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POTENCY OF ETHANOL EXTRACT OF DAYAK ONION (*Eleutherineamericanamerr*) AS PROTECTOR OF TESTOSTERONLEVEL, DIAMETER AND THICKNESS OF SEMINIFEROUS TUBULE IN MICE INDUCED WITH LEAD ACETATE

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ABSTRACT

Lead is a heavy metal that affects the health, one of them are male reproductive organ. Lead exposure leads to the formation of ROS (Reactive Oxygen Species) and decreases antioxidant reserves in the body. Lead toxicity in male reproductive system can be prevented with antioxidants. Dayak onion is a potential plant as a source of natural antioxidants.The aim of this study was to determine the potential of dayak onion ethanol extract to maintain testosterone level, diameter and thickness of seminiferous tubules in mice induced with lead acetate. The type of this study was purely experimental laboratory (true experimental) study with Posttest Only Control Group design. Subjects of the study were 30 male (Mus musculus) mice of Balb/C strains induced with lead acetate of 0.75 mg/KgBW/day and divided into 5 treatment groups, negative control group (K0), positive control group (K1), and treatment groups that received dayakonion extracts of 30 mg/KgBW/day (K2), 60 mg/KgBW/day (K3), and 120 mg/KgBW/day (K4). On day 39, the mice were sacrificed for measuring testosterone level, diameter and thickness of seminiferous tubule epithelium. Results showed significant differences in the levels of testosterone hormone in groups K0 with K1, K1 with K2 and K3. There was an increase in diameter of the seminiferous tubules of groups K2, K3, K4 compared to K1. Significant differences were obtained in the thickness of seminiferous tubular epithelium between groups K0 with K1, K1 with K2, K3, K4. In conclusion, dayakonion ethanol extracts are capable of maintaining testosterone and thickness of the seminiferous tubule epithelium, but are unable to maintain the diameter of the seminiferous tubules induced lead acetate

Keywords: ethanol extract of *dayak*onion, testosteronehormone level, diameter and thickness of seminiferous tubule, lead acetate.

INTRODUCTION

Air pollution contains a variety of heavy metals. One of the heavy metals is lead which is sourced from the burning of motor vehicle fuel and industrial emissions (Mardiani, 2008). Research results of National Aeronautics and Space Agency (LAPAN) on urban air pollution proves that

transportation emission is the highest contributor of air pollution in Indonesia, which is about 85% (Gusnita, 2012). Motor vehicle exhaust is one of toxic heavy metals (Golub, 2006). In men, lead toxicity leads to infertility (Panggabean*et al*., 2008). Lead exposure below the WHO-defined threshold (400µg/dl) decreases spermatozoa concentration, although clinically still within normal limits (I'tishom*et al*., 2011). Animal studies attempted to prove that lead exposure suppresses the hypothalamus-pituitary-testis axis (Garu*et al*., 2011). Al-Shaikh*et al*., (2013) mentioned that intraperitoneal injection of acetate in a dose of 8 mg/kgBW for 4 weeks resulted in damage to the seminiferous tubules, decreasing the number, activity and morphology of spermatozoa in treatment group. The negative effects of lead resulted in increased free radical formation and decreased antioxidant reserves (Flora *et al*., 2012). Increased free radicals that exceed normal results in decreased antioxidants that function as ROS (Reactive Oxygen Species) neutralizer (Shofia et al., 2013). Negative effects of free radicals on reproductive health can be overcome by providing antioxidants (Ernawati and Nurliani, 2012). Natural sources of antioxidants are preferred because they have few side effects (Flora *et al*., 2012).

Dayak onion is one of the plants that has potential as a natural antioxidant (Ernawati and Nurliani, 2012). This Central Kalimantan plant contains phenolic compounds of naphthoquinone group (Alves*et al*., 2003; Hara *et al*., 1997; Han *et al*., 2008; Nielsen &Wege, 2006). Naphthoquinone has bioactivity as an antioxidant and anti-cancer (Firdaus, 2006). Strong antioxidant activity on *dayak* onion ethanol extract is proved by IC_{50} value of 25,33 µg/ml (Kuntorini and Astuti, 2010). The content of phenolic compounds in *dayak* onions is thought to form a more stable and harmless antioxidant compound binding for body cells (Prior and Schaich, 2005).

