The preventive effect of Rome beauty (*Malus sylvestris Mill*) apple peel’s extract towards the malondialdehyde (MDA) levels of rat (*Rattus norvegicus*) stomach exposed to plumbum acetate

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Abstract. Plumbum (Pb) is one of heavy metals that can turn into free radicals, resulting in the descent and alteration of cell structures of the stomach. Apple of Rome beauty contains high flavonoids which act as antioxidant agents by supplying hydrogen ions to the free radicals.

This research utilized white rat (*Rattus norvegicus*) divided into 5 treatment groups, each group consisted of 4 white rats. They are negative control, positive control which were given 10 mg of Pb acetate for each rat/day in 14 days, and preventive control group which were given the extract of Rome Beauty apple peel within the dosage of 28 mg/200 g, 56 mg/200 g, and 112 mg/200 g for 21 days. The parameter observed were MDA levels of stomach. The result was then analysed with One Way ANOVA which was followed by Turkey. The result of this research exhibited the preventive group which was given Rome Beauty apple peel within the dosage of 112 mg/ 200 g was able to halt the increase of MDA levels significantly (p<0.05), which was 82.16 ± 1.40 µg/ml. The research concluded that Rome Beauty apple peel contain antioxidant that can prevent the damage of stomach tissue resulted by Pb acetate.

1. Introduction
Plumbum (Pb) is a heavy metals which is used in human activities, such as Pb in vehicle fuel, cosmetic, and pesticide [1]. Pb contamination in livestock can be influenced by the livestock food source which originating from agricultural land where grass can be contaminated with Pb from pesticides and fertilizers. Pb concentration in animal feed can cause chronic poisoning in adult cattle that consume 7 mg / kg / day feed and 600-800 mg / kg cause acute poisoning [2].

Plumbum (Pb) which entering the body orally can affect the digestive organs, one of the stomach. Plumbum (Pb) can increase the amount of Reactive Oxygen Species (ROS) and can cause lipid peroxidation in membrane cell which is composed of Poly Unsaturated Fatty Acid (PUFA), which contain double chains bound of Carbon (C=C) [3]. The continuous reaction of lipid peroxidation can cause damage to membrane function and tissue permeability [4]. The end result of lipid peroxidation is malondialdehyde (MDA) which can be set as indicator of free radical in the body [5].

The body needs antioxidant to protect it from damage which caused by free radical. Antioxidant is compound which can decrease the negative effect of free radical by giving one electron into free
radical compound, so that the activity can be inhibited [6]. One fruit that has antioxidant content is apple. Apples contain flavonoid and vitamin C. Phenolic compound in 100 g apple peel of Rome Beauty equals to 500.2 ± 13.7 mg [7]. This study aims to determine the preventive effect of Rome Beauty apple peel extract on MDA levels of the gaster in rat (Rattus norvegicus) exposed to Pb acetate.

2. Materials and methods
Materials for experiment are Pb (II) acetate (powder form), 0.2 gram of gastric organ of rat (Rattus norvegicus) strain male Wistar, and Apple peel varieties Rome Beauty. This experimental design use complete randomized trial design because the sample and environmental factor in this experiment is assumed same or homogenous. This trial is divided into 5 groups and 4 times of repeat. It is needed as much as 20 rat and each group contains 4 rat. (A) Negative Control / K-: without intervention; (B) Positive Control / K+: Induction Pb acetate 10 mg/mouse/day; (C) Intervention 1 (P1): Extract of apple peel Rome Beauty 28 mg/200 g BW + Pb acetate 10 mg/mouse/day; (D) Intervention 2 (P2): Extract of apple peel Rome Beauty 56 mg/200 g BW + Pb acetate 10 mg/mouse/day; (E) Intervention 3 (P3): Extract of apple peel Rome Beauty 56 mg/200 g BW + Pb acetate 10 mg/mouse/day. Plumbum acetate was given in form of white powder which is diluted with 1 cc aquadest and given per orally with feeding tube for 14 days start from day 15 until day 28. Apple peel extract was given for 21 days in day 8 until day 28 orally with feeding tube which diluted with 1 cc aquadest. Data analysis of gastric MDA levels was observed quantitatively by the One Way ANOVA analysis method, and continued using Tukey Test with α = 5% to analyze the difference between intervention group.

