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Mercury Exposure Effects to Skin Tissue of *Mus Muscullus* at Fibroblasts Cell Proliferation and Collagen quantity.

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ABSTRACT

The effects of heavy metals mercury on the cell proliferation and collagen synthesis from *Musmuscullus* tissue had the results. This research used laboratory animals Musmucullus as objects of the research. The skin of test animals would exposure to mercury. Mercury material will be included in a pharmaceutical skin cream and placebo treated to control group of test animals. From the description of the dangers of mercury that can cause the tissue damaged. The texture of cross sectional slices the skin of the test animals were exposed to mercury than control is different. The skin animals with mercury exposed has a number of muscle tissue less than control, epidermis and dermis are thinner and fragile than control. While the fat tissue of mercury exposed group had a thicker than the control. The skin animals exposed to mercury had a number of skin fibroblast cells which is smaller than the control at each area. Similarly, percent area collagen of test animals exposed to mercury which is smaller than the control.

Keywords: mercury, skin, fibroblast, collagen, tissue

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INTRODUCTION

Mercury has not been allowed to use in cosmetics in many countries include Indonesia. But in the reality, there are many cosmetics with mercury still to be found in the community. It was happening because the regulations are not working, and also public awareness of the importance of health care is very small [1]. This study aims to provide a rationale education for public about the dangers of mercury exposure on the skin. The dose-dependent effects of heavy metals including mercury on the cell proliferation, collagen synthesis, and non-collagen protein synthesis were studied in early passage cultures of human synovial cells exposed to 1-100 µM concentration of mercury for 5 days [2].

Determination of mercury species in liquid cosmetic samples is described. Hg^{2+}, $MeHg^{+}$ and $EtHg^{+}$ were obtained from only 5.00 mL sample solution. The detection limits of the analytes (as Hg) were 1.3 ng L^{-1} for Hg^{2+}, 7.2 ng L^{-1} for $MeHg^{+}$ and 5.4 ng L^{-1} for $EtHg^{+}$, respectively. The relative standard deviation (n = 10) of 0.5 ng mL^{-1} Hg^{2+}, $MeHg^{+}$ and $EtHg^{+}$ were 7.4%, 5.2% and 2.3%, respectively [3].

Women who use mercury-containing skin lighteners often have elevated mercury levels in their hair, blood and urine. Several studies have found hair mercury levels greater than 100 ppm in women using these products, compared with a ―normalǁ range of below 10 ppm [4,5]. Similarly far above normal levels of mercury have been measured in urine of women in Kenya and Tanzania who used mercury-containing skin lighteners, and users of these cosmetics in Hong Kong had elevated mercury levels in their blood and urine [5]. The mechanism might be involved in mercury induced toxicity and it is suggested that one of the well-known mechanism is a mercury induced reactive oxygen species [6]. Reactive oxygen species (ROS) have long been known to be a component of the killing response of immune cells [7].

The toxicology of mercury and its compounds, special attention is paid to those forms of mercury of current public health concern. Human exposure to the vapor of metallic mercury dates back to antiquity but continues today in occupational settings and from dental amalgam. Health risks from methyl mercury in edible tissues of fish have been the subject of several large epidemiological investigations and continue to be the subject of intense debate. Ethyl mercury in the form of a preservative, thimerosal, added to certain vaccines, is the most recent form of mercury that has become a public health concern. The review leads to general discussion of evolutionary aspects of mercury, protective and toxic mechanisms, and ends on a note that mercury is still an “element of mystery” [8].

Mercury (Hg) occurs in nature as ionic and elemental mercury. Natural sources include the weathering of cinnabar (HgS) deposits and volcanic and geothermal emissions. Man-made sources include dental amalgams, pharmaceuticals, cosmetics, the exploitation of geothermal fields, chloralkali plants, and other industrial activities such as manufacture of electrical products and paper and pulp mills. Synthetic organic mercury has been banned for decades.

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Today, the major source of human exposure to Hg is through the diet from consumption of fish and fish products [9].