The aim of this study was to prove that *dayak* onion (*Eleutherineamericanamerr*) ethanol extract is able to maintain testosterone hormone level, diameter and thickness of epithelium of seminiferous tubule in Balb/C-mice (*Musmusculus*) induced with lead acetate.

MATERIAL AND METHODS

Production of *dayak* onion extract

The skin of *dayak* onion as much as 10 kg was peeled off, dried, and then mashed. Then, the *dayak* onion powder weighed a total of 500 grams was put into a maceration device, added with 97% ethanol while being stirred until the ethanol solution was submerged 1 cm above the sample surface. The extraction was done for 3x24 hours. Substitution of solvent was done once a day. The solvent was evaporated using a vacuum rotary evaporator at a temperature of 40 degrees C until it did not evaporate again. The filtrate was evaporated back over the waterbath and the weight of the resulting extract was weighed (Ernawati&Anni, 2012).

Experimental animals and treatment

Subjects were 30 male mice (*Musmusculus*) Balb/C strain with criteria of 6-8 weeks old, 25-35 gram weight, healthy physical condition as characterized by agile movement and good appetite. The mice were divided into 5 groups, ie 2 controls and 3 treatments, each of which amounted to 5 mice. The treatments given to the animals were:

a. Negative control group (K0) receiving Na-CMC 0.5% for 38days

b. Positive control group (K1) receiving 0.5% Na-CMC for 3 days then induced with 0.075 g/KgBW/day dose of lead acetate for 35 days

c. Treatment group 1 (K2) receiving *dayak* onion 30 mg/KgBW onion extract for 3 days then induced with 0.075 g/KgBW/day dose of lead acetate. One hour later *dayak* onion extract

was given 30 mg/KgBW for 35 days.

d. Treatment group 2 (K3) receiving *dayak* onion extract 60 mg/KgBW for 3 days then induced with 0.075g/KgBW/day dose of acetate lead. One hour later *dayak* onion extract was given as much as 60 mg/kg for 35 days

e. Treatment group 3 (K4) receiving *dayak* onion extract of 120 mg/KgBW for 3 days, then induced with acetate lead in a dose of 0.075g/KgBW/day. One hour later *dayak* onion extract was administered in a dose of 120 mg/KgBW for 35 days.

Dayak onion and lead acetate extracts were administered on an oral basis using sonde. On day 39 all research subjects were sacrificed. The mice were terminated with anesthesia using chloroform and then performed surgery to take the testicles and blood in the heart. This study has earned the certificate of ethical eligibility from the Research Ethics Committee, Faculty of Medicine, Airlangga University, Surabaya.

Testosterone level measurement

The blood of the mice that has been taken from the heart is then centrifuged for its serum. Testosterone levels were measured using the ELISA method. All reagents were left at room temperature (18-25 degrees C) before use.

Measurement of seminiferous tubule epithelium thickness

Epithelial thickness was measured from the longest distance and the shortest distance from seminiferous tubules that was round or rounded and then averaged (Suciati, 2012). Staining of testicular histopathology preparations was done using hematoxylin-eosin (HE). The preparations were observed using a microscope with an ocular micrometer in 400x magnification.

Statistic analysis

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 ≥ 0.05. Normally distributed data were then analyzed by one-way ANOVA test to know the significant difference between control group and treatment group.

