Effect of Heavy Metal on Malondialdehyde and Advanced Oxidation Protein Produtcs Concentration: A Focus on Arsenic, Cadmium, and Mercury

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***Abstract*—Heavy metal and their salts are considered as very important group of environmental pollutant which in small quantities may be essential nutrients that protect your health, yet in larger quantity it become toxic and dangerous to human being. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. This study aimed to invetigate the effect of Arsenic (As), Cadmium (Cd), and Mercury (Hg) on Malondialdehyde (MDA) and Advanced Oxidation Protein Products (AOPP) concentration *in vitro*. MDA and AOPP level are increased during the exposure of As, Cd, and Hg. Furthermore MDA level positively correlated with AOPP level. It can be concluded from presented study that Arsenic, Cadmium and Mercury caused the increasing of MDA and AOPP levels. This study also suggested that the exposure of Arsenic, Cadmium and Mercury can caused oxidative stress and inflammation.**

***Index Terms*—arsen, AOPPs, cadmium, malondialdehyde, mercury**

I. Introduction

Forensic toxicology is one of the branches of forensic science. The science of toxicology is the science that examines the work and the harmful effects of chemicals or toxic to the biological mechanisms of an organism including heavy metal [1]. Heavy metals include arsenic, lead, copper and mercury are the oldest toxins known to humans, having been used for thousands of years [2], [3].

Arsenic is the number one substance in the most recent Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances published by the Agency for Toxic Substances and Disease Registry (ATSDR) (M.F. Hughes, 2011). In the Middle Ages, arsenic gained notoriety as an effective homicidal and suicidal agent, both because of the frequency of its use and because of its involvement in many high-profile murders [4]. In fact, arsenic is often referred to as the ''king of poisons'' and the ''poison of kings'' because of its potency and the discreetness, by which it could be administered, particularlywith the intent of removing members of the ruling class during the Middle Ages and Renaissance [5]. For example, it is well documented that arsenic was among the poisons in the death of Napoleon Bonaparte in 1851, which some conspiracy theorists claim was a political assassination [6]. In Indonesia arsenic poisoning is occur in the death of munir in 2004 [4].

Arsenic, a metalloid, occurs naturally, being the twentieth most abundant element in earth's crust and is a component of more than 245 minerals. The inorganic forms consisting mostly of arsenite and arsenate compounds are toxic to human health. Humans are exposed to arsenic primarily from air, food and water. Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals. However, elevated arsenic level in drinking water is the major cause of arsenic toxicity in the world [7].

Besides arsenic, another heavy metals which are toxic for human being are cadmium and mercury [8]. Cadmium is one of the most toxic substances in the environment caused its toxic effects on multiple organ systems and long elimination half-time. This metal is used in many occupations, including semiconductor manufacturing, welding, soldering, ceramics and painting [9]. Once absorbed, Cd irreversibly accumulates in the human body, in particularly in kidneys and other vital organs such the lungs or the liver [10].

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Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders [11]. Its wide industry related effects on human and animal biosystem have been well documented and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food [12].

The three metals toxicity is very well reported in the literature. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. In-depth studies in the past few decades have shown metals like iron, copper, cadmium, mercury, nickel, lead and arsenic possess the ability to Reactive Oxygen Species (ROS), resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNA [13]. Many studies confirmed the generation of various types of ROS during arsenic metabolism in cells [14]. Oxidative stress has been linked with the development of arsenic related diseases including cancers [8]. In a recent studies by E. Suhartono *et al*, it was shown that cadmium caused oxidative stress in kidney [15]. Several *in vivo* and *in vitro* studies suggested that exposure of experimental animals to inorganic or organic forms of mercury is accompanied by the induction of oxidative stress [14].

The production ROS during heavy metal poisoning can react with lipids, proteins, pigments and nucleic acids, causing lipid peroxidation. MDA, a well-known secondary product of lipid peroxidation after exposure to ROS [16]. Besides MDA, ROS were involved in production of advance oxidation protein products (AOPP) [9]. AOPP is dityrosine containing cross linked protein products, a definition that is important as it excludes protein aggregates that are formed by disulphide bonds as a result of oxidative stress [15].