Mercury can form a bond with the thiol group which formed very strong bonds and stable that caused by the high constant stability of mercury-thiol. In the formation of mercury complexes with thiol groups (from glutathione, albumin, cysteine and others) mercury binds to the free thiol groups available. The presence of mercury is bound to the thiol group on the cysteine residue is causing the function of cysteine is not properly. It is caused that thiol groups play an important role in the metabolism of the body, such as the active center of the enzyme. The presence of mercury atoms causes the enzyme does not work because the enzyme has done at specifically site. The incorporation of [3H]thymidine into trichloro acetic acid insoluble material was inhibited 50% by each of the heavy metals at concentrations between 1 and 10 µM. Mercury 10 µM decrease the DNA content of the cultures by less than 15%, which was attributed to cytotoxicity. A dose-dependent inhibition of [3H]proline incorporation into bacterial collagenase resistant (non-collagen) protein was observed after incubation with 10µM mercury [2].

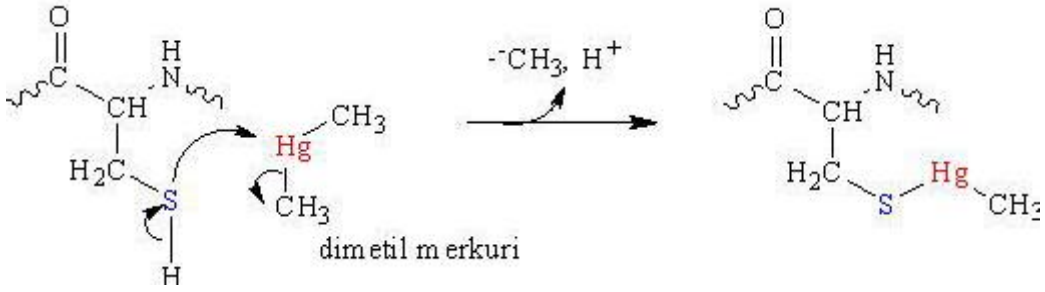

Figure 1.Cysteine structural damage caused by dimethyl mercury.

The other mercury's bond is the bond between mercury with disulfide. Effect of mercury on the disulfide bonds can cause two problems. The first is that methyl mercury causes breaking disulfide bonds. Disulfide bond is forming the tertiary structure of a protein. This disulfide bond rupture resulting protein loses its biological properties (protein denaturation). The second problem is that mercury is forming a disulfide bond replaces previous bridge. Although it seems no effect on the structure initially, the body will naturally detect that there are foreign proteins in the body. Adverse reactions may occur due to the influence of elemental mercury in the protein. Furthermore, this complex can cause damage to proteins that have been formed. The mechanism of its formation can be observed in Figure 2 [10]. Mercury has electron-sharing affinities that can result in formation of covalent attachments [11]. These attachments are mainly formed between mercury and sulfhydryl groups of protein [12].

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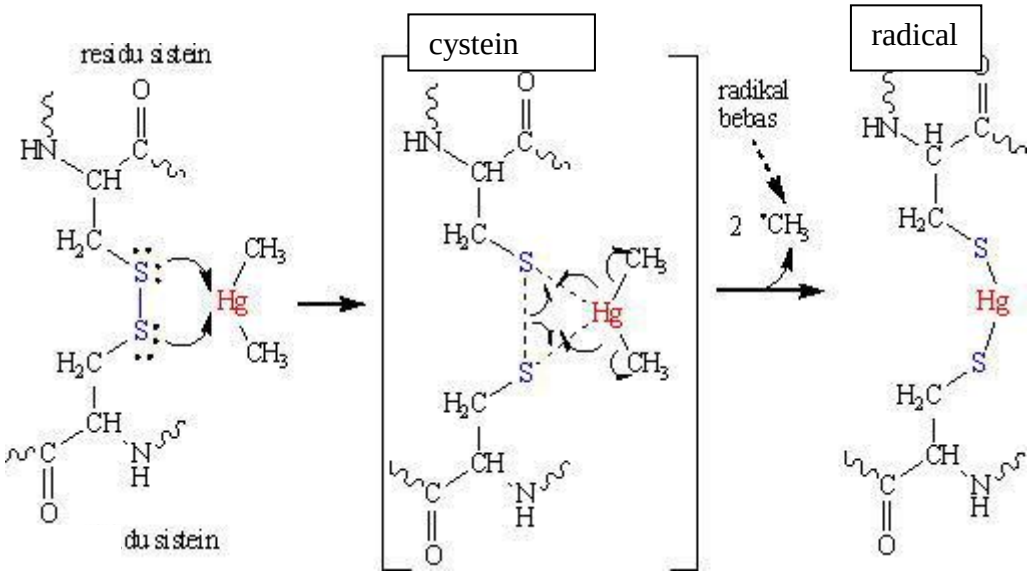

