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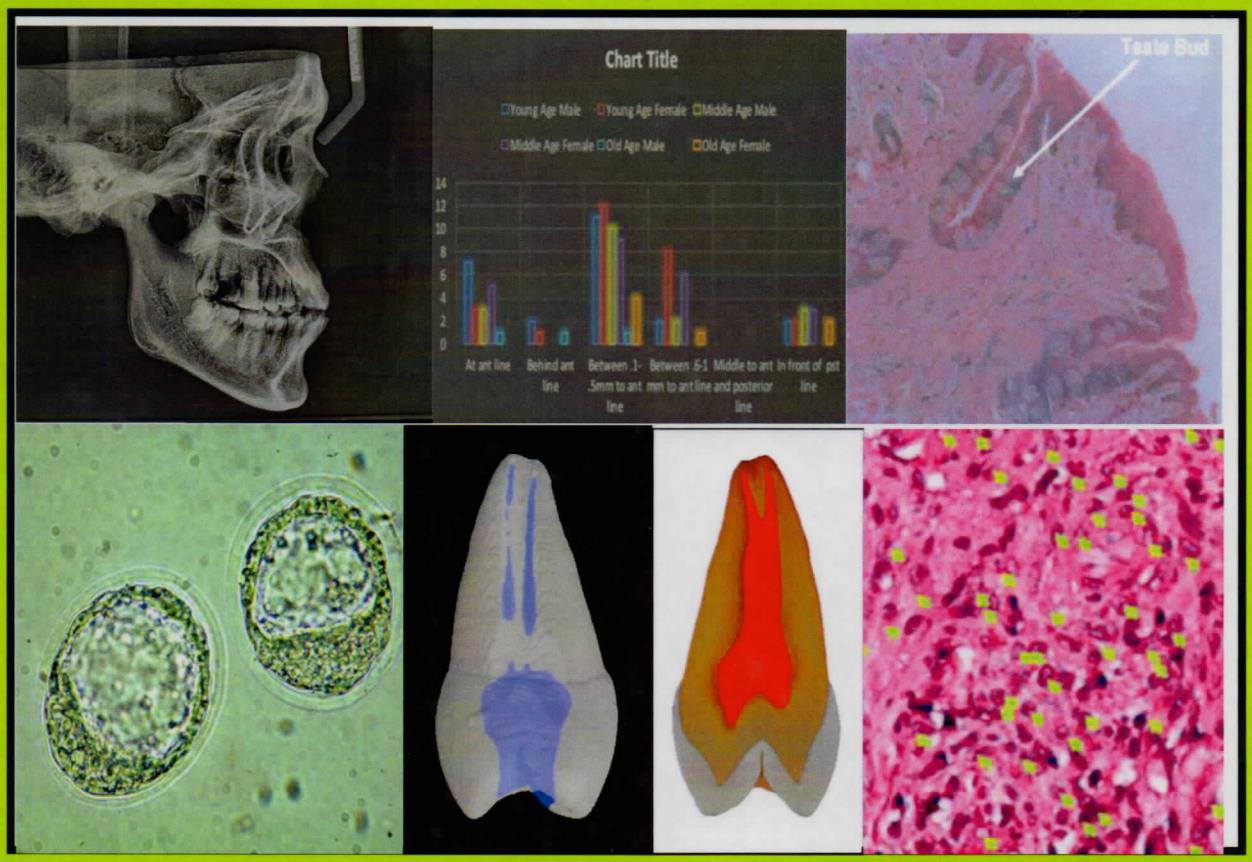


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# Protective Effect of Propolis Extract in Kidney Male Mice (Mus musculus) Induced by Lead Acetate

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**Abstract**

The aim of this research was to investigate the protective effect of propolis extract from Apis mellifera, on histopathological changes of the kidney induced by lead acetate in mice (Mus musculus). 25 male mice were randomly divided into five groups and administered orally with different treatments for 35 days. The treatment were Group C- (CMC-Na 1.5% and Tween 80 0.5% continued with aquadest one hour after the first administration), Group C+ (CMC-Na 1.5% and Tween 80 0.5% and continued with 20 mg/kg bw of lead acetate), and treatment group T1, T2, T3 (200, 400, 800 mg/kg bw propolis extract and continued with 20 mg/kg bw of lead acetate). After treatments, tissues were processed, and histopathological evaluation were examined using Arshad Scoring method. The result showed necrosis and tubular cast in the treatment group of T3 (1.44±0.09a and 1.04±0.43ab) have no significant difference with group of C- (1.24±0.33a and 0.96±026a). The mean value of necrosis and tubular cast in group T3 is the lowest, while the mean value of hydropic degeneration is the highest. It indicates the reversible cell injury. Thus, the effective dose of propolis extract used in this research is 800 mg/kg bw.

