The effect of inhaling mercury (Hg) on the hepar cells and the role of green tea extract as antioxidant

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ABSTRACT

This research aimed to identify the effect of inhaling mercury on the hepar cells of white mice and the role of green tea extract as antioxidant on such hepar cells having exposed to mercury histopathologically. It was an experimental research by using 48 male white mice (*Mus musculus*) as the sample. The sample was divided into 8 groups i.e.: A, B, C, and D, which were given treatment for 3 and 6 weeks. Each group consisted of 6 mice. A group was a negative contol which did not get any treatment. Group B as a positive control group was exposed by mercury. Group C was exposed to mercury and was given green tea extract at dosage 0.52 mg/20 gr body weight. Group D was exposed by mercury and was given green tea extract at dosage 1.04 mg/20 gr body weight. All white mice in the group B, C, and D were exposed by mercury through inhalation for 4 hours daily. To identified the effect of mercury, the hepar cells in all 4 groups were examined at the 3rd and 6th week by making histopathologic preparation in the Histopathology Laboratorium Faculty of Medicine Universitas Andalas. Then, the preparation were examined through Binocular Light Microscope in ordered to see the defficient occured. The data obtained were analyzed by ANOVA method and independent T-test with confidence level=95%. It was revealed that the hepar cells that was being exposed by mercury regularly were being degenerates. Then, the amount of green tea extract given reduces the degeneration occured.

Key words: Mercury (Hg), inhalation, green tea extract, antioxidant, hepar cells

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh menghirup merkuri pada sel hepar tikus putih dan peran ekstrak teh hijau (Camellia sinensis) sebagai antioksidan terhadap sel hepar tersebut setelah terkena merkuri secara histopatologis. Penelitian ini merupakan penelitian eksperimental dengan menggunakan 48 tikus putih jantan (Mus musculus) sebagai sampel. Sampel dibagi menjadi 8 kelompok, yaitu: A, B, C, D yang masing-masing diberikan pengobatan selama 3 dan 6 minggu. Setiap kelompok terdiri dari 6 tikus. Kelompok A merupakan kontrol negatif yang tidak mendapatkan perlakuan apapun. Kelompok B merupakan kontrol positif terpapar Mercury. Kelompok C terpapar Merkuri dan diberi ekstrak teh hijau dengan dosis 0,52 mg/20 gram berat badan. Kelompok D terpapar merkuri dan diberi ekstrak teh hijau dengan dosis 1,04 mg/20 gr berat badan. Semua tikus putih di Kelompok B, C, D terpapar merkuri melalui inhalasi selama 4 jam setiap hari. Untuk mengetahui pengaruh merkuri, sel-sel hepar dari empat kelompok sampel diperiksa pada minggu ke-3 dan empat kelompok diperiksa pada

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minggu ke 6 dengan membuat preparat histopatologi di Laboratorium Histopatologi Fakultas Kedokteran Universitas Andalas. Kemudian, preparat diperiksa dengan Mikroskop Cahaya Binokular untuk melihat terjadinya defisiensi. Data yang diperoleh dianalisis dengan metode ANOVA dan independen T-test dengan tingkat kepercayaan = 95%. Hasil penelitian menunjukkan bahwa sel-sel hepar yang terpapar merkuri secara teratur mengalami kemerosotan. Kemudian, jumlah ekstrak teh hijau yang diberikan mengurangi degenerasi yang terjadi.

Kata kunci: *Merkuri (Hg), inhalasi, ekstrak teh hijau, antioksidan, sel-sel hepar*

INTRODUCTION

Mercury (Hg) is a chemical that has been used extensively in the field of dentistry as restorative materials as a mixture of the amalgam fillings.[1-6] Patients and dental personnels are at greater risk of mercury poisoning, but dental personnels are more at risk because they are exposed continuously. Mercury can be absorbed through skin or swallowed, but the main risk of dental health personnel is the absorption through inhaling the fumes. Mercury is extremely hazardous in liquid form. Mercury vapors can be inhaled or absorbed through the alveoli in the lungs to 80%.[1,2,7-11] Preliminary research had been done on the students of the Faculty of Dentistry, Universitas Baiturrahmah, the result showed that there were 73.33% of students with mercury-contaminated blood.[12]