Figure 2: Mercury form a disulfide bridge bond trigger further damage to the tissue.

In this study, test animals (mice) was exposed to mercury that can cause denaturation of proteins. This research used laboratory animals Musmucullus as objects of the research. The skin of test animals would exposure to mercury. Mercury material will be included in a pharmaceutical skin cream and placebo treated to control group of test animals. From the description of the dangers of mercury that can cause the tissue damaged then further defined research problem as follows:

1. How sectional cross-sectional differences in the texture of the skin of test animals that were exposed to mercury than control?
2. How is a qualitative difference muscle tissue, fat tissue, epidermis and dermis skin test animals were exposed to mercury and control?
3. How do differences in quantitative collagen tissue and fibroblast cell?

The purpose of this study was to obtain data supporting the influence of mercury on the development of animal tissue test Musmuscullus. Tissue damage in the test animals also occur in human tissue, especially face skin tissue when using cosmetics containing mercury. Thus, the benefits of this research can provide education to the public about the dangers of cosmetics containing mercury.

MATERIAL AND METHODS

Material

Material used in this experiment is mercury in the cation form (Hg^{2+}). Cation Hg^{2+} included in the basic cream. Other material is *Musmuscullus.* Animal testing *Musmuscullus* is obtained from farmers assisted Brawijaya University in Malang. A total of animals test were 20. They are divided into 2 groups, each consist of 10 mice. They were randomly taken to be the

control group and the treatment group using mercury. Adaptation period for each group is 2 weeks. After the adaptation period is completed the animals test entered the treatment period.

Method

Embedding and staining of tissue

Skin or tissue was fixed in formalin removed and immersed in graded alcohol concentration of 10%, 20%, 30%, 50%, 70%, 90% and absolute alcohol. Furthermore dipped in Xylol and embedded in liquid paraffin which will soon solidify at room temperature. Paraffin blocks were made in blocks for further prepared thin slicing. Thin slices made with a thickness of 4 micron with microtome. This slice prepared for histological chemistry staining of skin or tissue. Staining using Hematoxylin-eosin stain (HE) to get a lot of information in the skin or tissue. Van Geisonstain to get a lot of information about collagen quantity.

Qualitative analysis of skin

Qualitative analysis of tissue taken to distinguish the skin tissue in the treated group and the control group linked to the characteristics of the epidermis, dermis and structure of tissue. This analysis used information histological chemistry staining with HE.

Experimental

Mercury Exposure

Animals test from the treatment group get mercury exposure by apply at skin cream with mercury and without mercury at the control group. Treatment was done every morning for 7 days.

Preparation of tissue fixative

After treatment for 7 days, the animals test prepared tissue fixative. The part of skin with size 1x2 cm2 prepare to fixative. This tissue soaked in 4% formalin before further treatment.

Quantitative analysis of tissue

Quantitative analysis performed on the amount of the number of existing fibroblasts in the skin tissue and collagen quantity. Fibroblast cells are very numerous and small in size so it is limited in a narrow area. Fibroblast cells computed at microscope field of view is taken. The number of fibroblast cells was calculated by making five cropping with a particular area in the HE staining. Collagen quantity calculated as percent of field collagen area by cropping red area in the Van Gieson staining.

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RESULTS

The structure of the skin tissue of test animals (*Musmuscullus*) can be observed in Figure 3, the left image shows the structure of normal skin and the right image shows the structure of skin exposure to mercury. Epidermis part of the normal group was thicker and it has good texture, while the mercury group seemed thin and broken. Similarly, the normal skin dermis denser with fibroblast cells, while the cell density of mercury group is reduced, so that there are empty spaces.