**Experimental article (J Int Dent Med Res 2017; 10(3): pp. 1060-1065)**

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## Introduction

In this industrial revolution, environmental pollution had became a real problem. It can damage the environment itself, which can come in the form of chemical substances, or energy such as noise, heat, or light. Lead (Pb) is the most common pervasive environmental pollutant which have diverse and deleterious effects rather than other heavy metals. It has been known as one of the most encountered toxic metal that induces a wide range of physiological, biochemical and behavioural dysfunction[14]. It caused cell damage by enhance lipid peroxidation. It also can induce over production of reactive oxygen species (ROS) that resulting oxidative stress in some cells[13].

ROS are reactive molecules containing oxygen in the body, which is usually a by product of their unpaired electron, that will effect the regular metabolism by damaging the cellular components. The failure of controlling the reactive oxygen species accumulation will lead to lipid peroxidation, enzyme inactivation, and even cell death.

Kidney as one of the most important organ of the body, will be the first target organ of lead toxicity, because of its role in the absorb and reabsorb the accumulation of divalent metals in the body[1]. The reactive oxygen species (ROS) content in the lead will caused stress injuries and also rapid cellular damage[13]. Tubular alterations also reported occured from hydraulic changes, then produce tubular swelling, causing necrosis due to its injury exceeds certain limits[24].

Acute renal failure is commonly due to acute tubular necrosis (ATN) involved by ROS that caused reversible loss of renal function occurred from ischemic or nephrotoxic[15,19,22]. Ischemia will caused the hypoxia tissue, and it will caused hypoxic stress on cells and tubules. The mechanism of ATN is initiated from the precipitous decrease in glomerular filtration rate (GFR), that also caused by ischemia and attendant hemodynamic alterations and also by

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evolving sublethal and lethal tubular epithelial injury. In the maintenance phase of ATN, sublethal alterations include brush border loss, vacuolization, and flattening proximal tubules, and dilation of tubules will be found, because the persistent reduction of GFR. The loss of brush border also caused the intratubular cast formation as the early structural changes followed by ischemia[13,21].

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. Besides that, antioxidant also can act as conjugators to remove the pollutants from the body[12]. World Health Organization (WHO) advocates that researchers investigate the possibility of using natural products, such as plant extracts and herbs, as alternatives [11].

Propolis have been known for its antioxidant properties[6,23]. Flavonoids is the powerful antioxidant, that play a great role in propolis immunomodulatory function. It has been proved that propolis had strong antioxidant activity by increasing the cellular immune response through the increase of mRNA for interferon-γ and activates the production of cytokines[25]. Previous study also have been proved that propolis is a powerful scavenger of reactive oxygen species (ROS)[8]. Besided that, propolis has antibacterial activity, antifungal, antiviral, anti-inflammatory, anti-tumor, and has the ability to modulate the immune process [17,18,20]

## Materials and Methods

*Ethical Issue* - This research was conducted under the ethical use of laboratory animals and also was approved by the ethical committee of Faculty of Veterinary Medicine, Universitas Airlangga

*Experimental Animals* - A total of 25 animals were at age 7-8 weeks old strain BALB/C male mice *(Mus musculus)*. The mice were kept under the same condition in well ventilated room, natural light cycle and they were allowed free acces to tap water and fed on the standard basal diet. Mice were divided into five groups and each group contained five animals. They were adapted for seven days then continued with preliminary treatment for three days, which was only the treatment groups gavaged by propolis as its doses. After that, the treatment will be given orally for 35 days as follows:

Group C- : 1.5% of CMC Na+0.5% Tween 80 for the first administration, continued with aquadest one hour after the first administration
Group C+ : 1.5% of CMC Na+0.5% Tween 80 continued with lead acetate (20 mg/kg bw)
Group T1 : Propolis extract (200 mg/kg) bw continued with lead acetate (20 mg/kg bw)
Group T2 : Propolis extract (400 mg/kg) bw continued with lead acetate (20 mg/kg bw)
Group T3 : Propolis extract (800 mg/kg) bw continued with lead acetate (20 mg/kg bw)

After treatments, the mice were euthanized to collect the kidney tissues, then the tissues were fixed in fixation solution.

