

***Hylocereus polyrhizus* Peel Extract Increase Testosterone Levels of Balb/C Mice (*Mus musculus*) Exposed Lead Acetate**

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ABSTRACT

Lead is a heavy metal, the main polluting material in the environment, which comes from gasoline, batteries, paint, pipes, soil, cosmetics, household appliances, and decorations. Lead is a free radical causing oxidative stress. Lead can accumulate in the body due to its slow elimination process. Lead increases oxidative stress by forming reactive oxygen species (ROS) such as superoxide ion (O_2^-), hydroxyl radical (OH^\cdot), and nitric oxide (NO), and direct suppression of antioxidant reserves such as Superoxide Dismutase (SOD), Catalase, and Glutathione Peroxide (GPx). *Hylocereus polyrhizus* peel is a natural antioxidant that can overcome the toxic effects of lead. The aim of this study was to analyze the treatment of *Hylocereus polyrhizus* peel extract orally once in a day for 40 days to increase testosterone levels of Balb/C mice (*Mus musculus*) exposed to lead acetate. The type of this study was true experimental study with Post Test Only Control Group design. Forty mice aged 8-10 weeks and the range of the body weight 30-40 grams were divided into 5 groups (each group of 8 mice). K- group was the control group without lead acetate and *Hylocereus polyrhizus* peel extract. K+, P1, P2 and P3 group was given 100 mg/kgBW lead acetate orally on the 1st day until day 14th. P1, P2 and P3 group was continued by giving *Hylocereus polyrhizus* peel extract orally on the 15th day until 39th day. P1 with dose 250 mg/KgBW, P2 with dose 500 mg/KgBW, and P3 with dose 1000 mg/KgBW. The results showed significant differences in testosterone levels between the K+ groups with P1 and P2. The conclusion of this study is *Hylocereus polyrhizus* peel extract can increase the testosterone levels of mice exposed to acetate lead.

Keywords: Lead Acetate, *Hylocereus Polyrhizus* Peel Extract, Testosterone Levels

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INTRODUCTION

Lead or Plumbum (Pb) is a heavy metal contaminant in the environment with increasing levels due to mining, smelting and various uses in the industry.¹ Lead in the form of tetraethyl lead is widely used as a mixture for fuel, which is the main source of lead pollution through vehicle exhaust gas (about 70-80%).² Lead is also one of the metals widely used in industries such as batteries, paint, rubber, ceramics, cable coatings, printing, water pipes, insecticides and cosmetics.³

Lead and its compounds enter the body through respiration, digestion, and skin exposure.⁴ 95% of the incoming lead in the body binds to the erythrocytes in the blood, to the liver, kidneys, brain, bone marrow and eventually leads to health problems including affecting the male reproductive system.⁵ The study of I'tishom et al (2007) showed that lead exposure below the WHO threshold in men exposed to motor vehicle exhaust emissions affects male fertility.⁶ The air intake threshold set by WHO is 0.5 µg / m³.⁷

Lead is a pollutant that can be toxic to male reproduction. Lead acetate given to mice orally can also lead to a significant decrease in testosterone.⁸ Testosterone is produced by Leydig cells, approximately 97% of the weak bonds with plasma albumin. Testosterone binds strongly to beta globulin which is called sex binding globulin hormone. Testosterone will circulate in the blood for 30 minutes to an hour. Testosterone is transferred to the tissues or degraded into an inactive product and is excreted. Testosterone is important for spermatogenesis.⁹

Testosterone is responsible for the differences in the masculine character of the body. Fetal testes are stimulated by chorionic gonadotropin from the placenta to produce testosterone during fetal development and until 10 weeks or more after birth, after which testosterone is not produced during childhood until approximately 10-13 years of age. Production of testosterone will increase rapidly under the stimulus of gonadotropin hormones produced by the anterior pituitary as the onset of puberty and lasts throughout life.¹⁰

Lead causes oxidative stress. Lead forms reactive oxygen species (ROS) (hydroperoxide, single oxygen and hydrogen peroxide).¹¹ Lead also directly suppresses antioxidant reserves (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione (GSH)).¹² Increased ROS as a result of lead exposure can be identified in the lungs, endothelial tissue, liver, brain, testes, and sperm.¹³

Antioxidants prevent the toxic effects of oxidative stress. Natural antioxidants are preferred over synthetic antioxidants. This is because natural antioxidants are cheaper and fewer side effects.¹⁴ *Hylocereus polyrhizus* peel has antioxidant activity with IC₅₀ value of 43.836 microgram / mL.¹⁵ *Hylocereus polyrhizus* peel contains phenolic or polyphenolic compounds.¹⁶ The antioxidant content of *Hylocereus polyrhizus* peel is five times larger than its flesh.¹⁷ The phenolic function is to stabilize free radicals by supplementing the electron deficiencies possessed by free radicals and inhibiting the chain reaction of free radical formation.¹⁸

The effect of *Hylocereus polyrhizus* peel extract on the testosterone levels of Balb / C mice exposed to lead acetate has not been studied. This study will examine the effect of *Hylocereus polyrhizus* peel extract on the testosterone level of Balb / C mice exposed to acetate lead.