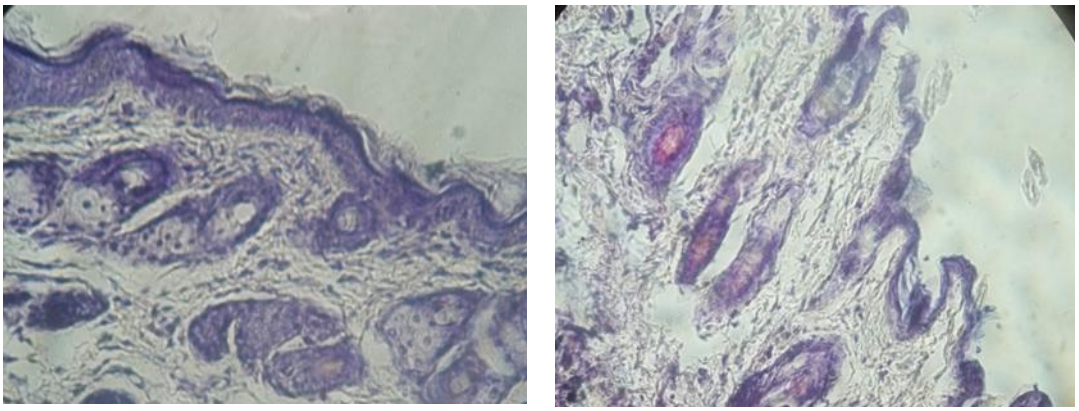

Figure 3. Skin tissue of MusMuscullus, normal group (left), mercury group (right)

In this study, exposure to mercury-containing creams do inorganic Hg for 7 days so that has been a change of the form of inorganic Hg M-Me-Hg characterized by damage to both fibroblast cells and collagen. Cell damage can be observed in Figure 4, while collagen breakdown can be observed in Figure 5.

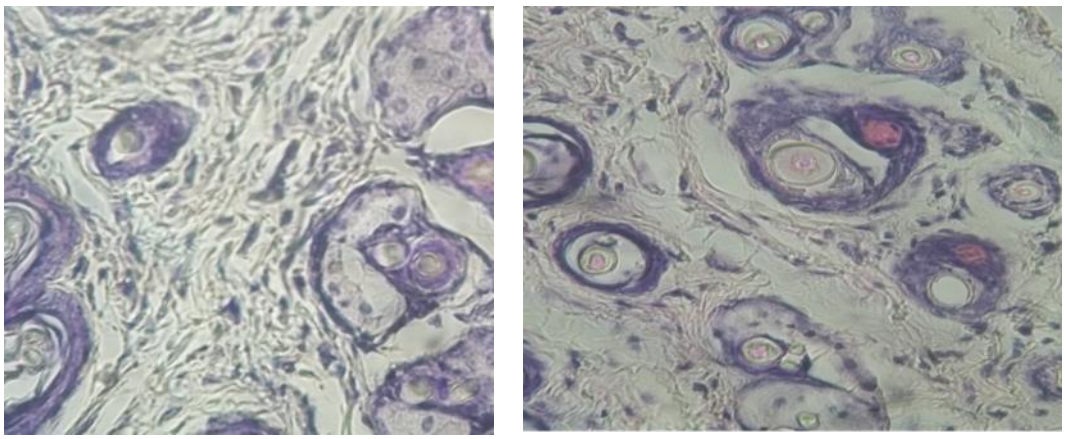

Figure 4. Fibroblast cell of Musmuscullus, normal group (left), mercury group (right).

The damage system of cell proliferation that occurs in the skin of test animals shown in Figure 4, the left image is the normal group and the right picture of mercury. Clearly fibroblast cell density of mercury smaller group than the control group. This indicates impaired fibroblast proliferation system. Mercury inhibits fibroblast cell proliferation.