*Preparation of Propolis Extract* – Raw propolis obtained from Agro Tawon Rimba Raya Bee Farm, Lawang, Malang with the species of *Apis mellifera*. Ethanolic extract of propolis was obtained using maceration method at Balai Penelitian dan Konsultasi Industri (BPKI) Ketintang, Surabaya. After that, the ethanolic extract of propolis was dissolved in mucilago of 1.5% CMC Na and 0.5% Tween 80 as the suspensator to make the propolis extract suspension.

*Preparation of Lead Acetate Solution* – The dose are made from 0.02 mg dissolved in 100 ml aquadest and given in 0.01 ml/g b of mice one hour after propolis extract administration.

*Hisopathology Analysis* – The fixed kidney tissues were sectioned, embedded in paraffin and stained in Hematoxylin and Eosin (H&E). Changes in experimental histopathologic parameters is based on previous study[3]. The parameters that we used: necrotic tubular cells, hydropic degeneration and tubular cast. They were graded as follows: (0) showing no changes, (1) changes less than 30% showing mild changes, (2) changes between 30%-50% showing moderate changes, (3) changes more than 50% showing severe changes.

*Data Analysis* – Histoptahological data of kidney were analyzed statistically using Kruskal-Wallis then continued with Mann-Whitney test to compare the treatment of each groups.

## Results

***Necrotic Tubular Cells*** – Treatment groups of C- are significantly different compared to treatment group of C+, T1, and T2 ($p<0.05$) but did not show significant difference with treatment group of T3 ($p>0.05$). Treatment groups of C+ did not show significant difference compared to treatment groups of T1 ($p>0.05$) but significantly different with treatment groups of T2 and T3 ($p<0.05$). Treatment groups of T1 significantly different with treatment groups of T2 and T3 ($p<0.05$). Treatment groups of T2 also showed significantly different with treatment groups of T1 and T3 ($p<0.05$) (Table 1, Figure 1). On the figure 2, on the treatment group of C+ some cells showed focal necrosis with rupture of the outer membrane, while on the treatment group of T3 the tubules cells were well arranged with the least value of necrosis compared to other group.

***Hydropic Degeneration*** – Treatment groups of C- are significantly different compared to treatment groups of T1, T2, and T3 ($p<0.05$), but did not show significant different with treatment groups of C+ ($p>0.05$). Treatment groups of T1 show significant difference with groups of C-, C+, T2, and T3 ($p>0.05$). Treatment groups of T2 also show significant dfference with treatment groups of C-, C+, T1, and T3 ($p<0.05$) (Table 1, Figure 1). We can see more swollen cells and pyknosis on the treatment group of T2 and treatment group of T3 showed the highest value of degeneration (Figure 2).

***Tubular Cast*** – Treatment groups of C- are significantly different to the groups of C+, T1, and T2 ($p<0.05$), but did not show significant difference of T3 ($p>0.05$). In the treatment groups of C+ are significantly different with treatment groups of C-, T2, and T3 ($p<0.05$), but did not significant difference with treatment groups of T1. Treatment groups of T1 insignificantly difference with groups of C+ ($p>0.05$), but showed significantly dfference with groups of C-, T2, and T3 ($p<0.05$). For the treatment groups of T2 insignificantly difference with T3($p>0.05$), but showed significant difference with C-, C+, and T1($p<0.05$) (Table 1, Figure 1). Histopathological features of kidney tubules shown on Figure 2.

## Discussion

Lead administration caused the accumulation of lipids, decreasing the activity of antioxidant enzymes, which could lead to lipid peroxidation and will cause the necrosis[13]. Proximal tubular cells are more suffer to get injury as its function for reabsorption and active transport and secretory.

Lipid peroxidation causing lead to substitute their bivalent cations like $Ca^{2+}$ and monovalent cations like $Na^{+}$. Influx of calcium ($Ca^{2+}$) in cytoplasm will increase the permeability of the cells to maintain the normal regulation of intracellular water. Thus the intracellular sodium ($Na^{+}$) will be increased and the water moves across cell membranes, causing cell swelling[16]. Tubular swelling can be reversible if the cause of injury is not exaggerate. But if the cells already passed the point of no return, the irreversible change occur and it becomes cell death[21].