3. Result and discussion
The MDA level of the positive control group was significantly different (p <0.05) from the negative control group, meaning that the administration of Pb acetate significantly (p <0.05) was able to increase the gastric MDA level of the rat (Rattus norvegicus). The average level of gastric MDA in the positive control group (B) showing increase of the amount, which was $133.28 \pm 11.88 \text{ \mu g} / \text{ml}$ when compared to the negative control group (A), which was $79.94 \pm 7.10 \text{ \mu g} / \text{ml}$. Group C, D, and E are a group of rat (Rattus norvegicus) who were given preventive measures of Rome Beauty (Malus sylvestris Mill) apple peel extract before being given of Pb acetate exposure. Group C who were given Rome Beauty (Malus sylvestris Mill) apple peel extract dose 28 mg / 200 g BW showed gastric MDA levels of $117.72 \pm 15.67 \text{ \mu g} / \text{ml}$. Group D who was given Rome Beauty (Malus sylvestris Mill) apple peel extract dose of 56 mg / 200 g BW showed gastric MDA levels of $114.11 \pm 17.65 \text{ \mu g} / \text{ml}$, while group E who was given Rome Beauty (Malus sylvestris Mill) apple peel extract dose 112 mg / 200 g BW shows the value of gastric MDA levels of $82.16 \pm 1.40 \text{ \mu g} / \text{ml}$, so that the group E is the preventive group that is most close to the MDA level value of the negative control group (A), which is $79.94 \pm 7.10 \text{ \mu g} / \text{ml}$. The results indicate that groups C, D were not significantly different compared to the control group (B), while the group E showed significantly different results compared to the control group (A).

Increased gastric MDA levels in the positive control group (K +) given Pb acetate exposure due to lipid peroxidation process. Lipid peroxidation occurs due to Pb compounds that enter the body will become Pb$^{2+}$ ions which can bind to sulfhydryl groups possessed by the body's antioxidant enzymes which causes the ability of enzymes to decrease thereby increasing the amount of ROS. The most reactive ROS group, the hydroxyl radical (-OH), which reacts with PUFA in cell membranes, this reaction is called lipid peroxidation [3]. Malondialdehyde (MDA) is the final product of lipid peroxidation that can be an indicator of free radicals inside the body [5].
Table 1. Result of gastric MDA levels of Rat (*Rattus norvegicus*) in treatment group

| Group | Average MDA ± SD (mg/dL) |
|-------|--------------------------|
| A - Control (-) | 79.94 ± 7.10 a |
| B - Control (+) | 133.28 ± 11.88 b |
| C - Dosage 28 mg/200 g BW | 117.72 ± 15.67 b |
| D - Dosage 56 mg/200 g BW | 114.11 ± 17.65 b |
| E - Dosage 112 mg/200 g BW | 82.16 ± 1.40 a |

Preventive administration of Rome Beauty (*Malus sylvestris Mill*) apple peel extract in groups P1, P2 and P3 was able to prevent the increasing levels of gastric MDA that were exposed to Pb acetate, because they contained flavonoids. The flavonoid content in Rome Beauty (*Malus sylvestris Mill*) apple skin extract acts as an antioxidant by giving one of its hydrogen ions (H⁺) to peroxyl lipid radicals (LOO⁻) which are the result of hydroxyl radical reaction (-OH) to PUFA in the lipid peroxidation process so the formation of free radicals and lipid peroxidation can be inhibited [8].

4. Conclusion

Preventive administration of Rome Beauty (*Malus sylvestris Mill*) apple peel extract at doses of 28 mg / 200 g BW, 56 mg / 200 g BW, and 112 mg / 200 g BW can prevent the increase in gastric MDA levels in rat (*Rattus norvegicus*) given Pb exposure acetate significantly (p> 0.05) with an effective dose of 112 mg / 200 g BW.

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