Environmental pollutant has become the major problems since the rapidly increased industrialization without adequate residue and waste control in many country especially in the developing country (Xianghua, 2000). Lead is the metal, which has been associated with human activities from the past and is considered as one of the major environmental pollutants (Aldamash and El-Nager, 2014). The use of lead had been started from 5000 years ago (Adikwu et al., 2013) and lead poisoning already occurs since the Roman Empire era (Rayner-Canham and Overton, 2006; Flora, 2012; Hegazy and Fouad, 2014). Global lead contamination, caused by the greatly increased circulation of lead in soil, water and air as a result of human activities, remains to terrace more years. (Tong et al., 2000).

Liver is the largest repository (33%) tissue of lead among other soft tissues followed by kidney. Lead induced hepatotoxicity was reported to be associated with the impairments of liver structure and function (Adikwu et al., 2013). Lead can cause oxidative stress by generating ROS throughcocination, caused by the greatly increased circulation of lead in soil, water and air as a result of human activities, remains to terrace more years. (Tong et al., 2000).

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affecting the heme metabolism (Miller et al., 2015) and scavenging the antioxidant enzymes through several mechanisms (Mudipalli, 2007). Oxidative stress can cause the accumulation of ROS that can induce lipid peroxidation (Miller et al., 2015) and can oxidize protein and DNA inside the cell that can cause cells necrosis (Gurer and Ercal, 2000).

Antioxidants can neutralize ROS and decrease oxidative stress (Poljsak et al., 2013). Antioxidant can inhibit the cellular damage through their free radical scavenging property. Their molecule is stable enough to donate an electron to neutralize and reducing the free radical capacity to damage the cell (Hassan et al., 2012).

Propolis has been known for its antioxidant properties. Propolis or bee glue is a dark-coloured resinous substance produced or collected by honey bees, containing beeswax, and sticky that collected from plants sources, mainly from flowers and leaves shoot or leaf buds, and then mixed with the saliva of the bees (Marcucci et al., 2001; Salatino et al., 2005; Gomez-Caravaca et al., 2006; Nakajima et al., 2009; Cottica et al., 2011). The benefit of propolis had been known since the ancient times (Sforcin and Bankova, 2014). Bhadauria et al. (2007) has proven the hepatoprotective effect of propolis. This hepatoprotective effect believed because of minerals, flavonoid and phenolic compound that can be found in the propolis because flavonoid and phenolic compound had high antiradical activity (Socha et al., 2014). Miguel (2014) also clearly stated that propolis has chelating metal ion activity. Because of the propolis potency in inhibiting the free radical substance by its antioxidant capacity and also chelate the presence of metal ion, it is believed that propolis can protect the liver from exposure of lead acetate.

MATERIAL and METHODS

Experimental design — Male mice (Mus musculus) BALB/C strain weighing 25 - 30 grams aged 7 - 8 weeks derived from PUSVETMA Surabaya were used for the experiment. Maintained at the same condition, provided balance diet and water ad libitum daily. Mice randomly divided into five groups, then adapted for 7 days in cages size of 36x28x12 cm, each consisting of five animals that will be given treatment orally or by gavage as follows: Bahan penelitian menggunakan bahan dasar basis krim meliputi white beeswax pellet, paraffin liquidum, natrii biborax, aquadest, serta alkohol 70%.

Negative control (C-) : 1,5% CMC-Na + 0,5% Tween 80 (38 days)

Positive control (C+) : 1,5% CMC-Na + 0,5% Tween 80 (3 days), then 1,5% CMC-Na + 0,5% Tween 80 + 20 mg/kg BW lead acetate (35 days)

First treatment (T1) : 200 mg/kg BW propolis (3 days), then 200 mg/kg BW propolis + 20 mg/kg BW lead acetate (35 days)

Second Treatment (T2) : 400 mg/kg BW propolis (3 days), then 400 mg/kg BW propolis + 20 mg/kg BW lead acetate (35 days)

Third Treatment (T3) : 800 mg/kg BW propolis (3 days), then 800 mg/kg BW propolis + 20 mg/kg BW lead acetate (35 days)

At the end of treatment, mice will be euthanized and dissected to collect the liver. The collected liver then stored in neutral buffer formalin 10% as fixation solution.