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Table 1. The date of fibroblast and collagen quantity of test animal Musmuscullus

Test animal group	The number of fibroblast cell at 80µm^2 cropping area (cells)	Percent area of collagen in the 80µm2 cropping area (%)
Normal(rep 1)	31 30 24 31 34 31 28 30 32 30	40.63; 40.50; 39.93; 39.75; 39.96; 34.45; 41.84; 41.51; 39.29; 39.95
Normal (rep2)	30 27 27 33 33 30 27 30 31 30	40.38; 40.13; 40.53; 39.88; 39.98; 39.94; 41.51; 37.95; 40.03; 39.40
Normal (rep3)	30 27 28 32 31 30 30 33 31 32	40.56; 40.23; 39.95; 39.23; 40.03; 39.58; 40.17; 39.39; 42.23; 39.40
Normal (rep4)	26 30 32 27 31 32 33 32 28 33	40.50; 40.32; 39.47; 39.84; 39.03; 40.12; 39.83; 42.12; 40.12; 40.10
Merkury(rep1)	8 10 9 11 11 7 8 9 10 12	18.05; 18.00; 17.74; 17.67; 17.77; 15.31; 18.59; 18.45; 17.46; 17.76
Mercury(rep2)	11 10 9 10 8 10 11 7 7	17.94; 17.83; 18.01; 17.72; 17.77; 17.75; 18.45; 16.86; 17.79; 17.51
Mercury(rep3)	11 7 8 10 9 7 11 10 8 8	18.03; 17.87; 17.75; 17.43; 17.79; 17.59; 17.85; 17.51; 18.77; 17.51
Mercury(rep4)	8 11 10 9 7 11 8 9 10 7	18.00; 17.91; 17.54; 17.71; 17.34; 17.83; 17.70; 18.72; 17.83; 17.82

Results of calculating the number of cells per 80 µm2 area of skin tissue and collagen density in percent of the area can be seen in table 1. Statistical analysis of the fibroblast cell number and percent of land area in the group of test animals yield 0.00 with a confidence level of significance of 0.95. This indicates that there are significant differences fibroblast cell number and percent area between groups of test animals. It can be concluded that mercury causes a change (decrease) in the number of fibroblast cells and also decrease the density of collagen in the skin of test animals.

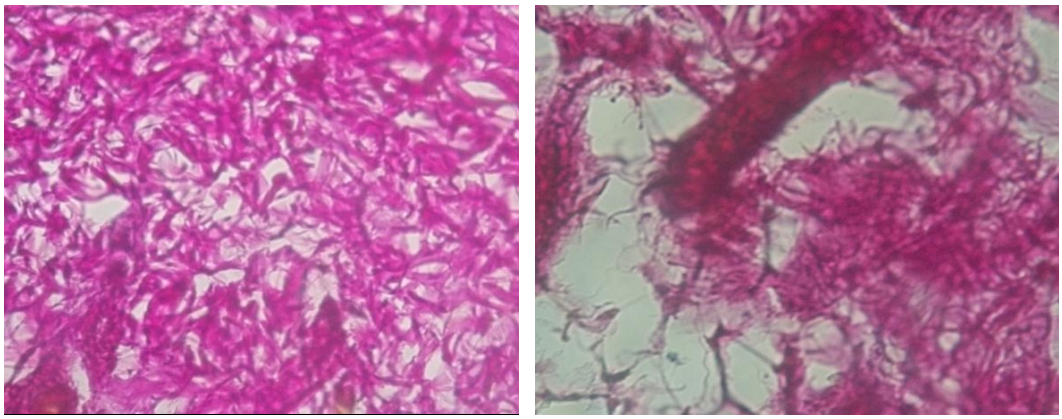

Figure 5. Collagen of Musmuscullus, normal group (left) and mercury group (right).

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DISCUSSION

Tissue qualitative analysis

Tissue damage that occurs due to changes the mercury compounds from inorganic mercury (contained in the cream) to the organic mercury attached in the tissue. The process of the reduction of Hg^{2+} to Hg(M-Me-Hg) followed by oxidation of the skin tissue around the Hg^{2+}. The oxidized tissue will experience a disconnection bond and damage. Termination bonding happens quickly cause to the formation of free radicals also caused continuous damage.