According to WHO, mercury is included to highly toxic. All Hg compounds are toxic to human beings when it exposes in sufficient quantities and for a long time, it can lead to inhibition of enzymes and damage the body's cells so that damage can occur permanently[11] Mercury can cause all sorts of diseases, including disorders of the immune system to the death.[1,2,5,9,13-19] Once absorbed, mercury tends to settle in organs such as liver, kidney, and brain.[2,5,9,20]

Mercury is a known as toxic metal that causes oxidative stress in tissues.[21] Mercury can enter the cells and damage the cells. Mercury can reduce the production of glutathione and it inhibits the glutathione peroxidase enzyme activity and causes oxidative stress in brain and other organs.[15,22] Mercury can trigger the formation of hydrogen peroxide, lipid peroxide, and hydroxyl radical damage to protein structures. This proves that mercury is the cause of the imbalance of oxidants (free radicals) and antioxidants, and this can lead to oxidative stress in tissues and organs. The ability of mercury produces free radicals is quite remarkable.[15,23,24] Hg is known to inhibit the synthesis of RNA and DNA and inhibit the rate of cell cycle in B lymphocytes.[24]

In normal condition without induction, the formation of $O_2^{\circ-}$, H_2O_2 dan OH° is able to be muffled by body antioxidant such as catalase and *superoxide dismutase* (SOD). However the continuous induction of this enzyme would lead to paralysis, thereby facilitating the formation of OH°. As a result of an imbalance between the formation of ROS with antioxidant enzym, body oxidative stress occurs. Exposure to oxidative stress in mammalian cells would result in glutathione oxidation and NADPH (nicotinamide adeninedinucleotide phosphate) followed by protein thiol homeostasis disruption and ATP depletion. ATP depletion causes sodium pump to reduce, so that ion of Na accumulates in the cell and causes water entry into the cell. That is characterized by cell swelling and endoplasmic reticulum dilatation. Morphologicaly, hydrophilic degeneration and fatty degeneration occur.[25]

ROS has target on three types of compounds that are essential to maintain the integrity of such as the cells lipids, proteins and DNA. Lipid peroxidation in the cells membrane leads to increased permeability of the membrane, resulting in passive swelling of mitochondria which would exacerbate the cell damage.[26]

Tea is a widely consumed drink in the world.[27] Green tea contains of poliphenol. Poliphenol antioxidant strength is 100 times more effective than vitamin C and 25 times higher than vitamin E. Polyphenol is useful to capture the free radicals so as not to oxidize fat, protein and DNA in cells and stop the proliferation of malignant cells(cancer).[27-32]

Flavonoids in polyphenol are powerful antioxidants which are scavengers for the radical

anion, singlet oxygen, lipid peroxy radicals, and its reactive power can also remove metal ions through attachment.[27-32]

Flavonoids (quercetin) as much as 47 µmol can reduce 50% of human lymphocyte DNA damage. Incubation of Jurkat cells for 24 hours with 15 µmol/L significantly reduced DNA damage. Epigallocatechin-3-gallate (EGCG) is able to increase the protection of the cells from lipid membrane peroxidation and DNA damage.[28]

Epigallocatechin-3-gallate may reduce the production of Reactive Oxygen Intermediate and has great effect as chemoprevention. Experiments on green tea polyphenols showed that it inhibits lipid peroxides. EGCG may provide protection from oxidative DNA damage, by penetrating the direct scavenging of ROS. This study showed that low concentrations of green tea polyphenols may reduce the hydroxyl radical that is the cause of base damage and single strand DNA damage, by the mechanism of electron transfer from catechins to radical side of the DNA. In vivo, green tea polyphenols also inhibit UVB, the cause of oxidative stress markers in animals prior to UVB radiation protection against depletion of glutathione: antioxidant enzyme glutathione peroxidase and catalase; reduce UV, the cause of lipid peroxidation, and inhibit UVB, the cause of protein oxidation. EGCG protects UV resistance, the cause of oxidative stress in humans as well.[33]

