PENGARUH SUPLEMENTASI VITAMIN C TERHADAP AKTIVITAS SITOKROM P450 1A1 (CYP1A1), GLUTATION-S-TRANSFERASE DALAM HEPAR DAN EMBRIOTOKSISITAS MENCIT DENGAN INTOKSIKASI TIMBAL

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ABSTRACT

Lead contamination occurs through air pollution and industry, enter the body through respiratory and digestive tract. High lead content will accumulate and affect adversely the cognitive function, causing neuropsychological dysfunction, encephalopathy, hyperactivity and other problems in children, disrupt the central nervous system and the immune system of children as well.

This experimental research is randomized control group post test only design. The experiment used of 27 pregnant mice, divided into three groups: negative control group, which were given distilled water, positive control group were exposed only to lead and the third group were exposed to lead and administered vitamin C. 25 mg / kg /day/ orally neutral lead acetate was given during gestation day 7 to 16, and vitamin C 64 mg / kg / day/ orally, started on gestation day 9 to 16.

Treatment group with vitamin C supplementation has the lowest CYP1A1 and glutathione S-transferase enzyme activity compared to positive and negative control groups. This results confirmed by the molecular weight of CYP1A1 enzyme ranges 53.7 to 59.2 kDa, and the western blotting test showed the same thin band both two groups. The lowest of the average lead concentration in the head of fetal mice and the blood of mice were found on the group of mice that treated with vitamin C. There was no effect of lead exposure on the fetuses survival, but only decreased fetal body weight. Supplementation of vitamin C can protect the liver, and fetus, by suspected mechanism that vitamin C could chelate the lead and excrete it via urine.

Key words: vitamin C, CYP1A1, GST, lead in mice blood, lead of fetal head

RINGKASAN

Pencemaran logam berat dapat terjadi melalui pencemaran udara maupun industri. Pencemaran udara di Indonesia sebagian besar disebabkan oleh emisi kendaraan bermotor. Laju pertambahan kendaraan bermotor meningkat dari tahun ke tahun sehingga konsumsi bahan bakar juga meningkat sedangkan bahan bakar bensin yang diproduksi saat ini belum bebas timbal.

Timbal yang masuk ke dalam tubuh melalui saluran pernafasan dan pencernaan diikat oleh eritrosit dan sebagian timbal plasma akan berdifusi ke jaringan lunak (sumsum tulang, system saraf, ginjal, dan hati) dan jaringan keras (tulang, kuku, rambut) dan sebagian timbal dieliminasi dari dalam tubuh melalui urine, feses, kulit, kuku dan rambut. Timbal akan disimpan di hati, ginjal, otak dan kulit serta jaringan lain yang bersifat toksis. Kadar timbal tinggi akan terakumulasi dan berpengaruh buruk terhadap fungsi kognitif, menyebabkan gangguan fungsi neuropsikologi, ada hubungannya dengan kehamilan abnormal, terjadinya anemia, gastro enteritis, ensefalopati, hiperaktivitas dan problema lain pada anak, mengganggu sistim saraf pusat, mengganggu sistim imunitas anak, penurunan fungsi ginjal, menghambat aktivitas enzim ferokelatase dan asam delta amino levulinat dehidratase. Timbal yang masuk dalam tubuh akan mengalami metabolisme xenobiotik di hati. Pada wanita dewasa, timbal mengganggu sistim reproduksi karena mengganggu daur menstruasi, meningkatkan resiko keguguran, bayi lahir dengan berat badan rendah dan gangguan pertumbuhan. Timbal yang ditimbun dalam tulang wanita, akan dimobilisasi pada saat mengandung, masuk kedalam peredaran darah ibu dan selanjutnya sampai ke tubuh janin.

Jenis penelitian ini adalah eksperimental laboratoris dengan rancangan *Randomized post test only control group design* . Sampel yang dibutuhkan adalah mencit bunting 27 ekor yang terbagi menjadi 3 kelompok, masing-masing kelompok terdiri dari 9 ekor. Kelompok kontrol negatif, sebagai plasebo yang diberi aquades saja. Kelompok kontrol positif, hanya dipapar Pb saja dengan pemberian plumbum asetat netral dengan dosis 25 mg/kg BB/hari melalui sonde, dimulai saat gestasi ke 7 sampai ke 16, diberikan pada pagi hari. Kelompok perlakuan, dipapar Pb saja dengan pemberian plumbum asetat netral dengan dosis 25 mg/kg BB/hari melalui sonde, dimulai saat gestasi ke 7 sampai ke 19 dan diberi vitamin C (kadar 99,5 % dari Fluka AG, *Chemische Fabril CH-9470 Busch SG*) dilarutkan dalam aquadest dengan dosis 64 mg/kg BB/hari melalui sonde, dimulai pada gestasi hari ke 9 sampai ke 16. Variabel bebas penelitian ini adalah suplementasi vitamin C pada induk mencit yang terintoksikasi timbal sedangkan variabel terikatnya adalah kadar timbal dalam darah induk mencit, profil CYP1A1, aktivitas glutation-S-transferase dalam hepar induk mencit, kadar timbal kepala fetus, embriotoksisitas dan gambaran histologi otak fetus. Rancangan analisisnya adalah deskriptif, manova atau Kruskal wallis. Penelitian ini dilaksanakan di Laboratorium Biokimia Universitas Brawijaya, dan pelaksanaan analisis parameter pemeriksaan bertempat di Laboratorium Biokimia Universitas Brawijaya, Balai Besar Laboratorium Kesehatan dan Laboratorium Patologi Anatomi RS Dr Soetomo Surabaya.

