Effect of Gamma Co\textsuperscript{60}-irradiated chitosan and vitamin E towards Pb acetate cytotoxicity on rat kidney

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Abstract. This research aims to assess the effectivity of the combination of Gamma Co\textsuperscript{60}-irradiated chitosan and vitamin E towards cytotoxicity of rat kidney which is exposed to Pb acetate. All six research groups, except the control group, were induced with Pb acetate. Negative control was treated with Pb acetate. Treatment 1 group was only treated with irradiated chitosan at the dose of 150 kGy. Treatment groups were treated with the combination of irradiated chitosan and vitamin E at several doses of 1.44, 2.16, and 3.00 mg kg\textsuperscript{-1}0 BW for 40 days. On the 41\textsuperscript{st} day, rats were terminated for renal tissue analysis. This research showed no pathological changes in the control and treatment 2 group. The group treated with only irradiated chitosan showed a minimum amount of damage. The group treated with a combination of irradiated chitosan and vitamin E on higher doses showed various pathological effects such as nephrosis, necrotic nephrosis, and interstitial nephritis. It was presumably affected by the prooxidant effect from abundant vitamin E content. In conclusion, the combination of Gamma Co\textsuperscript{60}-irradiated chitosan and vitamin E at the right dose effectively protects Pb acetate-exposed rats' renal tissue. Still, the higher dose of vitamin E will be ineffective.

1. Introduction
Lead (Plumbum/Pb) is a type of pollutant for the human body. Chronic Pb accumulation will disrupt the neural, hematopoietic, and reproductive system, kidney, and bone [1]. Bodily accumulation of lead will be naturally disposed of through the kidney. Continuous exposure to Pb\textsuperscript{2+} ions from lead will yield negative impacts on the kidney. Pb\textsuperscript{2+} ions will transform into free radicals that exert an oxidizing effect on specific tissues, especially adipose tissue. A Phospholipid is one of the primary components of the cell membrane. It also becomes the primary target for oxidation. Prolonged exposure from oxidation will increase lipid peroxidation that damages the cell membrane, which will lead to cell death (necrosis).

Damages in renal tissue due to Pb-acetate's chronic exposure have been reported [2-3]. The damages were considered relatively high, based on the Manja Roegnik score of 3-4 and Barthel Manja score of 29. Identified damages were parenchymatous degeneration (albumin), hydropic degeneration, necrosis, lysed cells, hyperplasia, and karyomegaly. Histopathological effect of long-term Pb-acetate exposure was also found on rabbits' renal tissue, which caused dilatation, congestion, heterochromatic nucleus effect, and an increase in renal tubule diameter urine barrier thickness [4]. Renal tissue damage was also detected by an increase in blood creatinine levels [5].
The preventive measure must be taken to lessen the detrimental effects of bodily Pb accumulation. These measures revolve around decreasing the activity of Pb$^{2+}$ ions. Several research types have reported chitosan potential as a chelating agent for Pb$^{2+}$ ions to reduce its free radical activity. However, the effectivity is still below expected results [2,6]. It is presumably caused by chitosan molecular weight, which is relatively large, which decreases the solubility rate. Chitosan has a molecular weight between 69.8 kDa with DD 78% to 126.2 kDa with DD 84% [7].

The effectiveness of chitosan can be optimized by reducing its particle size. Smaller particle size will enable chitosan to be transported to particular tissues and provides more controlled releasing therapy [8]. One of the most effective methods to reduce the size of the chitosan particle is ionizing irradiation. Irradiation on specific molecules can cut up glycosidic bonds [9]. The separation of several glycosidic bonds on chitosan will shorten the chain, which will make the chitosan particle become smaller and dissolve more easily. Irradiation of Gamma Co$_{60}$ on a dose of 150 kGy upon chitosan has been proven to decrease its molecular weight and viscosity compared to the dose of 50 kGy and 100 kGy [10]. As the molecular weight gets lower, the antioxidant activity from chitosan grows higher [11].

Detrimental effects from free radicals' exposure can be prevented with endogenous antioxidant activities from superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzyme. When the number of free radicals exceeds the capacity, an exogenous antioxidant can be administered. A combination of Vitamin C, as an exogenous antioxidant, with chitosan, was proven to escalate the enzymatic activities of SOD, CAT, and GPx on rats that were exposed to Pb-acetate [6].

Besides vitamin C, vitamin E is also known as an effective antioxidant to overcome free radicals' negative effects. Free radicals are widely known for the pathogenesis of the disease. Vitamin E is known to prevent lipid peroxidation that is caused by free radicals activity. By preventing lipid peroxidation, vitamin E provides protective effects towards free radicals-exposed tissue. Vitamin E also provides hydrogen ions (H$^+$) for the hydroxyl group (OH$^-$) on the ring structure of free radicals [12].

This research aims to assess the effectivity of Gamma Co60-irradiated chitosan combined with vitamin E in protecting rat kidneys exposed to Pb-acetate.