Research Location and Time -- This research was conducted at Laboratory of Animal Faculty of Veterinary Medicine Universitas Airlangga for the treatment of experimental animal. Tissue processing and histopathological
examination was done in department of Pathology Veteriner Faculty of Veterinary Medicine Universitas Airlangga. The experiment was carried out in April 2017 - May 2017.

Research Material and Equipment — The equipment used in this study include animal, balance, water container, spuit 1 ml, oral gavage tube, gloves, surgical instruments, microscope, a series of dehydration apparatus, microtome, water bath and hot plate. Materials used in this study were propolis ethanolic extract, lead acetate, aquadest, mice feed, ad libitum drinking water, Hematoxylin Eosin (HE) stain, buffer formaline, 70, 80, 90 and 96 % alcohol, xylol, paraffin, entellan and Hematoxylin Eosin.

Propolis Extract Preparation — Raw propolis produced by Apis mellifera were collected from Agro Tawon Rimba Raya Malang. Raw propolis then extracted using maceration method at Balai Penelitian dan Konsultasi Industri (BPKI), Ketintang, Surabaya. Ethanolic extract of propolis then given to mice as 200, 400 and 800 mg/kg BW (Zhao et al., 2009) with 1,5% CMC-Na and 0,5% tween 80 as suspensator.

Lead Acetate Preparation — The dose 20 mg/kg BW (Guang et al., 2009) preparation done by dissolving 0,02 mg lead acetate in 100 ml aquadest. Lead acetate were given one hour after propolis extract administration.

Liver Histopathological Preparation and Examination — The histopathological preparation procedure and liver histopathological examination was done at department of Pathology Veteriner Faculty of Veterinary Medicine Universitas Airlangga. Liver histopathological examination was done using Nikon Eclipse E100 microscope that connected to MTN-004-00348 optilab camera and optilab viewer 2.2 with 100x magnification followed by 400x magnification, then the examination was done by calculating the percentage of the lesion per five times from random fields of view area using Arsad scoring (Arsad, 2014).

Data Analysis — Data for each group were analysed statistically using Kruskal Wallis Test followed by Mann-Whitney Test to compare the treatment effect of each group. Statistical analysis for this experiment is using SPSS 24.0.

RESULT

The examination results obtained from each groups of C- as negative control, C+ as positive control, T1 as first treatment, T2 as second treatment and T3 as third treatment, then processed with Statistical Product and Service Solutions (SPSS) program using Kruskal-Wallis test. Kruskal-Wallis test showed the significant difference all of the parameters (p<0.05). After that, continued with Mann-Whitney U test to see the differences between each group. The data result can be seen in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sinusoidal dilatation Parameters (Mean±SD)</th>
<th>Cells degeneration</th>
<th>Cells necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C -</td>
<td>0,12a + 0,11</td>
<td>0,88b ± 0,27</td>
<td>0,96a ± 0,17</td>
</tr>
<tr>
<td>C +</td>
<td>0,68b + 0,11</td>
<td>0,32a ± 0,01</td>
<td>3,00d ± 0,00</td>
</tr>
<tr>
<td>T1</td>
<td>2,48d + 0,46</td>
<td>1,68c ± 0,54</td>
<td>1,80bc ± 0,63</td>
</tr>
<tr>
<td>T2</td>
<td>1,28c + 0,18</td>
<td>1,52c ± 0,33</td>
<td>1,16ab ± 0,17</td>
</tr>
<tr>
<td>T3</td>
<td>0,72b + 0,18</td>
<td>1,60c ± 0,14</td>
<td>2,23c ± 0,22</td>
</tr>
</tbody>
</table>

The data result and histopathological features of liver on mice are also shown on the Figure 1 and Figure 2 below.
Sinusoidal Dilatation Examination — The result showed that 35 days of lead acetate administration (C+) and T3 did not cause the occurrence of sinusoidal dilatation. Otherwise, T1 and T2 showed the occurrence of sinusoidal dilatation.