RESULT AND DISCUSSION

Protector effect of *dayak* onion (*Eleutherineamericanamerr*) on testosterone of miceBalb/C induced with lead acetate

The results showed significant differences in testosterone levels of K0 groups with K1, as well as K1 groups with K2 and K3. However.there was no significant difference between K2 and K3 groups with K4 (Table 1)

Table 1. Protector effects of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract ontestosterone level of miceBalb/C (*Musmusculus*) induced with lead acetate

Groups	Testosterone level (ng/dl) Mean ± SD
Negative control	$1.37^{a} \pm 0.57^{a}$
Positive control	$2.99^{b} \pm 1.18$
Dayak Onion Extract30mg/KgBW/days	$5.28^{a} \pm 2.3$

Dayak Onion Extract60mg/KgBW/days	$5.3^{a} \pm 0.71$
Dayak Onion Extract120mg/KgBW/days	$4.2^{ab} \pm 1.92$

Different superscript letters showing significant differences at p <0.05

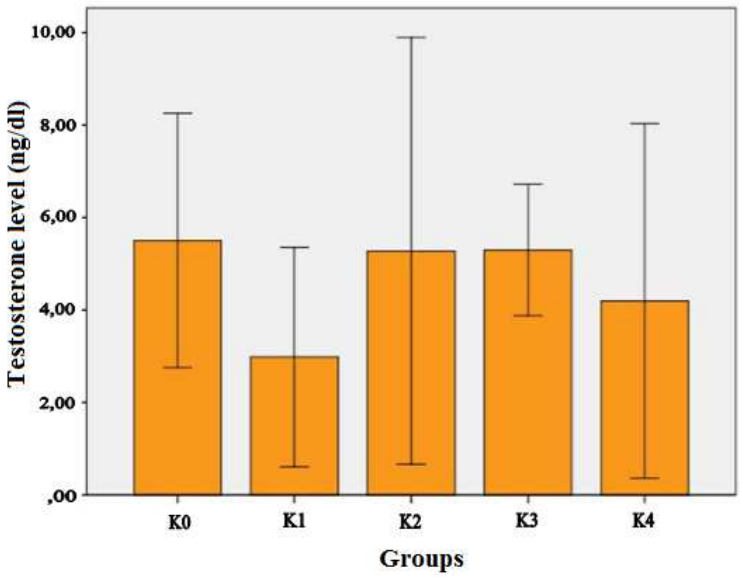
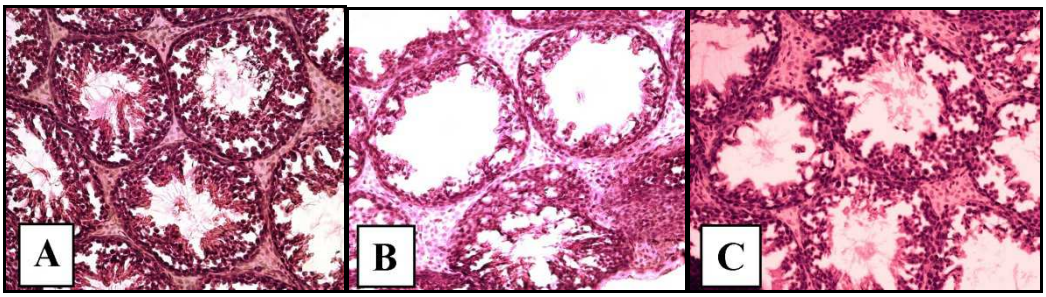


Figure 1. Protector effect of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract on testosterone level of lead-induced in miceBalb/c (*Musmusculus*). K0: negative control group; K1: positive control group; K2: group receiving *dayak* onion extract 30 mg/KgBW/day; K3: group receiving *dayak* onion extract 60 mg/KgBW/day; K4: group receiving onion extract 120 mg/KgBW.

Figure 1 shows that testosterone levels of mice in K1 decreased when compared with K0. This means that the administration of lead acetate of 0.075 g/KgBW/day for 38 days decreases the testosterone hormone levels of mice. Mice testosterone levels in all treatment groups (K2. K3, K4) increased when compared with K1. This means that administration of *dayak* onion extract for 35 days can maintain the testosterone hormone levels of the mice against the induction of lead acetate.