2. Methods
Chitosan has been irradiated with Gamma Cobalt$^{60}$ rays in the dose of 150 kGy [10]. Twenty-four male rats were randomly grouped into four treatment groups. Each group was provided with different treatments: control group (KK); negative control group, which was administered with Pb-acetate at the dose of 175 mg kg$^{-10}$ BW; treatment group 1, which was administered with Pb-acetate (175 mg kg$^{-10}$ BW) + irradiated chitosan (64 mg kg$^{-10}$ BW); treatment group 2, 3, and 4 which were administered with Pb-acetate (175 m kg$^{-10}$ BW) + irradiated chitosan (64 mg kg$^{-10}$ BW) + Vitamin E at the dose of 1.44, 2.16, and 3.00 mg kg$^{-10}$ BW respectively [13]. Treatment was provided for 40 days. On the 41st day, rats were terminated with chloroform and decapitation. Later, the kidneys were removed from the body and preserved using a 4% formalin. The kidneys were later processed into microanatomy slides with the paraffin method and Hematoxylin and Eosin (HE) stains. A descriptive analysis procedure was applied to assess the damage to kidney tissue.

3. Results and discussion
Histopathological examination of the control and different experimental groups shows control group showed no apparent transformation, Bowman's capsule, and glomerulus tissue normal appearance (Figure 1). In contrast, in the lead acetate treated group (negative control group), the kidney tissue showed necrotic nephrosis, a condition where tubular necrosis occurs (Figure 2).
Figure 1. The control group showed kidney tissue no apparent transformation, normal tissue appearance. X40 (HE).
1. bowman's capsule
2. glomerulus

Necrosis is apparent in renal tubule epithelial cells in treatment group 1, which was treated with irradiated chitosan. It is also marked with hydropic degeneration (Figure 3). Treatment group 4, treated with irradiated chitosan and vitamin E on the lowest dose, has normal and unchanging renal tissue.

Figure 2. Negative control group. Necrotic nephrosis is apparent; renal tubules have necrosis. X40(HE)
1. bowman's capsule
2. glomerulus

As for treatment groups 5 and 6, which were treated with irradiated chitosan and vitamin E on doses higher than group 4, the renal tubules exhibits hydropic degeneration or necrosis.

Cell death on tissues is considered a natural phenomenon. However, if the necrosis rate is relatively high, it can be considered not normal, and disruptions have presumably taken place. In this research, the Pb$^{2+}$ ions that enter the rats' body in the form of Pb-acetate have increased the necrosis rate on renal tissue. Pb$^{2+}$ ions act as free radicals that trigger oxidative stress. Increasing oxidative stress levels will damage adipose tissue as the cell membrane component, which leads to necrosis. Kidneys take the role of disposing toxins from the body through urine excretion. Due to this role, kidneys are prone to damage caused by toxins, including Pb$^{2+}$ ions.

Figure 3. Treatment group 1 has nephrosis, which is indicated from the hydropic degeneration on renal epithelial tubules X40 (HE).
1. bowman's capsule
2. enlarged glomerulus

Figure 4. Treatment group 2 has unchanging renal tissue X40(HE) 
1. bowman's capsule
2. glomerulus
Irradiated-chitosan treatment is proven ineffective in protecting kidneys in rats that are exposed to Pb-acetate. Nephrosis on renal tissue is indicated with necrosis on renal tubular epithelium tissue. The spreading damage to renal tubular epithelium tissue triggers renal failure [14]. It is presumably caused by the size of the chitosan. Even though chitosan had been previously irradiated with Gamma Co\textsuperscript{60} on the dose of 150 kGy and has lower molecular weight and viscosity, the size is still too large for effective absorption [10]. By such means, the solubility rate is still not optimal.

The protective effect on Pb-acetate-exposed renal tissue is shown in the treatment group that receives the combination of Gamma Co\textsuperscript{60}-irradiated chitosan and vitamin E on the lowest dose. The normal tissue appears normally as in the control group. It is affected by the synergistic activity between irradiated chitosan as the chelating agent for Pb\textsuperscript{2+} ions and vitamin E. The combination reduces the free radical activity from Pb\textsuperscript{2+} ions that will protect the cell membrane. This result shows that irradiated chitosan's function as a protective agent for the body, especially in preventing the negative effects from Pb\textsuperscript{2+} ions, can be optimized by combining the agent with vitamin E as an antioxidant. Vitamin E is widely known as an exogenous antioxidant due to its activity in scavenging free radicals. Vitamin E also protects polyunsaturated fatty acids (PUFAs) and other cell membrane components from free radical oxidation. Hydrophobic vitamin E is submerged between the double phospholipid layer on the cell membrane. It increases vitamin E's effectivity in preventing lipid peroxidation and a chain of chemical reactions that involve PUFAs oxidative damage. However, prooxidant activity from vitamin E is also found in this research. A higher dose of vitamin E is proven to increase renal tubules' damage level, as shown in treatment groups 5 and 6. It is allegedly caused by the bodily accumulation of vitamin E inside the tissue, which eventually transforms into prooxidant. Prooxidant activity emerges when oxidative stress in the body reduces but is still supplied with antioxidants. It transforms vitamin E into prooxidant. Three factors that affect the functional alteration of antioxidant to prooxidant. The factors are metal ions, the antioxidant concentration, and the redox potential [15]. This alteration will worsen the tissue condition due to increased reactive oxygen species (ROS) that damages the cell. By such means, using vitamin E as an antioxidant and protective agent for cell membranes requires utmost circumspection.

4. Conclusion
Accumulation of Pb\textsuperscript{2+} ions causes renal tissue damage. A combination of Gamma Co\textsuperscript{60}-irradiated chitosan and vitamin E at the right dose effectively protects the renal tissue of Pb acetate-exposed rats, but the higher dose of vitamin E will be ineffective.
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