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GREEN TEA LEAVES EXTRACT EFFECT ON HISTOPATHOLOGY OF MERCURY CHLORIDE INDUCED RAT'S LIVER

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ABSTRACT

This research was aim to observe the histopathology of mercury chloride induced rat's liver given green tea extract in different dosages. Thirty rats were divided into five groups with different treatments and administered through orally for 20 days. The treatment consist of negative control (CMC Na 1% solution + aquadest), positive control (CMC Na 1% solution + 8 mg/kg bw of mercury chloride), treatment 1, 2 and 3 (200, 400, and 800 mg/kg bw of green tea leaves extract respectively + 8 mg/kg bw of mercuric chloride). Scoring data was analyzed by Kruskal Wallis and continued with Mann-Whitney U test to see the significant difference ($p<0.05$) between all treatments. Result analysis is there are a significant difference between negative control and positive control that indicate mercuric chloride significantly decreased the necrosis and degeneration of liver cells and treatment 3 (800 mg/kg bw) gave the best prevention for histopathology necrosis and degeneration of liver cells. The conclusion is green tea leaves extract could protect rats liver from the damage effect of mercuric chloride. This research aimed to prove the preventive effect of green tea leaves extract (*Camellia sinensis*) on rats (*Rattus norvegicus*) that are induced by mercuric chloride.

KEY WORDS: Green tea leaves extract, Histopathology, Mercury chloride, Rat's liver

INTRODUCTION

The liver is an important organ that acts as a place for metabolism and detoxification of biological compounds. This organ functions in neutralizing the toxic substances that enter the body, one of which comes from mercury chloride (Saidi, 2013).

Mercury chloride is very dangerous for the body and the environment. In Indonesia the increase in industrialization is the main cause of increase in environmental pollution by mercury. That is because mercury can be used optimally for mining (Lestarisa, 2010). In the central and western districts of Lombok, mercury chloride pollution is increasing. The mercury content in grass and natural soil has exceeded the threshold that can be a risk to the health of livestock around the area (Astiti, 2014). Mercury chloride pollution also occurs in water areas which causes a lot of poisoning because the surrounding population often consumes fish that come from waters polluted with mercury chloride from industrial waste discharges. That indicates that mercury chloride is very dangerous for the body (Hadi, 2013).

Mercury chloride is very dangerous because it can produce free radicals so that it can cause lipid peroxidation so that it can cause cell damage and cause degenerative diseases, for example liver disease (Halliwell, 2007; Chen, 2001). The cause of permanent damage to the liver, kidneys, and brain is the entry of all components of mercury chloride continuously (Alfian, 2007). Mercury chloride that enters the body will circulate in the blood vessels. Specifically, mercury chloride will bind to sulfihydryl (-SH) groups, one of which is found in the Gluthation (GSH) compound. Irreversible bonding between two GSH molecules with mercury chloride will cause a reduction in GSH levels because mercury chloride inhibits GSH reductase which functions to regulate GSH and GSH synthetase which functions to synthesize new GSH (Nabi, 2014). With decreased levels of GSH as the

body's natural antioxidant, it causes free radical concentrations that are not balanced with antioxidants or called oxidative stress. Therefore the body needs antioxidants that come from outside to prevent oxidative stress or protect liver cells from mercury chloride toxicity, one of which is the use of green tea leaf extract.

Asian communities including Indonesia believe that green tea contains substances that are useful for the prevention and cure of various types of diseases (Chen, 2001; Yusni, 2015). Green tea herbal plants have long been known and used by the people of Indonesia to tackle health problems. The use of green tea plants as traditional medicine in Indonesia has been carried out by our ancestors since centuries ago (Sukandar, 2006).

Green tea contains polyphenol compounds namely catechins which are useful as antioxidants that can neutralize free radicals. The types of tea catechins are very varied consisting of epicatechin, epigallocatechin, epicatechin - 3-gallate and epigallocatechin-3-gallate. However, the main catechin content is epigallocatechin, epicatechin (EGCG 59%) which has a strong free radical capture activity with strength up to 4-5 times higher than vitamins E and C (Gramza, 2005).

Based on the 2014 USDA, the catechin content in green tea is ± 11.86 mg / 100 g. The largest catechin content is epigallocatechin-3-gallate of ± 3.96 mg / 100 g. Green tea is thought to have an effect as a hepatoprotector. Hepatoprotector is efficacious compounds protect cells and repair damaged liver tissue due to the influence of toxic substances (Panjaitan, 2008). Giving hepatoprotector can be done for prevention (preventive) or healing (curative) (Kumarappan, 2011). One of the ingredients needed as a hepatoprotector is antioxidants. Antioxidants are found in a variety of plants that are easily available to the public, inexpensive, and do not contain harmful chemicals (Situmorang, 2010).