The transformation of inorganic Hg to MMeHg by microbial methylation is a result of adaption against Hg toxicity. Enzymatic reduction of Hg^{2+} to Hg^{0} and confers high resistance to inorganic mercury salts. This narrow-spectrum of Hg resistance is located in plasmids in the inducible mer operon. The enzyme, mercuric reductase, is codified by the merA gene. Less common, but very important, are the broad-spectrum Hg-resistant bacteria, which cleave the C-Hg bond of organomercurials by the enzyme organomecuriallyase, codified by the merB gene. Other sulphydryl proteins are involved in the control, binding, and transport of Hg. Under anaerobic conditions, Hg toxicity is drastically reduced by organic and inorganic sulfides such as H_2S. The latter reacts with Hg+2 and MMeHg to produce the stable HgS and the volatile dimethylmercury (DMeHg). MMeHg is also formed and it accumulates to the top of the food chain. Importantly, bacteria convert toxic forms of Hg to harmless forms to protect themselves and in so doing detoxify the surrounding environment as well [9].

More than 90% of total Hg in muscle tissue of top marine predators is mono-methyl-mercury (M-Me-Hg), the most toxic species of Hg. M-Me-Hg crosses cell membranes by passive diffusion beginning with the intestinal wall. Its long half-life in biological tissues leads to accumulation of high concentrations at the top of the food chain. Minimal increases in M-Me-Hg content of autotrophic organisms produces an unexpectedly large accumulation of Hg (bio-magnification) in carnivores, as the terminal end of the food chain [9].

Tissue quantitative analysis

Mercury has not been characterized as essential for any biologic reaction. However, it is readily accumulated in the body due to many defense mechanisms. Based on sulphydryl binding inside the cell, mercury is trapped to minimize its general distribution and inhibition of essential biologic processes [9].

Me-Hg from the diet is almost completely absorbed following digestion. Distribution to the bloodstream and to other tissues is essentially completed within 4 days, but the maximum accumulation in the brain takes 4 to 6 days. At that point, the brain contains 6% of the total dose of Me-Hg given. Kidney, liver, lung and striated muscle tend to be relatively high in total mercury, whereas other organs such as heart, pancreas and spleen are relatively low [9].

The central nervous system (CNS) is the critical target organ in Me-Hg toxicity. The earliest symptoms of intoxication in adults are non-specific from the CNS including paresthesia and malaise. Subsequently, coordination problems, hearing impairment, and constriction of the visual field was represented. Specific areas of the CNS are damaged, such as the visual cortex of the cerebrum and the granular cells of the cerebellum. The developing brain is particularly sensitive to Me-Hg and the fetal brain may even be affected even when the mother shows no signs of poisoning Me-Hg may inhibit both cell proliferation and migration[9].

Other research areas presented in the book, included Hg-induced aberrant immune responses. Mercury can produce hypersensitivity reactions, systemic autoimmunity and nephrotic syndrome due to membranous glomerulonephritis. Animal models suggest deposition of immune complexes and complement as a feature of the Hg-induced tissue damage [9].

Mercuric ion is cytotoxic and mutagenic to cells; however, the mechanisms of mercuric ion-induced cytotoxicity are not well understood. Numerous studies have suggested that these effects may be due in part to the alteration and inhibition of a variety of cellular processes including DNA replication, DNA repair, RNA transcription, and protein synthesis. The use of an intact human cell multi protein complex (which we have termed the DNA synthesome) to carry out full-length DNA replication and DNA synthesis in the presence of Hg^{2+} ion in vitro. DNA replication and DNA polymerase activity, as well as DNA replication fidelity of the human cell DNA synthesome, are specifically inhibit by physiologically attainable concentrations of mercuric ion [13].

The process of collagen biosynthesis involves the role of DNA and RNA present in the cell nucleus. Mercury that is able to interact with nucleic acids in the cell nucleus fibroblasts causes impaired collagen biosynthesis. This can be observed in the figure is the right 5.bagian mercury that has the number density of collagen smaller than normal group right picture.

Mercury interacts strongly with nucleic acids and proteins. Inorganic mercury ions cause a specific inhibition of brain tubulin binding of GTP, and interacts strongly, yet reversibly, with the N-binding sites of purines and pyrimidines. Whereas, organic mercurial compounds produce irreversible damage to nucleic acids(9).