In the preliminary study conducted in 2008, there were significant differences in blood mercury levels in mice exposed to mercury in the group given green tea extract those not given green tea extract at a dose of 0.52 mg and 0.78 mg/20 g body weight. In mice that were given green tea extract, mercury levels in blood were found lower than the group who were not given green tea extract.[34]

As a result of continuous exposure to Hg, there will be a decrease in antioxidant activity in the body, especially Glutathione peroxidase (GPX). The decrease in antioxidant activity will lead to the formation of free radicals that may cause damage to cell membranes in the body. The cell membrane damage will cause cell damage as a whole. Therefore, extracellular antioxidant that is usually obtained from food such as vitamin C, vitamin E and green tea polyphenols is necessary.

Based on the background described, researchers wanted to know more about the influence of mercury to damage the liver cells and the effects of green tea extract on liver cells of mice exposed to mercury histopathologically.

The purpose of this study was to determine the effect of inhalation of mercury in the liver cells and the effects of green tea extract as an antioxidant against the mice liver cells exposed to mercury seen histopathologically

METHODS

This study was purely experimental studies conducted on male white mice (*Mus musculus*) at the Laboratory of Pharmacy, Universitas Andalas. Sample of 48 male white mice were divided into 8 groups randomly. The green tea used was *Kepala Djenggot* brand, which was extracted in West Sumatra Provincial Health Laboratory. Mercury used was a type of metal mercury commonly used as amalgam fillings, with brand of Kasadental Japan. Liver cells were seen histopathologically. Histopathologic preparations were made with HE (haematoxylin eosin) staining, assessed by Binocular light microscope at magnification of 400x, then examined in the Pathology Laboratory Faculty of Medicine Universitas Andalas. The research was conducted from February to June 2011.

In this study, white mice (*Mus musculus*) weighing 20-25 g were used. Mice were randomly selected. Forty eight mice were divided into 8 groups randomly such as A, B, C, and D groups (with 3 and 6 weeks of treatment). Each group consisted of 6 mice. A group was a negative control in which the samples were not given any treatment. B group was the positive control that exposed only with mercury without administration of green tea extract. C group was exposed to mercury and was given a green tea extract at a dose of 0.52 mg/20 gr body weight. D group was exposed by mercury and was given green tea extract at a dose of 1.04 mg/20 gr body weight.

Mice in B, C and D groups were exposed to mercury through inhalation for 4 hours a day. Mice were placed in a cage with a specific design, given the same treatment and feed. In the third and sixth weeks, the liver cells of each group was

Table 1. Differences in the average number of mice liver cell degeneration at various dose and duration of green tea extract

Duration of Dose (mg/20 gr BW)	Degeneration A	B	C	D	ρ (Anova)
3 Week	5.25±0,5	40.00±11.55	20.00±0.00	17.5±5.00	0.000*
6 Week	5.25±0,5	85.00±4.08	62.5±5.00	70.00±8.16	0.000*
ρ (T test)	1.000	0.002	0.000*	70.00±8.16	0.000*

Note:* significance at ρ <0.05

examined by making histopatologic preparation at Anatomical Pathology Laboratory Faculty of Medicine Universitas Andalas. The preparation was then examined with Binocular light microscope. Damage that occured in liver cells was visible. The data obtained were processed and analyzed using ANOVA and independent T test with 95% confidence level.

RESULT

The research result can be seen in Table 1-3. Histopathologic feature of liver cells in the control group (A) can be seen in Fig. 1. Liver consisted of lobules in which, central vein located in the middle, hepatic cells arranged in a plate with between sinusoids, definite cell boundaries, and polygonal-shaped cells.

Histopathologic feature of liver cells in group exposed by mercury without given green tea extract (group B) for three weeks: hepatic cells seem greater, eosinophylic cytoplasm, partially degenerated cells, indefinite cell boundaries, some cells with intracytoplasmic vacuola, and indefinite sinusoid (Fig. 1 B3).