Several studies have shown that antioxidants can protect lipid membranes from oxidation (Amic, 2003). A study of 1371 men over the age of 40 proved that consuming more than 10 cups of green tea per day would reduce the concentration of enzymes marking liver damage, namely aspartate amino transferase, alanine transferase, and ferritin. Based on other studies, the administration of green tea extract (*Camellia sinensis*) dose of 60 mg/ 200gBW in rats has an effect to protect the liver as indicated by a significant decrease in the level of liver cell nucleus damage in isoniazid-induced mice (Amelia, 2008).

Based on the background description, further research is needed regarding the effectiveness of green tea leaf extracts (*Camellia sinensis*) in white rats (*Rattus norvegicus*) induced by mercury chloride with histopathological features of the liver.

MATERIALS AND METHODS

This research was a laboratory experiment with the design used to completely randomized design (CRD) using 30 male rats were divided into 5 experimental groups. Each group consisted of 6 white rats.

The experimental animals used in this study were male white rats (*Rattus norvegicus*) aged 3 months with a body weight of 120-150 g obtained from the Faculty of Medicine, Airlangga University, Surabaya. The sample used in this study was the liver organ. The sample size used in this study is based on the formula of Federer (Kusriningrum, 2008):

$t (n-1) \geq 15$
$5 (n - 1) \geq 15$
$5 n - 5 \geq 15$
$n \geq 4$
t = treatment group
n = number of samples

This study consisted of 5 treatment groups that had a number of replications at least 4 so that the whole requires a minimum of 20 male white rats (*Rattus norvegicus*). The correction factor in this study was 50% of the minimum total rat. 50% x 20 mice = 10 mice. So that the number of rats used in this study were 30 male white rats (*Rattus norvegicus*).

Data is arranged in tabular form and then analyzed statistically using the Kruskal-Wallis test. If there are significant differences between the study groups ($p < 0.05$), followed by the Mann-Whitney U test. Statistical analysis in this study uses the SPSS (Statistical Program of Social Science) program.

RESULTS AND DISCUSSION

Liver cell examination is carried out by observing 10 visual fields on a microscope with 400x magnification. Pathological changes observed include degeneration and necrosis of liver cells.

The results of observations of liver cell degeneration and necrosis are presented in Table 1.

Statistical analysis of liver cell degeneration in Table 1 shows that the K- (1% CMC-Na + Aquades) treatment group had significant differences with all the K +, P1, P2 and P3 treatment groups. The K + treatment group (CMC-Na 1% + mercury chloride 8mg/kg BW) showed significant differences with all the treatment groups K-, P1, P2 and P3. The treatment group P1 (EDTH 200 mg/kg BW + mercury chloride 8mg/kgBW) showed significant differences with all the treatment groups K-, K +, P2 and P3. P2 treatment group (EDTH 400 mg/kg BW + 8 mg/kg BW mercury chloride) showed significant differences with all treatment groups K-, K +, P1 and P3. The P3 treatment group (EDTH 800 mg/kg BW + mercury chloride 8 mg/kgBW) showed significant differences with all the treatment groups K-, K +, P1 and P2.

Table 1. Degeneration and Necrosis of Liver Cells in White Mice induced by mercury chloride (*Rattus norvegicus*).

Treatment	Liver cell degeneration (Mean ± SD)	Liver cell necrosis (Mean ± SD)
P1	2,25a ± 0,05	2,75a ± 0,17
P2	1,87b ± 0,15	2,55a ± 0,54
P3	0,97c ± 0,15	1,05b ± 0,41
K-	0,52d ± 0,12	0,62b ± 0,18
K+	2,62^{c} ± 0,09	3,22c ± 0,26

Note: The difference in superscript in the same column shows a significant difference ($p < 0.05$).

The results of statistical analysis of liver cell necrosis in Table 1 show that the K-treatment group had significant differences in K^{+}, P1, and P2, but did not differ significantly in P3. The K + treatment group showed significant differences with all the K^{-}, P1, P2 and P3 treatment groups. The treatment group P1 showed significant differences in the K-, K + and P3 groups, but did not show a significant difference in P2. The P2 treatment group showed significant differences in the K-, K + and P3 groups, but did not show a significant difference in P1. The P3 treatment group showed a significant difference to the K +, P1 and P2 groups, but did not show a significant difference to the K-.