Various antioxidants have been used in combination with chelators. Some experts have suggested that more than one antioxidant is needed to reach the clinical effectiveness so that molecules work effectively in a network to remove the oxidant stress and regeneration the oxidant defenses simultaneously[5,22]. Propolis is one of the free radical scavengers with several bioactive compounds present to protect the oxidative damage by neutralizing the reactive oxidants.

Antioxidant contained in propolis, such as: CAPE, flavonoids, isoflavon, and other phenolic compounds are capable to modulate SOD. The SOD enzyme will remove super oxides ($O^{2-}$) as the free radical by converting it into $H_2O_2$. After that it will be converted become $H_2O$ and $O_2$ by Catalase (CAT) enzyme[26]. Whereas, GPx enzyme causing glutathione (GSH) donates its electron and combine with another molecule of glutathione to form glutathione disulfide (GSSG), which is the oxidized form of glutathione[9].

CAPE is a phenolic compound with potent scavenging activity by diminishing ROS amount and improve the efficiency of enzymatic mechanisms. CAPE also have better bioavailability in lipophilic environment because of its partition coefficient[7]. Flavonoids also provide protection against oxidative stress by terminating the free radical chain reaction[9]. The

antioxidant activities related to flavonoids and its phenolic compounds is caused by their ability to chelate metal ions and scavenge the free radicals species, such as singlet oxygen, superoxide anions, hydroxyl radicals, and etc.

On the treatment group of T1, the cells showed less necrosis than in the treatment group of C+, but we still can find some karyolysis and karyorhexis on the nucleus of the tubules cells which indicates the necrotic tubular cells. Treatment group of T2, the tubules cells were well arranged, but the highest value of degeneration is in the treatment group of T3, that shows reversible changes because it didn't pass the point of no return.

The insignificant difference between treatment group of C- and C+ are might because of the difference of the value of necrosis. In the treatment group of C- showed the normal condition of the cells, thus the value of necrosis and degeneration are less. While in the treatment group of C+ showed the dominance of the histopathological damage is the necrosis, so the hydropic degeneration become less. Tubular casts are derived from tubular epithelium as the marker of tubular damage[10]. Thus, the least necrosis caused by tubular damage, will caused the less value of tubular casts.

## Conclusions

The dose of 800 mg/kg bw is the most effective dose of propolis extract in protecting kidney tubules from histological damage caused by necrosis, hydropic degeneration, and tubular casts induced by lead acetate.

## Acknowledgments

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## Declaration of Interest

The authors declare that they have no competing interest.

| Treatment | Parameters (Mean±SD) | | |
|---|---|---|---|
| | Necrotic Tubular Cells | Hydropic Degeneration | Tubular Cast |
| C- | 1.24 ± 0.33$^{a}$ | 0.80 ± 0.37$^{a}$ | 0.96 ± 0.26$^{a}$ |
| C+ | 2.80± 0.14$^{c}$ | 1.12 ± 0.41$^{a}$ | 2.60 ± 0.24$^{c}$ |
| T1 | 2.52 ± 0.22$^{c}$ | 1.40 ± 0.32$^{b}$ | 2.40 ± 0.14$^{c}$ |
| T2 | 1.88 ± 0.30$^{b}$ | 1.88 ± 0.30$^{c}$ | 1.52 ± 0.18$^{b}$ |
| T3 | 1.44 ± 0.09$^{a}$ | 2.56 ± 0.26$^{d}$ | 1.04 ± 0.43$^{ab}$ |