Previous studies by H.V. Patel *et al* in arsenic exposed rats, AOPP and PCO were increased significantly in both studied tissue compared to control [17]. Other studies showed a dose-dependent increase in MDA production in breast and lung carcinoma cell lines, with increasing doses of arsenic trioxide [18]. exposure to Cd caused an increasing of AOPP level that kidney rats and increasing MDA level in ovarian rats [15], [16]. However there is few studies on the mechanism of oxidative stress in blood exposed to heavy metal such as arsen, cadmium and mercury.

Since advanced oxidation protein products are not only a markers of oxidative stress but also act as inflammatory mediators, and MDA act as an marker for cellular damage. Thus our study aim to investigate the effect of arsen, cadmium and mercury *in vitro* by determined the levels of AOPP and MDA.

II. Material and Methods

A. Preparation of Blood Sample

The blood samples were collected under aseptic conditions for the analysis of MDA and AOPP level after heavy metal exposure. Blood was collected without any anticoagulant and allowed to clot for 1 h. Clotted sample was centrifuged at 3500 rpm 30 min at 4^{O}C (in cold centrifuge). Serum was separated and stored at 20^{O}C for further analysis.

Then blood was prepared for exposure to arsenic, cadmium and mercury. Samples were divided into three treatment groups with different concentrations of metals. For arsenic and mercury the concentrations are 0, 0.001, and 0.002 mg / l, whereas for cadmium at concentrations 0, 0,003 and 0,006 mg / l.

B. Malondialdehyde (MDA) Determination

MDA was measured by the method of Buege and Aust [19]. For the first take a serum from blood sample. Then add 1 mL aquadest then disposed of in thee pendorf. After that added 100% TCA 100 uL, 1% Na-Thio 100 uL and 250 uL of 1 N HCl. The solution is heated at a temperature of 1000°C for 20 minutes. Then centrifuged to 3500 rpm for 10 minutes. Supernatant was taken. After that, add distilled water up to 3500 uL. The result is read by a spectrophotometer with a maximum wavelength of 500-600 nm, in day 0, 2, 4,and 6.

C. Advanced Oxidation Protein Products (AOPPs) Determination

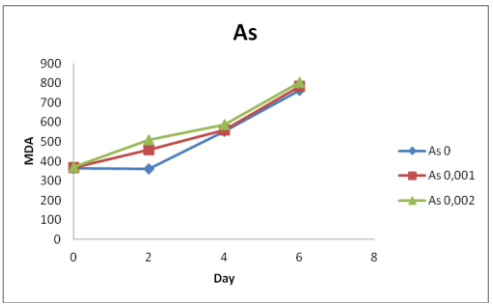
Serum AOPP measurement were made by spectrophotometric methods as describe by Witko-Sarsat *et al* [20].

D. Statistical Analysis

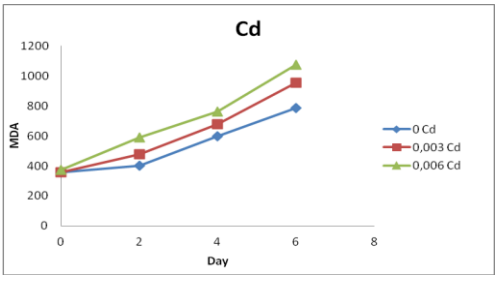
The Data of MDA and AOPP levels displayed in the linear graph. Furthermore to analyze the relationship between AOPP and MDA levels in each metal exposure, we use a linear correlation. For analyze the data and draw the graphic, we used Microsoft Excell 2007.

III. Results

MDA concentration in serum, in relation to the heavy metal (As, Cd, and Hg) concentration and time presented in three figures (Fig. 1 (a), (b), and (c)).



(a)



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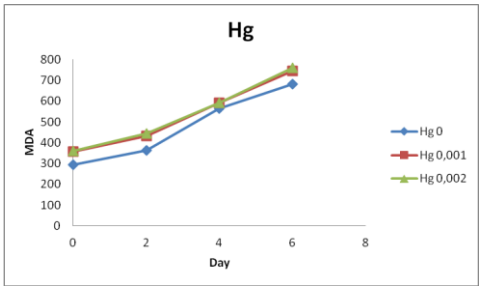


(c)

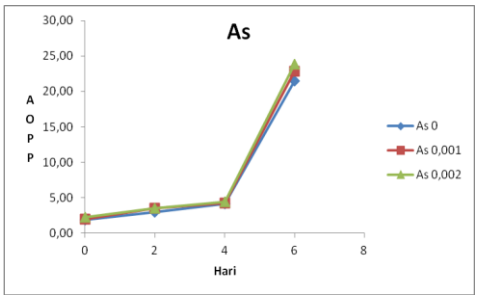
Figure 1. MDA Levels after (a) As (b) Cd (c) Hg exposure in different concentration and time

That Fig. 1 (a), (b), and (c) revealed that the heavy metal exposure increase the level of MDA. The Fig. 1 (a), (b), and (c) also indicated that the increasing of heavy metal concentration lead to an increase in MDA level.