CONCLUSSION

Green tea leaf extract (*Camellia sinensis*) is effective in reducing the level of degeneration and necrosis of white rat (*Rattus norvegicus*) liver cells induced by mercury chloride. The optimal dose of green tea leaf extract (*Camellia sinensis*) in this study was 800mg/kg BW.

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REFERENCES

Alfian, Z. 2006. Mercury: Between the Benefits and Effects of Its Use for Human Health and the Environment [Thesis]. Faculty of Math and Science. University of Northern Sumatra. [Text in Indonesia].

Amelia, N. 2008. Hepatoprotective Effects of Green Tea Leaf Extract (*Camellia sinensis*) in White Rat (*Rattus norvegicus*) induced by isoniazid [Thesis]. Medical School. Sebelas Maret University. accessed January 6, 2011. [Text in Indonesia].

Amic, D., Davidovic, A. D., Besto, D. and Trinajstic, N. 2003. Structure-radical scavenging activity. Relationship of flavonoids. *Croatia Chemical Acta.* 76 (1): 55-61.

Astiti, L.G.S and Sugianti, T. 2014. The Impact of Traditional Gold Mining on the Environment and Animal Feed on Lombok Island. *Animal Science*. 12 (2): 101-106. [Text in Indonesia].

Chen, Z.Y., Zhu, Q. Y., Tsang, D. and Huang, Y. 2001. Degradation of Green Tea Catechins in Tea Drinks. *J. Agric. Food Chem.* 49 (1): 477-482.

Gramza, A., Pawlak-Lemañska, K., Korczak, J., Wsowicz, E. and Rudzinska, M. 2005. Tea Extracts as Free Radical Scavengers. Polish *Journal of Environmental Studies*. 14 (6) : 861-867.

Hadi, M. 2013. Danger of Mercury in Our Environment. Health Environment Poltekkes, Denpasar. [Text in Indonesia].

Halliwell, B. and Gutteridge, J. M. C. 2007. *Free Radicals in Biology and Medicine* 5th Edition. Oxford University Press. Oxford. 896.

Kumarappan, C., Vijayakumar, M., Thilagam, E., Balamurugan, M., Thiagarajan, M., Senthil, S., Das, S.C. and Mandal, S.C. 2011. Protective and curative effects of polyphenolic extract from *Ichnocarpus frutescense* leaves on experimental hepatotoxicity by carbon tetrachloride and tamoxifen. *Ann of Liver*. 10 (1): 63-72.

Kusriningrum, R.S. 2008. *Trial Design*. Airlangga University Press. Surabaya. [Text in Indonesia].

Lestarisa, T. 2010. Factors Related to Mercury Poisoning (Hg) in Unlicensed Gold Mining (PETI) in Kurun District, Gunung Mas Regency, Central Kalimantan.

[Thesis] Postgraduate Program. Diponegoro University. Semarang. [Text in Indonesia].

Nabi, S. 2014. Toxic Effects and Remedy. *Toxic Effects of Mercury*. 19-27.

Panjaitan, R. G. P. 2008. Testing of hepatoprotector activity of the earth's peg roots (*Eurycoma longifolia* Jack.). [Dissertation]. Bogor Agricultural Institute Graduate School. [Text in Indonesia].

Saidi, S.A., Azaza, M.S., Windmolders, P., Van Pelt, J. and El-Feki, A. 2013. Cytotoxicity evaluation and antioxidant enzyme expression related to heavy metals found in tuna by-product meal: An *In vitro* study in human and rat liver cell lines. *Exp Toxicol Pathol*. 1-9.

Sukandar, E. Y. 2006. Trends and Paradigms in the World of Pharmacy, Industry-Clinics-Health Technology. http://itb.ac.id/focus/ focus_file / oration-scientific-dies 45.pdf. [January 2006].

Situmorang, T.S. 2010. Effect of papaya juice (*Carica papaya* L.) as a hepatoprotector on the liver of mice exposed to paracetamol. [Thesis]. Sebelas Maret University School of Medicine. [Text in Indonesia].

Sukandar, E. Y. 2006. Trends and Paradigms in the World of Pharmacy, Industry-Clinic-Health Technology. http://itb.ac.id/focus/ focus_file / oration-scientific-dies 45.pdf. [January 2006]. [Text in Indonesia].

Yusni, Husni, T. T. R and Achmad, T. H. 2015. Activities of Green Tea Polyphenols (*Camellia sinensis* (L) O. Kuntze) As Immunomodulators through Immunoglobulin E (IgE) Suppression Response in Allergic Rhinitis. *MKB*. 47 (3): 160-166. [Text in Indonesia].