Protector effect of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract on epithelium thickness of seminiferous tubule in miceBalb/C (*Musmusculus*) induced with lead acetate

The epithelium thickness of mice seminiferous tubule was measured using a microscope with an ocular micrometer in 400x magnification (Fig. 2).



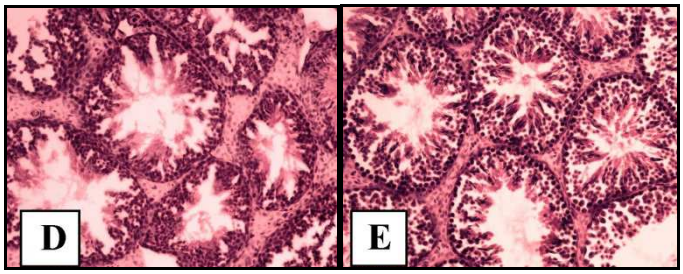


Figure 2. Protector effect of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract on epithelium thickness of seminiferous tubule in miceBalb/C (*Musmusculus*) induced with lead acetate: (A) negative control group; (B) positive control group; (C) group of *dayak* onion extract 30 mg/KgBW; (D) group of *dayak* onion extract of 60 mg/KgBW; (E) group of *dayak* onion extract of 120 mg/KgBW

The results showed significant differences in epithelium thickness of seminiferous tubular in mice between group K0 with group K1 and group K1 with K2, K3, K4 group. However, there was no significant difference between group K3 and K4 (Table 2).

Table 2. Effects of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract on testosterone level of miceBalb/C (*Musmusculus*) induced with lead acetate

Groups	Epithelial thickness of seminiferous tubule (µm) Mean ± SD
Negative control	$54.64^{a} \pm 5.21$
Positive control	$41.29^{b} \pm 4.43$
Dayak Onion Extract30mg/KgBW/days	$48.42^{c} \pm 3.38$
Dayak Onion Extract60mg/KgBW/days	$53.23^{a} \pm 2.63$
Dayak Onion Extract120mg/KgBW/days	$54.11^{a} \pm 1.65$

Different superscript letters, showing significant differences at $p < 0.05$

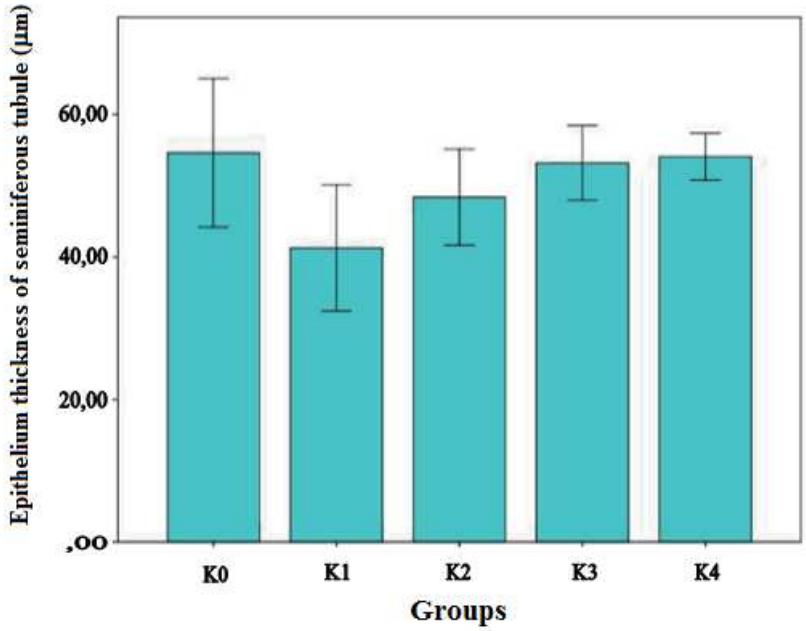


Figure 3. Effect of *dayak* onion (*Eleutherineamericanamerr*) ethanol extract on testosterone level in miceBalb/c (*Musmusculus*) induced with lead acetate. K0: negative control group; K1: positive control group; K2: group of *dayak* onion extract 30 mg/KgBW/day; K3: group of *dayak* onion extract 60 mg/KgBW/day; K4: group of *dayak* onion extract 120 mg/KgBW/day.