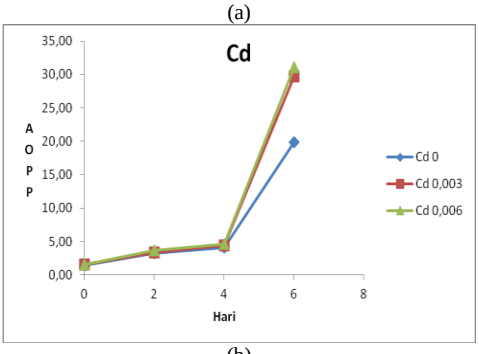
Fig. 2 (a), (b), and (c) presented the levels of AOPP in relation to heavy metal concentration (As, Cd, and Hg) and time.

Similar to MDA, AOPP levels are increased with the increasing of time and heavy metal concentration (As, Cd, and Hg).

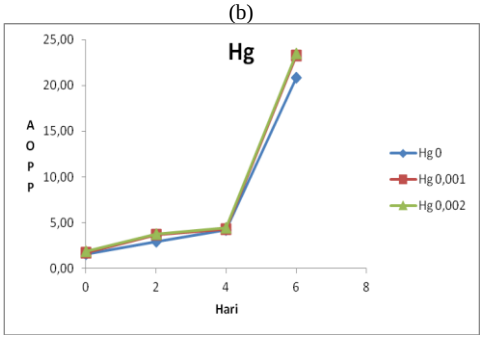
The correlation between MDA and AOPP level during the exposure of heavy metal (As, Cd, and Hg) in different concentration are presented in Table I.



(a)



(b)



(c)

Figure 2. AOPP Levels after (a) As (b) Cd (c) Hg exposure in different concentration and time

TABLE I: CORRELATION BETWEEN MDA AND AOPP DURING THE EXPOSURE OF ARSENIC, CADMIUM AND MERCURY

Heavy Metal	R^2
Arsenic (As)	0,87
Cadmium (Cd)	0,81
Mercury (Hg)	0,79

From Table I revealed, there are positive correlation between MDA and AOPP levels during the exposure of As, Cd, and Hg. It means the increasing of MDA levels are followed by the increasing of AOPP levels.

IV. DISCUSSION

Metal induced toxicity is very well reported in the literature. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress [13]. Oxidative stress is an unavoidable aspect of aero bic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences in living organisms [21].

In this study there are three heavy metal was used to determined the level of MDA as a marker for oxidative stress induced by heavy metal. The three heavy metal are arsen (As), Cadmium (Cd) and mercury (Hg).

Arsenic is an important contaminant, whose occurrence in environmental media (air, soil, water) results from natural sources and from anthropogenic activities. Humans are mainly exposed to inorganic arsenic species (arsenite, $AsO3^{3-}$, and arsenate, $AsO4^{3-}$) [22]. Ingestion of contaminated drinking water along with industrial emissions is the major routes for human exposure to arsenic. Most ingested and inhaled arsenic is well absorbed through the gastrointestinal tract and lungs into blood stream. Then, it is distributed and metabolized in large number of organs including the liver, kidney, blood, skin, adipose tissue, liver, skeletal muscle and pancreas [17].

Many studies confirmed the generation of various types of ROS during arsenic metabolism in cells [14]. Oxidative stress has been linked with the development of arsenic related diseases including cancers. In addition to ROS, reactive nitrogen species (RNS) are also thought to be directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic. Many recent studies have provided experimental evidence that arsenic-induced generation of free radicals can cause cell damage and death through activation of oxidative sensitive signalling pathways [23].

