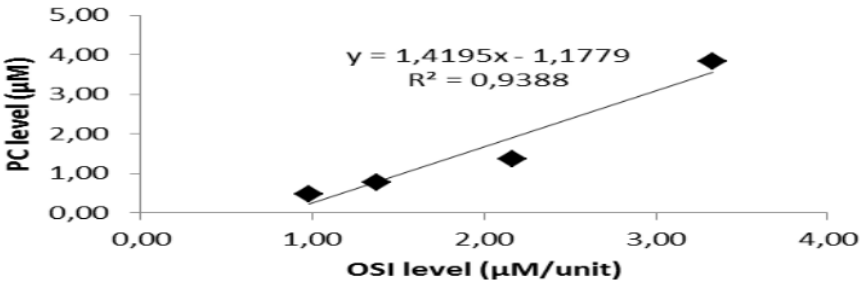

Figure 5: Correlation between OSI level and PC level in liver homogenate after iron exposure.

DISCUSSION

Fe is crucial to biologic functions, including respiration, energy production, DNA synthesis, and cell proliferation. The human body has evolved to conserve Fe in several ways, including the reycling of Fe after the breakdown of red cells and the retention of Fe in the absence of an excretion mechanism[26]. However, although Fe is vital for so many metabolic reactions, free Fe is toxic to tissue, damaging cellular membrans, proteins, and DNA[27]. The toxicity of Fe was known related to the property to generate ROS. Results of this present study revealed an oxidative stress in liver homogenate exposed to Fe in different concentration. It can be seen from the level of TO, TA, and OSI. However, to best of our knowledge, there have been no investigations of association between Fe exposure with OS in liver cells homogenate with this paremeters. However, several reports have documented a relationship between excessive Fe diets and OS in human and animal model[28-29].

The level of TO was significantly increased especially if the liver exposed to Fe in higher concentrations (3000 μg/dl). This is because the measurement of TO only measured H_2O_2, and not measured the other ROS such as, radical superoxide and radical hydroxyl. It is well known that Fe induced the formation of ROS via both Fenton and Haber-Weiss reaction. The ROS that produced during this two reactions were H_2O_2, radical superoxide, and radical hydroxyl[30].

Besides as a result of increased levels of ROS, OS can occured as a result of reduced physiological activity of antioxidant defenses against ROS[31]. It was supported by the result of this present study. The result revealed that Fe exposure in all concentrations could significanlty decreased the TA in liver homogenate. From this results, we can also assumed that Fe exposure affect all enzymatic antioxidant. From this point of view, we can also assumed that Fe not only increased the level of H_2O_2, but also increased another ROS such as radical superoxide and hydroxyl radical. Again, this result is in line with TOS level result that mentioned earlier. Recently, OS is evaluated not only by measuring the levels of oxidants and antioxidants. OS can also evaluated by measuring the ratio between them. This first appoarch was made in human medicine by Sharma et al.[15] In this study we used that appoarch but with slight modification. The result revealed that Fe exposure increased OSI. This increasing indicated that the increasing risk of OS because of the increase in ROS production or defensive antioxidant consumption[17].

OS elicited oxidative damage to several biomolecules, including protein[32]. Protein damage by OS can be evaluated with several marker, such as PC. PC is a type of protein oxidation that can be promoted by ROS and PC is the most widely used biomarker for oxidative damage to protein[20]. It also in line with this results of this present study. According to the result, Fe exposure increase OS as can bee seen by the increasing of OSI. OS then induced the protein damage that can be seen by the increasing of PC level and strong correlation between OSI and PC level in liver cells. However, as far as we know, there have been no investigations of association between OS and PC and their correlation induced by Fe in liver cells homogenate.