Histopathologic feature of liver cells in the group exposed by mercury and given green tea extract as much as 0.52 mg/20 g BW for 3 weeks (Group C): the size of liver cells appeared normally, eosinophilic cytoplasm, some cells degenerate, indefinite cell boundary, definite sinusoid, histiocytes containing cells, and intracellular edema did not occur (Fig. 1 C3).

Histopathologic feature of liver cells in the group exposed by mercury and given green tea extract as much as 1.04 mg/20 g BW for 3 weeks (D) the size of normal liver cells appear, eosinophilic cytoplasm, some cell degenerate, definite cell borders, definite sinusoid, widened, histiocytes cell proliferation. Intra-cellular edema does not occur (Fig. 1 D3).

Histopathologic feature of liver cells in the group exposed by mercury only without given green tea extract (B) for 6 weeks: liver cells appear swollen, eosinophilic cytoplasm, partially degenerate cells, cells boundaries blurred, sinusoid containing histiocytes cells, and vacuola intra-cells (+) (Fig. 1 B6).

Histopathologic feature of liver cells in the group exposed by mercury and given green tea extract (C) 0.52 mg/20 g BW for 6 weeks: visible intracellular edema, eosinophilic cytoplasm, partially degenerate cells, cell borders vague, indefinite sinusoids, intra cytoplasmic vacuoles (+) (Fig. 1 C6).

Histopathologic feature of liver cells in the group exposed to mercury and given green tea extract (D) 1.04 mg/20 gr BW for 6 weeks: liver cells edema appear, indefinite boundaries cells, the majority of cells degenerate and suffered from necrosis (Fig. 1 D6).

Table 3. The results of analysis of liver cell degeneration in various groups based on the dose of green tea extract for 6 weeks

Group	A6	B6	C6	D6
A6	-	0.000*	0.000*	0.000*
B6	-	-	0.000*	0.009*
C6	-	-	-	0.387
D6	-	-	-	-

Table 2. The results of analysis of liver cell degeneration in various groups based on the dose of green tea extract for 3 weeks

Group	A3	B3	C3	D3
A3	-	0.000*	0.037*	0.105
B3	-	-	0.004*	0.002*
C3	-	-	-	1.000
D3	-	-	-	-

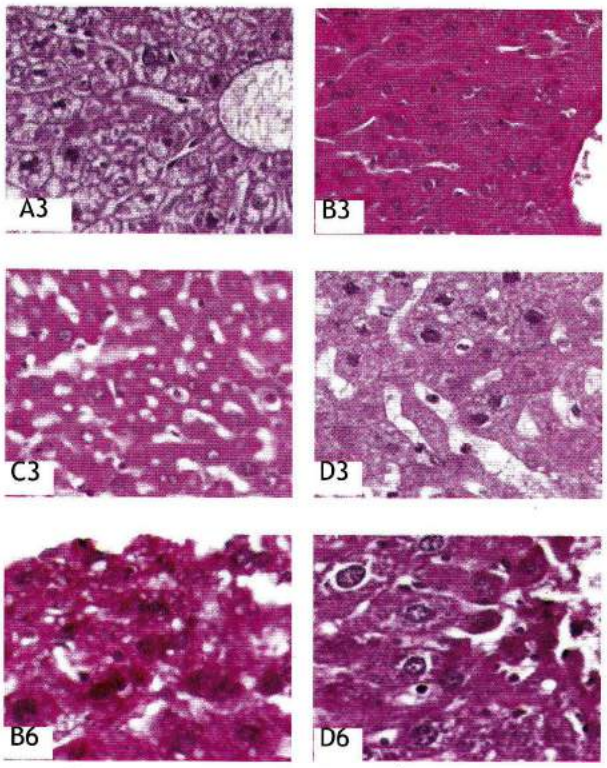

Figure 1. Histopatologic feature of liver cells of white mice exposed by mercury and given green tea extract in different doses and duration.