MATERIAL AND METHODS

Tools and material

The research tool includes cage pen, sonde, scales, and disposable syringe. The research materials included mice (*Mus musculus*) Balb/c males aged 8-10 weeks weighing 30-40 grams obtained from Biochemistry Laboratory, Faculty of Medicine, Airlangga University, solution of red dragon fruit peel extract obtained from Laboratory Phytochemical UPT Materia Medica Stone, lead acetate solution, ether, sterile aquadest, diluent solution (NaCl 0.9%), NaCMC 0.5%, ELISA kit.

Experimental animals and treatment

Subjects were 40 male mice (*Mus musculus*) Balb/C strain with criteria of 8-10 weeks old, 30-40 gram weight, and healthy physical condition. The experimental animals before the study were acclimatized by placing the mice in cages for 1 week. Unhealthy animals whose body weight fell <10% were excluded from the study. The cage is conditioned to a certain humidity, light and temperature so that it is homogeneous and constant. Mice were given food and drink regularly, cleanliness and comfort of the cage were maintained during the research process.

The mice were divided into 5 groups, 2 controls and 3 treatments, each of which amounted to 8 mice. Experimental animals were acclimatized by placing mice in the cage for 1 week. K- group was the control group without lead acetate and red dragon fruit peel extract administration, K + group was given 100 mg/KgBW lead acetate orally on the 1st day until day 14th. The treatment group was the group treated as a positive control and continued by giving the red dragon fruit peel extracts orally on 15th day until 39th day with a dose of 250mg / kgBW (P1), 500mg / kgBW (P2), and 1000mg / kgBW (P3).

When sacrificing, mice are given ether and guarded so as not to harm the mice. Mice were sacrificed on the 40th day, blood was taken from the heart of the mice by spiraling directly into the heart and being sucked slowly. A blood-filled tube is placed on the

test tube rack and stays for about 10 minutes. After centrifuge of 3000 RPM for 15 minutes to separate the blood serum. The blood serum is separated into a new tube and measured testosterone levels. Measurements of testosterone levels were performed at the Regional Health Laboratory of Surabaya using the Enzym Linked Immunosorbent Assay (ELISA) method. This research has earned the certificate of ethical eligibility from the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya.

Statistic analysis

The type of this study was true experimental study with Post Test Only Control Group design. Data of the results were presented in mean and standard deviation, also in histogram. Assessment of data distribution used Kolmogorov-Smirnov test. Data distribution was normal if p value \geq 0.05. The data is not normally distributed so that it is analyzed by Kuskal-Wallis test. The Mann-Whitney Post-Hoc analysis was performed to determine which groups differed significantly.

RESULT

The results of this study showed significantly different testosterone levels (p <0.05) were group K- with P1, K + with P1, K + with P2, and P1 with P3 (table 1).

Table 1. Effect of *Hylocereus polyrhizus* peel extract to testosterone levels on Balb/C mice (*Mus musculus*) exposed to lead acetate

Groups	Testosterone levels (ng/dl)
	Mean \pm SD
Negative control	2,85 ^a \pm 4,65
Posistive control	2,16 ^a \pm 5,60
<i>Hylocereus polyrhizus</i> peel extract 250 mg/kgBW	12,06 ^b \pm 7,29
<i>Hylocereus polyrhizus</i> peel extract 500 mg/kgBW	7,27 ^c \pm 7,88
<i>Hylocereus polyrhizus</i> peel extract 1000 mg/kgBW	2,34 ^a \pm 5,55

^{a,b,c} Different superscript within each column differ significantly (p <0.05)

Table 1 showed testosterone levels of the positive control group (lead acetate treatment group) decreased in comparison with negative control group. It means that administration of lead acetate 100 mg/kgWB on the 1st day until day 14th can lower testosterone levels. Treatment with 250 mg/kgBW *Hylocereus polyrhizus* peel extract markedly increased testosterone level which is significantly different from the positive control. It means that *Hylocereus polyrhizus* peel extract at dose 250 mg/kgBW is optimal dose to increase testosterone level of mice exposed to lead acetate 100 mg/kgBW.

DISCUSSION

The results of this study showed that lead acetate exposure orally could lower the levels of testosterone and administration of *Hylocereus polyrhizus* peel extract at dose 250 mg/kgBW/day orally can significantly increase testosterone levels.