Cells Degeneration Examination — Table 1 represent the effect of propolis ethanolic extract on histopathological changes of liver. The cells degeneration results of C+ group that were administered with lead acetate showed significant difference compared to C- group, also T1, T2 and T3 that were administered lead acetate and propolis ethanolic extract showed significant difference compared to C- group. This result showed that propolis extract could not protect the liver from cells degeneration caused by lead acetate.

Cells Necrosis Examination — The necrosis cells were significantly increased after lead acetate administration, while the administration of propolis ethanolic extract can reduce the necrosis cells. Even T2 with dose 400 mg/kg BW showed similar result with C- group, show the evidence that propolis ethanolic extract can protect the liver from lead acetate intoxication.
DISCUSSION

Lead that enter the body orally will interact with bacteria then will produce endotoxin. This endotoxin will cause inflammation of the Kupffer cell so that the Kupffer cell will produce several substance including cytokine (Ercal et al., 2001) and transforming growth factor-β (TGF-β) (Shimizu et al., 2012). Lead in the liver will produce reactive oxygen species (ROS) in which reactive oxygen species (ROS) will induce lipid peroxidation and will produce malondialdehyde (MDA) as the end result of lipid peroxidation (Miller et al., 2015). Lipid peroxidation can destroy the hepatocytes, and these destroyed hepatocytes will also release transforming growth factor-β (TGF-β) (Shimizu et al., 2012). Cytokines, reactive oxygen species (ROS), malondialdehyde (MDA) and transforming growth factor-β (TGF-β) that present in the liver can activate the stellate cell then become activated stellate cell or myofibroblast like cell. These activated stellate cells then produced a huge amount of collagen and cause sinusoid vasoconstriction (Bataller and Brenner 2005) that we can see in the result of C+ group that showed low result of sinusoidal dilatation.

High result on T1 may be due to an inflammatory process that occurs due to lead exposure, but dose 200mg/kgBW of ethanolic extract of propolis in this group has not been able to chelate lead optimally so that the inflammatory process in this group will continue to occur. The result of second treatment group T2 is not as high as T1 may because the inflammatory process in this group has begun to subside. This result indicates that dose 400mg/kgBW able to chelate lead optimally. The third treatment group T3 showed no significant different with positive control group C+. This result might happen because large amount of antioxidant that cannot chelate the lead, but act as pro-oxidants (Skibola and Martyn, 2000), resulting sinusoidal constriction.

Endotoxine that produced due to lead acetate intoxication can cause Kupffer cells produce thromboxane and prostaglandin. Thromboxane leads to sinusoid vasoconstriction resulting in reduced oxygen supply to the hepatocytes. While prostaglandins will increase hepatocyte metabolism which will increase oxygen demand. So that the condition can lead to ischemic hepatocytes (Thurman et al., 1997; Agarwal and Seitz, 2001). Ischemic condition can disrupt the oxidative phosphorylation process in mitochondria that cause lack of ATP as energy (Kumar et al., 2014).

Lead in the body can cause the accumulation of ROS (Ercal et al., 2001). Establishment of ROS by lead is due to the inhibition of Amino Levlulic acid synthase (δ-ALAS) that cause auto-oxidation of Amino Levlulic acid (ALA) then generate ROS (Miller et al., 2015). Lead also can scavenge the antioxidant enzyme in the body that can cause accumulation of ROS (Haleagrahara et al., 2010; Miller et al., 2015). Those ROS formation provoke lipid, protein, and DNA oxidation (Oda and Ibrahim, 2012).

According to McGavin and Zachary, (2017) lipid peroxidation can destroy plasma membrane that will cause increasing the permeability of Na+, H2O, and Ca2+ and disturb Na+-K+ ion pumps. Na+, H2O, and Ca2+ will move into the cells and lead to cell swelling or cells degeneration. But cells degeneration still considered normal, because this change is cells adaptation to injury.