**Table 1.** Data of kidney histopathological changes in kidney tubules of mice *(Mus musculus)*.

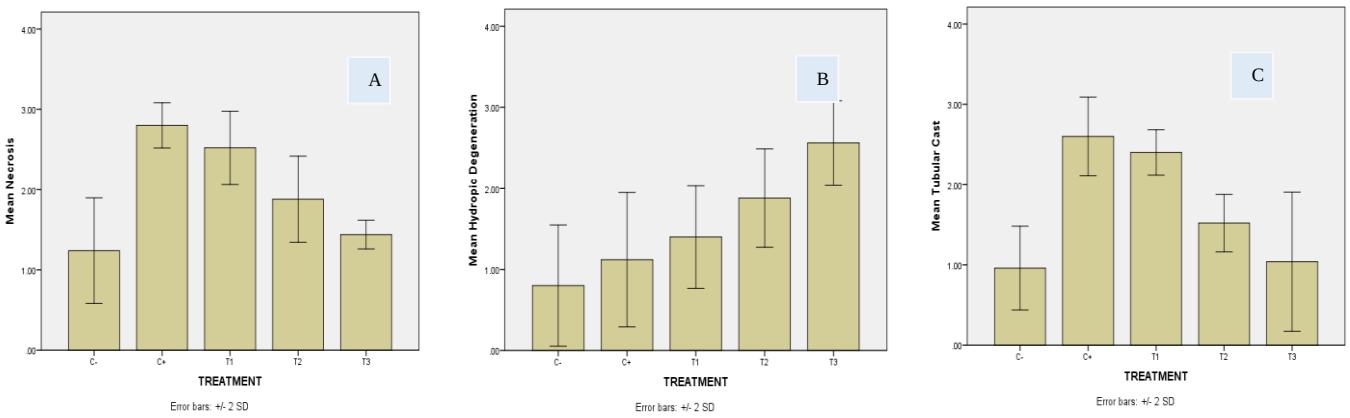


**Figure 1.** Graphic of data result of (A) necrotic tubular cells, (B) hydropic degeneration, and (C) tubular cast of kidney tubules of mice *(Mus musculus)*.

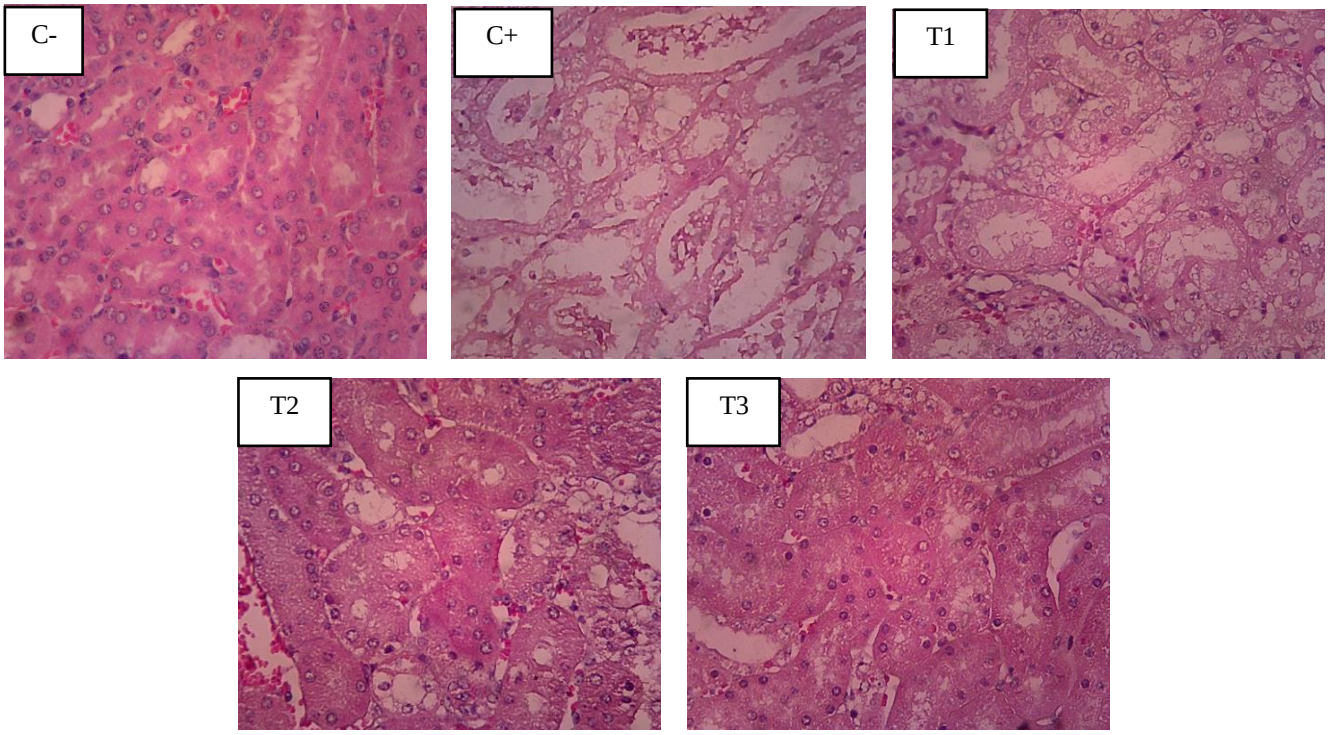


**Figure 2.** Histopathological features of kidney tubules on mice (400x magnification, stain: HE).

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