Timbal yang masuk dalam tubuh akan mengalami metabolisme xenobiotik di hati. Enzim yang bekerja pada metabolisme xenobiotik terbagi menjadi 2 fase yaitu fase I, dikatalisis oleh enzim CYP1A1, fase II, dikatalisis oleh enzim Glutation S-transferase. Profil enzim CYP1A1 diketahui dengan cara mengukur aktivitas enzim CYP1A1 dan menganalisis berat molekul enzim CYP1A1 untuk melihat kualitas enzimnya.

Suplementasi vitamin C menurunkan aktivitas enzim CYP1A1 pada hepar induk mencit yang terintoksikasi timbal dan hasil pemeriksaan berat molekul enzim CYP1A1 berkisar 53,7-59,2 kDa. Aktivitas enzim CYP1A1 pada kelompok perlakuan dengan vitamin C terendah bila dibandingkan kelompok kontrol, sesuai dengan peneliti Chang (2009) maupun Ueta (2003) bahwa suplementasi vitamin C mempengaruhi ekspresi gen

penyandi CYP1A1 secara tidak langsung pada tingkat transkripsi. Vitamin C memiliki efektivitas yang tinggi untuk menekan induksi enzim CYP1A1.
Aktivitas terendah enzim Glutation S-transferase didapatkan pada kelompok mencit yang terintoksikasi timbal dengan suplementasi vitamin C. Suzuki *et al* (1996) menyebutkan ada hubungan antara timbal dengan aktivasi gen yang menyandi Glutation S-transferase melalui *Gluthathion S-transferase-placental enhancer I (GPEI)* sehingga keberadaan timbal dalam tubuh dapat meningkatkan aktivitas enzim Glutation S-transferase, seperti yang terjadi pada kelompok kontrol positif yang hanya dipapar oleh timbal saja.
Pada kelompok perlakuan dengan vitamin C memiliki kadar timbal terendah pada kepala fetus dibandingkan kelompok lainnya. Adanya *corpus callosum* dan *cerebral cortex* yang lebih tebal dibandingkan kelompok kontrol positif. Paparan timbal dapat menginduksi ekspresi neurothropin dan berpengaruh pada pertumbuhan saraf , *brain-derived neurothropic factor neurothropin-3,* serta reseptor tirosin kinase dan reseptor neurotropin (P75NTR) (Nemoto *et al*, 2000). Penelitian yang dilakukan oleh Rodrigues *et al* (1999) menyimpulkan bahwa timbal akan berinteraksi dengan protein G dan sub unit katalitik dari *cerebral cortical* untuk menghambat aktivitas enzim *adenylil cyclase* sehingga gambaran *cerebral cortex* didapatkan lebih tipis dibandingkan kelompok kontrol.
Timbal masuk ke dalam tubuh fetus melalui sirkulasi umbilical dan plasenta. Plasenta pada primata dan *rhodent* dapat diinduksi untuk memproduksi protein terikat metal dengan kadar tinggi. Pengaruh paparan timbal terhadap embriotoksisitas, pada jumlah fetus hidup tidak ada perbedaan antar kelompok, tetapi pada kelompok kontrol positif yang hanya diberi timbal saja memiliki nilai resorpsi embrio sebesar 7,8% dan keadaan ini tidak didapatkan pada 2 kelompok lainnya. Untuk pengaruh terhadap berat badan fetus antar kelompok, memiliki beda yang signifikan dimana pada kelompok kontrol positif memiliki rerata berat badan fetus paling kecil, disusul oleh kelompok perlakuan dan kelompok kontrol negatif. Ini sesuai dengan penelitian yang dilakukan oleh Singh (1993) pada kelompok dengan paparan timbal tinggi terjadi reduksi ukuran fetus, reduksi berat badan fetus dan terjadi peningkatan resorpsi embrio. Pada penelitian ini jumlah fetus pada kelompok kontrol positif tidak ada perbedaan dengan kelompok lainnya dengan dosis timbal yang dipakai adalah 25 mg/kg BB mencit.
Suplementasi vitamin C memberikan proteksi pada hepar, dengan dugaan bahwa vitamin C mengkhelat logam timbal sebelum masuk ke hepar induk dan diekskresi via urine. Suplementasi vitamin C memberikan proteksi kepada fetus yang ditunjukkan dengan gambaran histologi otak fetus yang lebih baik dibandingkan dengan kelompok kontrol positif dan rerata kadar timbal pada kepala fetus yang paling rendah dibandingkan kelompok lainnya.