CONCLUSION

- There were differences in the texture of cross sectional slices the skin of the test animals were exposed to mercury than control.
- There were differences qualitative test of skin animals. The skin animals with mercury exposed has a number of muscle tissue less than control, epidermis and dermis are thinner and fragile than control. While the fat tissue of mercury exposed group had a thicker than the control.
- There were differences quantitative test of skin animals. The skin animals exposed to mercury had a number of skin fibroblast cells which is smaller than the control at each

area. Similarly, percent area collagen of test animals exposed to mercury which is smaller than the control.

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REFERENCES

[1] The Analysis of Mercury in Farmaceutical Cream at The Biutycal Clinik in Surabaya. Taufikurohmah, Titik and Setiarso, Pirim. Surabaya : Unesa University Press, 2012. Chemistry National Conference 2012. pp. 112-120.

[2] Effect of heavymetals on humanrheumatoidssynovialcell proliferation and collagen synthesis. Goldberg, Ronald L, Kaplan, Stephen R and Fuller, George C. 2002, Elsevier Inc.Available online 5 November.

[3] Article Speciation of mercury in liquid cosmetic samples by ionic liquid based dispersive liquid–liquid microextraction combined with high-performance liquid chromatography-inductively coupled plasma mass spectrometry. Xiaoyu, Jia, et al., et al. 2011, Journal of Analytical Atomic Spectrometry Issue 7, 2011 ,26. , pp. 1380-1386

[4] The problem of the use of mercurials cosmetics in Senegal. Gras, G and Mondain, J. 1981, Recherche Europeene en Toxicologie 3(4), pp. 175-178.

[5] A Study of Mercury Levels in Fish and Humans in Mwakitolyo Mine and Mwanza Town in the Lake Victoria Goldfields,Tanzania. Kahatamo, J.m.J, Mnali, S.R and Akagi, H. Lars Lander(ed): Small-Scale Mining in African Countries : Dar es Salaam Tanzania, 1998. International Conference/Workshop Dar es Salaam.

[6] Toxic Metals and Oxidative Stress Part I: Mechanism Involved in Metal Induced Oxidative Damage. Ercal, Nuran, Gurer-Orham, Hande and Aykin-Burns, Nukhet. 2001, Current Topics in Medicinal Chemistry, pp. 529-539.

[7] Held, Paul.An Introduction to Reactive Oxygen Species. Highland Park, Winooski, Vermont o5404-0998 USA : Bio Tek Instruments, Inc., 2010.

[8] The Toxicology of Mercury and Its Chemical Compounds. Thomas W, Clarkson and Laszlo, Magos. 2006, Informa Health Care Vol. 36, No. 8 ,, pp. Pages 609-662 (doi:10.1080/10408440600845619).

[9] Metal Ions in Biological Systems, Vol. 34 Mercury and Its Effects on Environment and Biology, edited by Astrid Sigel and Helmut Sigel. Marcel Dekker, Inc., New York, 1997, 604 pages. Bidlack, W. R and Wayne, R. Bidlack. 2013, the American College of Nutrition Print ISSN: 0731-5724 Online ISSN: 1541-1087 whose registered office is Mortimer House, 37-41 Mortimer Street, London, W1T 3JH. Registered in England and Wales. Number 3099067. UK VAT Group: GB 365 4626 3, p. 604.

[10] The relativistic character of mercury is important factor in deteriorating the living system. Sumitro, Sutiman B and Zahar, Gretha. Orlando, Florida USA : ICEME Inc., 2010. ICEME, April 6-9.
[11] Bondy, S.C. Oxigen generation as a basis for neurotoxicity by metals. [book auth.] L.W Chang and Eds. Toxicology of Metals. Baco Raton : CRC Press, 1996, pp. 699-706.
[12] Cysteine Metabolism and metal toxicity. Quig, D. 1998, Alter.Med.Rev, 3, pp. 262-270.
[13] Mercuric Ion Inhibits the Activity and Fidelity of the Human Cell DNA Synthesome. Sekowski, Jennifer W, et al., et al. 2002, Elsevier Inc. http://dx.doi.org/10.1006/taap.1997.8185 cited by in scopus (32).