DISCUSSION

The results showed that degeneration of the liver cells of mice in groups B, C, and D in 3 and 6 weeks increased. The results were analyzed by ANOVA analysis to determine the effect of dose and duration of mercury and green tea against mice's liver cells degeneration (Tab. 1). Having analyzed statistically, the average increase in liver cells degeneration of mice showed significant difference in treatment of 3 weeks (ρ=0.000) and 6 weeks (ρ=0.000). The least liver cells degeneration occured to the group D, the group that was given the treatment of green tea extract administration 1.04 mg/20 gr BW in 3 week mercury exposed (17.5%). While, degeneration was most commonly seen in group B, the group in which mice were exposed by mercury for 6 weeks and was not given green tea extract (85%).

Research results also showed significant differences in group B who were inhaled mercury without given green tea extract between 3 weeks and 6 weeks. The higher rates of degeneration of the liver cells occured at 6 weeks of treatment (85%) than in 3 week treatment group (40%) (Tab. 1). Based on these results, the longer the exposure by mercury, the more the liver cells were damaged (degeneration).

Mercury is a heavy metal that is very dangerous, because the ability of mercury to produce free radicals is quite remarkable. Mercury can cause depletion of antioxidant enzymes in the body such as GPX, SOD and catalase. These enzymes play an important role in counteracting the formation of free radicals in the body. With the depletion of these enzymes, the formation of free radicals that may cause damage to body cells, including liver cells, may occur. The enzyme of catalase is widely available in the liver. Catalase enzyme plays an important role in the acquisition of additional adaptive response of cells against oxidative stress by converting H_2O_2 to H_2O dan O_2. Actually liver is an organ that has the best antioxidant protectors system compared to other organs. But due to continuous exposure to mercury, the production O_2°- and H_2O_2 will increase steadily. As a result, there is also an increased need for of SOD and catalase enzymes to neutralize ROS. Thus, the enzymes of SOD and catalase will increasingly depleted and will eventually decrease when compared to mice without any exposure to mercury. With the reduction in of SOD in the liver, the body's antioxidant activity is also reduced, resulting in an increase in free radicals that can damage cells.[16,23-25]

The results also showed that there were no significant differences between groups A and D (ρ = 0.105) (Tab. 2). So it can be concluded that administration of green tea extract at a dose of 1.04 mg/20 g BW in mice exposed to mercury (group D) for 3 weeks can suppress the degeneration of liver cells (there is no real difference with the degeneration of cells as a control group A).

A number of studies suggested that green tea polyphenols was associated with an effective scavenger of oxygen and nitrogen reactive species. Polyphenols also have the ability to bind (chelate) metal ions. Polyphenols can also inhibit the formation of reactive oxygen species (ROS) by inhibiting the provision of tea xantine oxidase in mice significantly increase GST activity in the liver and administration green tea polyphenols in drinking water of mice also significantly increased the activity of GST in liver and small intestine. Tea consumption may prevent the depletion of GSH caused by carbon-tetrachloride in male mice

liver, but not in female mice. Provision of tea and tea polyphenols have been reported to prevent the reduction of antioxidant enzyme activity of a number of animal suffering oxidative stress. Green tea extract in mice drinking water may prevent the reduction of SOD in liver and serum associated with ethanol as well as the activities of gluthatione peroxidase (GPX) and catalase liver.[29] Epigallocatechin gallate (EGCG) in green tea may increase the protection of cell membrane lipid peroxidation and DNA damage.[28]

In the body's defense against free radicals and ROS, SOD serves to destroy O_2^{o-} by converting it to peroxide that will be destroyed also by catalase or glutathione peroxidase. Radical nature of the high O_2^{o-} will be converted by SOD into less reactive H_2O_2.[25] Administration of green tea extract on mice exposed to mercury may prevent the reduction of antioxidants in the liver primarily SOD (superoxide dismutase). Thus, green tea extract in certain doses can prevent the damage (degeneration) liver cells.

CONCLUSION

According to the research results, it can be concluded that exposure with mercury continuously affect the damage (degeneration) of liver cells. The longer the exposure to mercury, the more the liver cells damaged. Green tea extract at a certain dose affect the liver cell damage arising from mercury exposure in mice, and green tea extract with a specific dose can reduce damage of liver cells.

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