Figure 3 shows that epithelium thickness of seminiferous tubule of K1 mice decreased when compared with K0. This means that administration of lead acetate 0.075 g/KgBW/day for 38 days may decrease epithelium thickness of seminiferous tubular in mice. Mice testosterone levels in all treatment groups (K2, K3, K4) increased when compared with K1. This means that administration of *dayak* onion extract for 35 days can maintain epithelium thickness of mice seminiferous tubule against the induction of lead acetate lead.

DISCUSSION

In this study, lead exposure could lower the levels of testosterone and epithelium thickness of seminiferous tubule in mice. This is consistent with the results of a study by Hamadouche*et al*., (2013) which showed that administration of lead acetate resulted in decreased brain weight of mice, increased residual lead in the mice brain, degenerative changes and hypertrophy of endocrine cells of the pituitary gland, atrophy of the seminiferous tubules, decreased the number of Sertoli and Leydig cells, and decreased testosterone levels significantly. Lead is a free radical that can cause the formation of ROS (Reactive Oxygen Species) and decreased body antioxidant reserve. ROS is one type of free radicals that damage cells (Setiati, 2003). The condition in which the ratio of free radicals or oxidants is greater than antioxidants is known as oxidative stress. Oxidative stress in the male reproductive system at central level causes an imbalance in the hormone axis of HPT (Hypothalamus-Pituitary-Testis). Disorders of the HPT axis affect the regulation of the hormone testosterone. Negative effects of lead that disrupt the regulation of testosterone affects the structure of the testes, including the diameter of the seminiferous tubules and spermatogenic cells (Aprilian*iet al*, 2013) because of the proliferation, differentiation and disrupted germ cell metabolism. The accumulation of ROS is also thought to contribute to cell damage, apopotosis and cell death (Handy *et al*., 2009). Lead can lower testis weight, and the degeneration of the seminiferous tubules that is marked by the absence of spermatogenic cells in tubular lumen and an increase in the expression of

caspase-3 (Algawish and Abdelrazek, 2014). Excessive apoptosis of the testes triggers the destruction of the seminiferous tubules.

The effects of ROS on male reproductive system due to lead exposure can be overcome by the administration of antioxidants. Antioxidants play an important role in inhibiting or delaying oxidative stress arising from an imbalance between the production of free radicals and antioxidants (Day, 2014). Natural sources of antioxidants are preferred because they have few side effects. *Dayak* onion is one of the plants that has the potential as a natural antioxidant. Phytochemical content in *dayak* onion plants include alkaloids, glycosides, flavonoids, phenolic, steroids, tannins (Galingging, 2007) and quinone (Nawawi*et al*., 2007). Naphthoquinone predominant contained in *dayak* onion is known to possess bioactivities as anticancer, antioxidant, antimicrobial, antifungal, antiviral, and antiparasitic (Babula*et al*., 2005; Robinson, 1995). The mechanism of naphthoquinone as an antioxidant is as a free-radical scavanger, a heavy metal tracer, and also an inhibitor of the enzyme responsible for the production of free radicals (Gutteridge and Halliwell, 2000). Therefore, the ethanol extract of *dayak* onion (*Eleutherineamericanamerr*) is able to maintain testosterone and epithelium thickness of the seminiferous tubules because these compounds may act synergistically with other metabolites that also functions as an antioxidant via free-radical scavengers enzyme production due to exposure to lead acetate.

CONCLUSION

Dayak ethanol extracts are capable of maintaining testosterone and thickness of the seminiferous tubule epithelium, but are unable to maintain the diameter of the seminiferous tubules in mice induced with lead acetate

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