Oxidative stress is a relatively new theory of arsenic toxicity. Since about 1990, additional data supporting this theory and scientific acceptance of this mode of action have continued to occur. The first oxidative theory of arsenic carcinogenesis that includes a detailed metabolic pathway was presented by Yamanaka et al. Dimethylarsine (a trivalent arsenic form) is a minor *in vivo* metabolite of DMA (a pentavalent arsenic form) produced by a process of reduction in vivo. Dimethylarsine can react with molecular oxygen form a $(CH_3)_2As.$ radicals and superoxide anions. This $(CH_3)_2As.$

can add another molecule of molecular oxygen and form the $(CH_3)_2$ AsO-radical [24].

Arsenic-mediates formation of the superoxide anion radical ($O2^{-\bullet}$), singlet oxygen ($^1O2$), the peroxyl radical (ROO•), nitric oxide (NO•), hydrogen peroxide (H_2O_2), dimethylarsinic peroxyl radicals ([(CH3)2AsOO•]) and also the dimethylarsinic radical [(CH3)2As•]. The exact mechanism responsible for the generation of all these reactive species is not yet clear, but some studies proposed the formation of intermediary arsine species [8].

Besides through the formation of free radical, as mentioned above, depletion of tissue glutathione level has been found to be a causative factor in arsenic-induced oxidative damage. It has been established by the observation of Rana et al. that arsenite binds with nucleophilic sulfhydryl groups and thereby reducing GSH content in tissue, aggravating the oxidative threat to tissue. Perturbation of glutathione content in cardiac tissue was reported earlier. They also reported that short-term arsenic toxicity in rats produces a significant decrease in cardiac GSH concentration associated with increased lipid peroxidation level [25].

The second heavy metal in this study is Cd. Occupational exposure to Cd has been associated with occurence of increased oxidative stress. An interesting mechanism explaining the indirect role of Cd in free radical generation was presented some years ago [13]. Cd itself is unable to generate free radicals directly, however, indirect formation of ROS and RNS involving the superoxide radical, hydroxyl radical and nitric oxide has been reported [26].

Some experiments also confirmed the generation of non-radical hydrogen peroxide which itself in turn maybe a significant source of radicals via Fenton chemistry. Cd can activate cellular protein kinases (protein kinase C) which result in enhanced phosphorylation of various transcription factors which in turn lead to activation of target gene expression [27], [28].

In this mechanism it was proposed that Cd can replace iron and copper in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), thus increasing the amount of unbound free or chelated copper and iron ions participating in oxidative stress. Displacement of copper and iron by Cd can explain the enhanced Cd-induced toxicity, because copper displaced from its binding site, is able to catalyze breakdown of hydrogen peroxide *via* the Fenton reaction. These results are supported by recent findings by Watjen and Beyersmann (2004). Displacement of copper and iron by Cd can explain the enhanced cadmium-induced toxicity, because copper, displaced from its binding site, is able to catalyze breakdown of hydrogen peroxide via the Fenton reaction [29].

The third heavy metal in this study is mercury (Hg). Mercury is a transition metal commonly named quicksilver due to its liquid and silvery characteristics. It is recognized by the symbol Hg, which comes from the Latin term *hydrargyrum*, meaning ''watery silver''. It is present in the environment due to both natural (earth's surface evaporation and volcanic eruptions) and anthropogenic (emissions from coal-burning power stations and incinerators) sources [30].

Several in vivo and in vitro studies suggested that exposure of experimental animals to inorganic or organic forms of mercury is accompanied by the induction of oxidative stress. The high affinity of mercuric ions for binding to thiols naturally suggests that following depletion of intracellular thiols (especially glutathione) either directly or indirectly causes, or predisposes, proximal tubular cells to oxidative stress. Lund et al. have demonstrated that the administration of mercury as Hg(II) in rats resulted in glutathione depletion and increased formation of H_2O_2 and lipid peroxidation in kidney mitochondria [14].

Based on the explanation above, the three heavy metals we use in this study are known to cause the formation of ROS, resulting in oxidative stress.

The accumulation of ROS like hydroxyl radical (HO•), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O2$) disturbs the oxidant-antioxidant balance These active species react with bio-molecules like lipids, proteins and DNA impairing their functional properties which in turn brings about alterations in the normal activities of cells, tissues, organs and ultimately organisms evident as disease symptoms, and other pathological conditions [31].