In conclusion, the present study demonstrated that Fe induced OS and protein damage as can be seen from the level of OSI and PC in liver cells. Also, the present study demonstrated that OS induced by Fe, related to protein damage in liver cells.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Resosudarmo BP, Resosudarmo IAP, Sarosa W, Subiman NL. Socioeconomic Conflicts in Indonesia's Mining Industry. The Henry L. Stimson Center 2009. ISBN: 978-0-9821935-0-1.
2. Joppe BC. Coal transport Kalimantan. Thesis Report 2011. Delft University of Technology: Delft.
3. Fatah L. The impacts of coal mining on the economy and environment of South Kalimantan province, Indonesia. Economy and Environment Program for South East Asia 2007. ISSN 1608-5434 ; 2007-RR2.
4. Aneja VP, Isherwood A, Morgan P. Characterization of particulate matter (PM_{10}) related to surface coal mining operations in Appalachia. Atmospheric Environment 2012; 54 (2012): 496-501.
5. Cui D, Baisheng N, Hua Y, Linchao D, Caihong Z, Fei Z, Hailong L. Experimental research on optimization and coal dust suppression performance of magnetized surfactant solution. Procedia Engineering 2011; 26: 1314-1321.
6. Chen W, Liu Y, Huang X, Rong Y. Respiratory diseases among dust exposed workers. Respiratory Diseases, Dr. Mostafa Ghanei (Ed.) 2012. ISBN: 978-953-307-964-6. InTech. Available from: http://www.intechopen.com/books/respiratory-diseases/respiratory-diseases-among-dust-exposed-workers.
7. Setiawan B, Kania N, Nugrahenny D, Nurdiana N, Widodo MA. Subchronic inhalation of particulate matter 10 coal dust induces atherosclerosis in the aorta of diabetic and nondiabetic rats. Biomarkers and Genomic Medicine 2014; 6: 67-73.
8. Guo W, Zhang J, Li W, Xu M, Liu S. Disruption of iron homeostasis and resultant health effects upon exposure to various environmental pollutants: A critical review. Journal of Environmental Sciences 2015; 34: 155-164.
9. Grazuleviciene R, Nadisauskiene R, Buinauskiene J, Grazulevicius T. Effects of elevated levels of manganese and iron in drinking water on birth outcomes. Polish Journal of Environmental Study 2009; 18 (5): 819-825.
10. Udipi S, Ghugre P, Gokhale C. Iron, oxidative stress and health, oxidative stress- molecular mechanisms and biological effects, Dr. Volodymyr Lushchak (Ed.), 2012, ISBN: 978-953-51-0554-1, InTech.
11. Kania N, Thalib I, Suhartono E. Chlorinative index in liver toxicity induced by iron. International Journal of Toxicological and Pharmacological Research 2016. Article in Press.
12. Suhartono E, Nijka JA, Anhar VY, Sari RA, Edyson, Marisa D. Anti-lipid peroxidation activities of three selected fruits juices against cadmium induced liver damage *in vitro*. Journal of Tropical and Life Sciences 2015; 5 (2): 75-79.
13. 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.
14. Aslan M, Horoz M, Celik H. Evaluation of oxidative status in iron deficiency anemia through total antioxidant capacity measured using an automated method. Turkish Journal of Hematology 2011; 28: 42-46.
15. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ, Agarwal A. The reactive oxygen species vs total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Human Reproduction 1999; 14: 2801–2807.
16. Dilek F, Ozceker D, Ozkaya E, Guler N, Tamay Z, Kesgin S, Yazici M, Kocyigit A. Oxidative stress in children with chronic spontaneous urticaria. Oxidative Medicine and Cellular Longevity 2016; 2016: 1-8.
17. Abuelo A, Hernandez J, Benedito JL, Castillo C. Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 2013; 7 (8): 1374-1378.
18. Yalcin S, Ulas T, Eren MA, Aydogan H, Camuzcuoglu A, Kucuk A, Yuce HH, Demir ME, Vural M, Aksoy N. Relationship between oxidative stress parameters and cystatin C levels in patients with severe preeclampsia. Medicina (Kaunas) 2013; 49(3): 118-123.
19. Fedorova M, Bollineni RC, Hoffmann R. Protein carbonylation as a major hallmark of oxidative damage: update of anlytical strategies. Mass Spectrometry Reviews 2013. DOI: 10.1002/mas.
20. Pradeep AR, Ramchandraprasad MV, Bajaj P, Rao NS, Agarwal E. Protein carbonyl: An oxidative stress marker in ginggival crevicular fluid in healthy, ginggivitis, and chronic periodontitis subjects. Contemporary Clinical Dentistry 2013; 4 (1): 27-31.
21. Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A. Protein carbonylation, cellular dysfunction, and disease progression. Journal of Cellular Molecular Medicine 2006; 10 (2): 389-406.
22. 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.
23. 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 Phamrmacological Research 2016; 8 (1): 18-22.

24. Yunanto A, Gunawan P, Iskandar, Suhartono E. Effect of antibitiotic applications on salivary amylase and catalase kinetic parameters on neonatal at risk of sepsis in vitro. International Journal of Toxicological and Pharmacological Research 2015-2016; 7 (6): 269-273.
25. Abdolsamadi HR, Goodarzi MT, Mortazavi H, Robati M, Ahmadi-Motemayel F. Comparison of salivary antioxidants in healthy smoking and non-smoking men. Chang Gung Medical Journal 2011; 34: 607-611.
26. Camaschella C. Iron deficiency anemia. The New England Journal of Medicine 2015; 372: 1832-1843.
27. Drygalski AV, Adamson JW. Iron metabolism in man. Journal of Parenteral and Enteral Nutrition 2013; 37: 599.
28. Morgan JLL, Ritchie LE, Crucian BE, Theriot C, Wu H, Sams C, Smith SM, Turner ND, Zwart SR. Increased dietary iron and radiation in rats promote oxidative stress, induce localized and systemic immune system responses, and alter colon mucosal environment. FASEB Journal 2014; 28: 1-13.
29. Sevcikova M, Modra H, Slaninova A, Svobodova Z. Metals as a cause of oxidative stress in fish: a review. Veterinarni Medicina 2011; 56 (11): 537-546.
30. Das TK, Wati MR, Shad KF. Oxidative stress gated by Fenton and Haber Weiss reactions and its association with Alzheimer's disease. Archive of Neuroscience 2014; 2 (3): e20078.
31. Poljsak B, Suput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative Medicine and Cellular Longevity 2013; 2013: 1-11.
32. Rahman T, Hosen I, Islam MMT, Shekhar HU. Oxidative stress and human health. Advances in Bioscience and Biotechnology 2012; 3: 997-1019.

3._Oxidative_Stress_and_Protein_Carbonylation.pdf

ORIGINALITY REPORT

17%	12%	11%	12%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universitas Brawijaya Student Paper	5%
2	Submitted to CVC Nigeria Consortium Student Paper	4%
3	www.contempclindent.org Internet Source	4%
4	japsonline.com Internet Source	4%

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		