Furthermore, that lipid peroxidation will also damage some organelles membrane such as mitochondria and lysosome. The damage of mitochondrial membrane induces Ca2+ efflux to the cytosol,
depletion of oxidative phosphorylation and ATP. Increasing intracellular Ca2+ activates number of enzymes such as ATPases that also resulting depletion of ATP, proteases that will cause cell membrane damage and endonuclease that will degrade the chromatin cells. Injury to lysosome membrane results in leakage of lysosome enzymes into the cytoplasm, leading to enzymatic digestion of protein, RNA, DNA, glycogen, and the cells die by necrosis. Lack of ATP will induce anaerobic glycolysis that will produce lactic acid then decrease pH. This pH decrease can cause the clumping of nuclear chromatin (Kumar et al., 2014).

Our ethanolic extract of propolis contain Caffeic Acid Phenethyl Ester (CAPE), polyphenol, flavonoid, saponin, quersetin and terpenoid. Caffeic Acid Phenethyl Ester (CAPE) works to maximize scavenger activity against free radical by inhibiting hydroxyl activity (*OH) become less reactive (Cadenas and Packer, 2002). Carreno et al. (2017) stated that Caffeic Acid Phenethyl Ester (CAPE) showed protective effect against hydrogen peroxide (H2O2), can diminishing reactive oxygen species (ROS) levels and improving enzymatic mechanism efficiency. Phytochemicals reported to exhibit antioxidant activity against lipid peroxidation and to inhibit the production of intracellular reactive oxygen species (ROS) (Shirai et al., 2015). Saponin increase intracellular superoxide dismutase (SOD), mitochondrial superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) activity and increase glutathione (GSH) content. Saponin inhibited NADPH oxidase whereas NADPH oxidase produces reactive oxygen species (ROS). Saponin also reduce intracellular H2O2 and O2-, and mitochondrial reactive oxygen species (ROS) marked by decrease of malondialdehyde (MDA) level (Fan et al., 2016). Quercetin can prevent lipid peroxidation that helps to preserve membrane integrity, donate electrons, and increase the production of antioxidant enzyme such as superoxide dismutase, catalase, glutathione reductase, and glutathione-peroxidase (Banjarnahor and Nina, 2014). Even at low concentration, quercetin can decrease lipid peroxidation (Shirai et al., 2015).

The cells necrosis result on the present study of T2 with dose 400 mg/kg bw of propolis ethanolic extract showed significant difference with C- and no significant difference with C+, indicates the antioxidant activity from propolis ethanolic extract on that dose was sufficient to protect against ROS. Whereas T1 with dose 200 mg/kg BW of propolis ethanolic extract showed significant different with C+ and C-, but it showed no significant different with T2 whereas T2 showed no significant different with C-. This indicates that this dose of ethanolic extract of propolis can protect the liver against lead acetate but not in the optimal level. Maybe it is due to lack of the antioxidant activity from propolis ethanolic extract on 200mg/kgBW dose. So this dose was not sufficient to against ROS formation. Treatment group of T3 with 800mg/kg bw dose of propolis ethanolic extract showed significant difference with C-, C+, T1 and T2. Indicates there was the reduction of antioxidant activity. Skibola and Martyn, (2000) reported that excessive intake of flavonoid especially quercetin can act as pro-oxidants that generate free radicals, as mutagens, and as inhibitors of key enzymes involved in hormone metabolism. Wang et al. (2016) also stated that excess intake of polyphenols can cause toxicity.

**CONCLUSION**

Based on this research, it could be concluded that ethanolic extract of propolis with dose 400 mg/kg BW could reduce the damage of
histopathological changes in mice’s (Mus musculus) liver with lead acetate exposure.

REFERENCES


Haleagrarahara, N., T. Jackie, S. Chakravarthi, M. Rao and A.


