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Effect of Gamma Co^{60}-irradited chitosan and vitamin E towards Pb acetate cytotoxicity on rat kidney

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Abstract. This research aims to assess the effectivity from the combination of Gamma Co^{60}-irradiated chitosan and vitamin E towards the 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 only 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^{-10} BW for 40 days. On the 41st day, rats were terminated for renal tissue analysis. This research showed that the control group and treatment 2 did not provide any pathological changes. The group treated with only irradiated chitosan, showed a minimum amount of damage, while the group treated with the 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^{60}-irradiated chitosan and vitamin E at the right dose is effective to protect the renal tissue of Pb acetate-exposed rats, but 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 of Pb2+ ions from lead will yield negative impacts on the kidney. Pb^{2+} ions will transform into free radicals that exert an oxidizing effect on certain tissues, especially adipose tissue. A Phospholipid is one of the primary components of the cell membrane. It also becomes the main 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 chronic exposure of Pb-acetate has 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 the renal tissue of rabbit, which caused dilatation, congestion, heterochromatic nucleus effect, and an increase in renal tubule diameter and 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 types of research have reported the potential of chitosan as a chelating agent for Pb^{2+} ions to reduce its free radical activity. However, the effectivity is still below expected results [6][2]. 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 effectivity 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 certain molecules can cut up glycosidic bonds [9]. 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].

Aside from vitamin C, vitamin E is also known as an effective antioxidant to overcome the negative effect of free radicals. 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 that are exposed to Pb-acetate.

2. Methods

Chitosan has irradiated with Gamma Cobalt^{60} rays in the dose of 150 kGy [10]. Twenty four male rats were randomly grouped into 4 treatment groups. Each group were 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 on, 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 (Fig 1), In contrast, in the lead acetate treated group (negative control group) the kidney tissue showed necrotic nephrosis is a condition where tubular necrosis takes place (Fig 2).

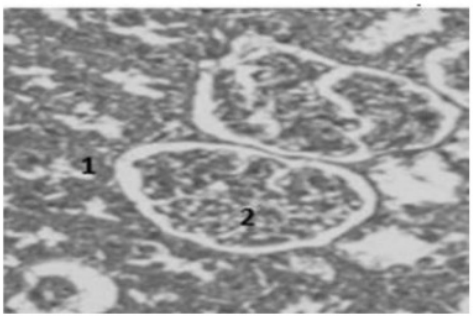

Fig 1. Control group, showed kidney tissue no apparent transformation, tissue normal appearance. X40 (HE).
1. Bowman's capsule
2. glomerulus

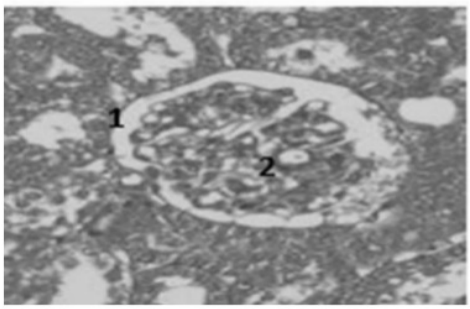

Fig 2. Negative control group. Necrotic nephrosis is apparent; renal tubules have necrosis. X40(HE)
1. Bowman's capsule
2. glomerulus

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

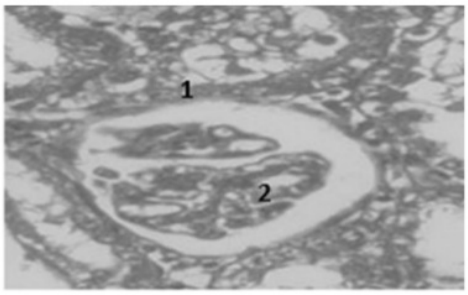

Fig 3. Treatment group 1 has nephrosis, which is indicated from the hydropic degeneration on renal epithelial tubules X40 (HE).
1. Bowman's capsule
2. enlarged glomerulus

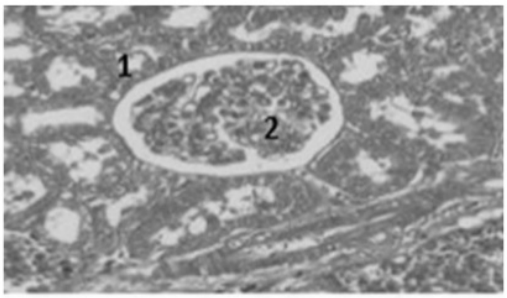

Fig 4. Treatment group 2 has unchanging renal tissue X40(HE)
1. Bowman's capsule
2. glomerulus

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.

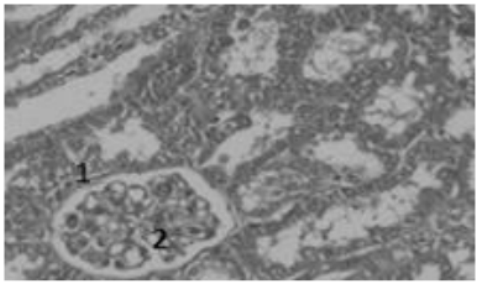

Fig 5. Treatment group 5; epithelial tissue on renal tubules have hydropic degeneration or nephrosis. X40 (HE)
1. Bowman's capsule
2. enlarged glomerulus

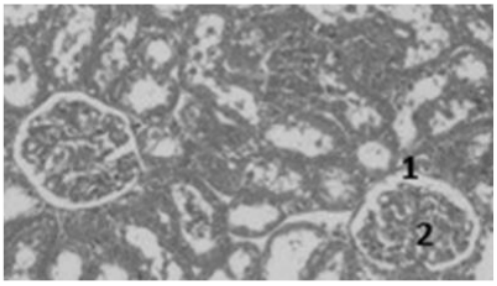

Fig 6. Treatment group 6; epithelial tissue on renal tubules have nephrosis. X40 (HE)
1. Bowman's capsule
2. enlarged glomerulus

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 component of the cell membrane, 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.

Irradiated-chitosan treatment is proven ineffective to protect 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 chitosan. Even though chitosan had been previously irradiated with Gamma Co^{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^{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^{2+} ions and vitamin E. The combination reduces the free radical activity from Pb^{2+} ions that will protect the cell membrane. This result shows that the function of irradiated chitosan acts as a protective agent for the body, especially in preventing the negative effects from Pb^{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 the effectivity of vitamin E 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 the damage level on renal tubules, 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 membrane requires utmost circumspection.

4. Conclusion

Accumulation of Pb^{2+} ions causes renal tissue damage. A combination of Gamma Co^{60}-irradiated chitosan and vitamin E at the right dose is effective to protect the renal tissue of Pb acetate-exposed rats, but the higher dose of vitamin E will be ineffective.

5. Acknowledgments

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