Lead acetate can lower testosterone levels because it can enter the blood and cross the testicular blood barrier. It can damage Leydig cells which play a role in the formation of the hormone testosterone. Leydig cells are located in the space between one seminiferous tubule and the other seminiferous tubule. These are cells that are very sensitive to toxic chemical compounds and free radicals.¹⁹

Lead can trigger oxidative stress by increasing the lipid peroxidation potential in the testes and causing interference with the hypothalamus-pituitary-testis axis resulting in a decrease in GnRH secretion which has an impact on decreasing FSH and LH hormones which in turn can interfere with the function of Leydig cells.²⁰ This study is consistent with the results of a study showed that the administration of lead acetate 10 mg/kgBW and 15 mg/kgBW in mice for 20 days intraperitoneally causes a decrease in testosterone levels.²¹ This study is consistent with the results of a study showed that the administration of lead acetate with a dose of 40 mg and 80 mg orally in mice significantly increase testosterone.²² Biswas and Ghosh (2004) showed that the administration of lead

acetate dose 8 mg/kg/day by peritoneal injection in mice for 14 days may indirectly affect steroidogenesis by lowering serum gonadotropin levels (FSH, LH and testosterone levels).²³

Lamia *et al.* (2008) and Haouas *et al.* (2015) showed that oral exposure to lead acetate in mice significantly lower testosterone levels.^{24,25} Mokhtari and Zanboori (2011) showed that lead acetate given orally in mice as much as 50 and 100 mg/kgBW for 28 days resulted a significant decrease in testosterone.²⁶ Testosterone is produced by Leydig cells and it is important for the development of spermatogenic cells so that there is a significant relationship between intratesticular testosterone and the number of Leydig cells per gram of testis.⁹

Lead is a free radical causing damage to the Leydig cell mitochondrial membrane. Damage of mitochondrial cell membrane of Leydig cells due to free radical reactivity will inhibit cholesterol conversion to pregnenolon catalyzed by P-450cc cytochrome. Pregnenolone is an ingredient in the biosynthesis of testosterone. This causes the biosynthesis of the hormone testosterone disrupted so that testosterone levels decrease.²⁷

Hylocereus polyrhizus peel contains natural antioxidant compounds inhibiting free radicals. The study showed that red dragon fruit peel (1 mg/kg) inhibit 83.48 (1.02%) of free radicals.²⁸ The study showed that red dragon fruit peel has antioxidant activity with an IC₅₀ value of 43.836 µg/mL.¹⁵ It is higher than the flesh. The study showed that the antioxidant activity in the extract of red dragon fruit ethanol with concentration of 0.0625; 0.125; 0.25; 0.5; and 1 gram/100 mL gave a percentage of antioxidant activity with an average of 6.468%; 9.738%; 12,286%; 13,141% and 20,867% and IC₅₀ of 3,14 gram / 100 ml.²⁹

Antioxidants have two functions, they are primary and secondary. The primary function gives the hydrogen atom rapidly to the lipid radical (R *, ROO *) or converts it to a more stable form, while the antioxidant radical derivative (A *) has a more stable state than the lipid radical. The secondary function slows down the rate of auto-

oxidation of the autooxidation chain termination mechanism by altering the lipid radicals to a more stable form. The low concentration of primary antioxidants (AH) inhibits or prevents the reaction of fat and oil autooxidation. The addition may inhibit oxidation reactions at initiation and propagation stages. The antioxidant radicals (A*) formed in the reaction are relatively more stable. It does not have enough energy to react with other lipid molecules and form new lipid radicals.³⁰

The administration of *Hylocereus polyrhizus* peel extract causes antioxidants in the body increased so oxidative stress due to lead stops. This causes the hypothalamus gradually improve and GnRH secretion increases. Increased GnRH secretion causes LH and FSH secretion by the pituitary to increase. The LH function stimulates Leydig cells to produce testosterone. FSH interacts with Sertoli cells to stimulate Androgen Binding Protein (ABP). ABP is a receptor for binding testosterone in the blood. Increased secretion of LH and FSH causes ABP and testosterone to increase.

This study showed that a dose of 250 mg / kgBW / day is the maximum dose of red dragon fruit peel can increase testosterone levels. This is not in accordance with the study of Aziz et al. (2010) showed that a dose of 500 mg / kgBW / day is the maximum dose of red dragon fruit peel extract can increase the number of spermatozoa.³¹ This may be due to this study using serum testosterone levels. Testosterone in the body is divided into two, namely plasma testosterone or testosterone serum and intratesticular testosterone; and which affects spermatogenesis is intratesticular testosterone. Intratesticular testosterone levels were 3 times higher than serum testosterone levels.³²

CONCLUSION

Hylocereus polyrhizus peel extract can increase testosterone levels of mice exposed to lead acetate.

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