SUMMARY

Contamination of lead occurs through air and industrial pollution. Air Pollution in Indonesia is caused by vehicle emissions, because the fuel has not been freed of lead.
Lead enter the body through the respiratory and digestive tract bind erythrocytes and in the part of lead will diffuse into the soft tissue (bone marrow, nervous system, kidneys, and liver) and hard tissue (bone, nails, hair) and some of lead eliminated from the body through urine, feces, skin, nails and hair. Lead is stored in the liver, kidney, brain and

skin and other tissues that are toxic. Lead will accumulate and adversely affect cognitive function, causing neuropsychological dysfunction, abnormality in the pregnancy, anemia, gastro enteritis, encephalopathy, hyperactivity and other problems in children, interfere with CNS, disrupting the child's immune system, decreased kidney function, inhibit ferochelatase enzyme activity and d-amino acid levulinic dehidratase activity. Lead in the body will influence xenobiotic metabolism in the liver. In adult women, lead interferes with the reproductive system by interrupting the menstrual cycle, increasing the risk of miscarriage, a baby born with low weight and stunted growth. Lead is deposited in the bones of women, will be mobilized at the time of conception, into the maternal circulation and on to the fetus.

This experimental research is randomized control group post test only design. The experiment used of 27 pregnant mice, divided into three groups: negative control group, which were given distilled water, positive control group were exposed only to lead and the third group were exposed to lead and administered vitamin C. Neutral lead acetate, 25 mg / kg /day/ orally was given during gestation day 7 to 16, and vitamin C (99% from Fluka AG, Chemische Fabril Bush SG CH-9470) 64 mg / kg / day/ orally , started on gestation day 9 to 16.

The independent variable was supplemented vitamin C in mice that intoxication lead and the dependent variable were the lead content in the blood of mice, the profile of CYP1A1, the activity of glutathione-S-transferase in mice liver, lead level of fetal head, embriotoxicity and description fetal brain histology. The research's results were analyzed with descriptive, Manova or Kruskal Wallis. This research was done at the Laboratory of Biochemistry UB, and analysis of parameters located in Brawijaya University's Laboratory of Biochemistry, Central Health Laboratory, Pathology Laboratory Dr. Soetomo Hospital.

Lead in the body will influence xenobiotic metabolism in the liver. Enzymes in the metabolism xenobiotic divided into 2 phases which first phase, catalyzed by the enzyme CYP1A1, and second phase, catalyzed by the enzyme Glutathione S-transferase. CYP1A1 enzyme profile was determined by measuring the activity of the enzyme CYP1A1 and molecular weight of CYP1A1. Supplementation of vitamin C reduces the activity of CYP1A1 enzyme in the mice's liver of intoxication lead and molecular weight of CYP1A1 enzyme was 53.7-59.2 kDa. The lowest CYP1A1 enzyme activity in treatment groups with vitamin C than other groups, according to researcher Chang (2009) and Ueta (2003) that Vitamin C supplementation affected the expression of CYP1A1 gene encoding indirectly at the level of transcription. Vitamin C is highly effective to suppress the induction of CYP1A1 enzyme

The lowest activity of Glutathione S-transferase enzyme was found in groups of mice that intoxication lead with supplementation of vitamin C. Suzuki et al (1996) concluded that the lead activated of genes that encode Glutathione S-transferase via Glutathione S-transferase-placental enhancer I (GPEI) so that the presence of lead in the body increased the activity of Glutathione S-transferase enzyme, also happened in positive control group exposed lead only.

Treated with vitamin C group had the lowest lead in the fetus's head than other groups, it also indicated the existence of the corpus callosum and cerebral cortex that is thicker than the positive control group. Exposure to lead may induce the expression neurothropin and effect on nerve growth, brain-derived factor neurothropic neurothropin-3, and the

receptor tyrosine kinase and receptor neurotropin (P75NTR) (Nemoto et al, 2000). Rodrigues et al (1999) concluded that the lead will interact with G protein and the catalytic subunit of cerebral cortical activity to inhibit the enzyme adenylic cyclase that thinner cerebral cortex than the control group.

Lead enters the body of the fetus through the umbilical and placental circulation. Rhodent placenta in primates can be induced to produce proteins with high levels of metal bound. The effect of lead exposure on embriotoxicity there were no differences, the number of living fetuses between groups, but on the positive control group which given only lead alone has a value of embryo resorption by 7.8% and it was not found in the other two groups. For the effect on fetal body weight between groups, a significant difference where the positive control group had a mean weight loss fetal smallest, followed by treatment group and negative control group. Singh (1993) concluded that high lead exposure in the group occured fetal size reduction, reduced fetal weight and increased resorption of embryos. In this study the number of fetuses in the positive control group there were no differences with other groups with a dose of lead that is used is 25 mg / kg mice.

Supplementation of vitamin C will protect the liver through chelating reaction vitamin C and then lead will be excreted via urine. Supplementation of vitamin C provides protection to the fetus was showed by the fetal brain histology was better than the positive control group and the average concentrations of lead on fetal head is the lowest among the other groups.