If ROS react with bio-molecules such as lipids, proteins, nucleic acid, these can cause lipid peroxidation, protein denaturation and DNA mutation [32]. If the ROS react with lipid, it calls lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxidation degrades polysaturated fatty acids of cell membranes with consequent disruption of membranes. Previous studies showed that cadmium induced histopathological changes and lipid peroxidation in the liver and kidneys of rodents. Occupational exposure to elemental mercury leads to increased lipid peroxidation in erythrocytes in humans [33]. Flora *et al*. (2002) reported that GaAs induced lipid peroxidation in blood, liver and kidney of rats [34].

The overall process of lipid peroxidation consists of three stages: initiation, propagation and termination. Initiation, the first stage, involves the attack of a ROS capable of abstracting a hydrogen atom from a methylene group in the lipid. The presence of a double bond adjacent the methylene group weakens the bond between carbon and hydrogen so the hydrogen can be more easily removed from the fatty acid molecule. Fatty acids with no double bonds or with one double bond can undergo oxidation but not a chain lipid peroxidation process [15].

The process of hydrogen abstraction leaves behind a fatty acid having one unpaired electron. When oxygen is present in the surrounding tissues, the fatty acid radical can react with it leading to the formation of lipo-peroxyl radicals (ROO•). Once formed, lipo-peroxyl radicals (ROO•) can be rearranged *via* a cyclization reaction to endoperoxides (precursors of malondialdehyde) with the final product of peroxidation process being MDA [32].

MDA is a polar molecule of small molecular mass. MDA may be measured in different biological samples

and, even though it is not the only indicator of oxidative stress, it is often used due to its procedure simplicity [35]. In this study, we investigated the effect of oxidative stress in serum *in vitro* treated with As, Cd, and Hg by evaluating MDA production. We found a dose and time-dependent increase in MDA production in serum. This result indicated that the As, Cd, and Hg inducec oxidative stress in serum *in vitro* [35].

Oxidative stress lead to formation of glycoxidation products, including advanced glycation endproducts (AGEs - among them Nε- (carboxymethyl)lysine (CML) is best known), and advanced oxidation protein products (AOPPs). AOPPs can be formed in vitro by exposure of serum albumin to hypochlorous acid. In vivo, plasma AOPPs are mainly carried by albumin and their concentrations are closely correlated with the levels of dityrosine [36].

The result of this study suggest that the three heavy metals As, Cd, and Hg induced the formtaion of AOPPs. Furthermore there are positive correlation between the level of MDA and AOPP on the exposure of As, Cd, and Hg. It means, during exposure to As, Cd, and Hg will increase both MDA and AOPP.

AOPP is dityrosine containing cross linked protein products, a definition that is important as it excludes protein aggregates that are formed by disulphide bonds as a result of oxidative stress. Therefore, AOPP is a good oxidative stress marker, which originates under oxidative and carbonyl stress and increase global inflammatory activity [15].

AOPPs measurements reflect the reactive species generation and the degree of protein oxidation [37]. It was reported that AOPPs generated by different oxidation patterns lead to the production of either hydrogen peroxide or nitric oxide [38]. Nitric oxide can interact with superoxide anion-radical forming reactive nitrogen species such as peroxynitrite. These reactive nitrogen species secondarily promote important reactions such as nitrosation, oxidation or nitration, leading to impaired cellular functions and enhanced inflammatory reactions [39], [40]. AOPPs are referred to as markers of oxidative stress as well as markers of neutrophil activation in chronic disease [41]. It has thus been shown that chlorinated oxidants of neutrophil origin may lead to oxidative stress, notably protein oxidation. In addition to increased formation, decreased removal/detoxification of AOPPs may contribute to the stress [36].

AOPPs are believed to be more closely related to inflammation. According to previous studies AOPP may represent a novel class of proinflammatory mediators acting as a mediator of oxidative stress and monocyte respiratory burst. The monocyte is thus, at the same time, the elective cellular target of AOPP and a potential source of oxidants inducing AOPP [20]. A close correlation was observed between AOPP and neopterin, the monocyte activation marker. This selective relationship between AOPP and monocyte activation was further established with positive correlations between AOPP and TNF-α and its soluble receptors, and, to a lesser degree, with IL-1Ra [41].

V. Conclusion

It can be concluded from presented study that Arsenic, Cadmium and Mercury caused the increasing of MDA and AOPP levels. This study also suggested that the exposure of Arsenic, Cadmium and Mercury can caused oxidative stress and inflammation.

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