Conference Paper

PROTECTIVE EFFECT OF PROPOLIS EXTRACT AGAINST LEAD ACETATE TOXICITY IN MICE (*Mus musculus*) TESTES

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Abstract

This study was aimed to investigate the protective effect of propolis extract from *Apis mellifera* that was obtained from Agro Tawon Rimba Raya Malang against the exposure of lead acetate 20 mg/kgBW orally. Twenty-five BALB/C mice were randomly devided into five groups. Negative control received only CMC-Na 1.5% and Tween 80 0.5% also aquadest an hour after the first administration; Positive control group that administered CMC-Na 1.5% and Tween 80 0.5% then continued received 20 mg/kgBW of lead acetate, Treatment group was received 200; 400 and 800 mg/kgBW of propolis first and then an hour after that received 20 mg/kgBW of lead acetate. The treatment was conducted for 7 days of adaptation and 35 days of treatment. At the end of the research all mice were sacrificed and testes were collected. Testes tissue were processed using Hematoxylin-Eosin staining. The result showed that an increase of spermatocyte, spermatid and sertoli cell compare to group that only received lead acetate, but thickness of seminiferous tubules epithelium showed slighly similar in all groups.

Keywords: propolis, lead, reactive oxygen species, free radical, testes.

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1. Introduction

Nowadays development of this country has become very rapid from year to year. This development can not be separated from establishment of many industrial companies. Lead has been used as material of production. (Elfiah, 2015). Lead contributes has been used since prehistoric times, distributed and mobilized widely all over the world. It remains a serious problem in developing and industrializing countries in all aspect, both in human health and to the environment (Tong *et al.*, 2000).

The harmful effect of lead is a term that should be concerned because it's proved that lead can cause fertility disorder in male (Brugh and Lipshultz, 2004). A study of Guang

et al (2009) proved that Pb with dose of 20 mg/kgBW can cause the decrease of testes weight, decrease of diameter of seminiferous tubules and decrease of seminiferous tubules thickness in male mice testes.Vaziri and Sica in Haouas *et al* (2015) reported that lead exposure stimulate the production of intracellular reactive oxygen species (ROS). ROS destruct the polyunsaturated fatty acids of phospholipids in cells membrane that also cause impairment of cellular function and gene mutation.

No specific drug of choice for testis damage especially seminiferous for tubules breakage. Medicinal treatment developement should grow well and better, therefore innovation of source of drug must be increased by new research. Propolis is honey bees product that has more than 300 chemical compounds, generally it contains resin and vegetable balsams, wax, essential and aromatic oils, pollens, and others (Frozza *et al*., 2013). Therefore it is believed to have effects in both protecting cell agains oxidative stress and its role in increasing male fertility like stated by Makhlouf *et al* (2008). Krell in Nugroho (2015) stated that flavonoids and phenolics are believed to have the main role in antioxidant activity. It also has chrysene and vitamins that play a role to advance fertility (Makhlouf *et al* (2008).

The present work was aimed to know the protective effect of propolis ethanolic extract due to the exposure of lead acetate.

2. Materials and Methods

2.1. Chemicals

Materials used in this research were lead acetate, propolis extract, CMC-Na, Tween 80, aquadest, mineral water, BR-1 feed, formalin 10%.

2.2. Experimental Preparation

Experimental animals used in this research were male mice (*Mus musculus*) strain BALB/C aged 7 - 8 weeks with an average weight of 25 – 30 g derived from Pusvetma Surabaya. Mice were adapted for 7 days in cages in size of 36x28x12 cm, feed and drink were given *ad libitum*. Experimental animals were received 20 mg/kgBW of lead acetate (Guang *et al*., 2009).

2.3. Experimental Design

All 25 mice were devided into five groups by simple random sampling. Propolis treated groups were given propolis for three days first then all groups were treated for 35 days.

Negative Control : Mice were administered 1.5% of CMC-Na and 0.5% of Tween 80 solution and aquadest; **Positive Control** : Mice were administered 1.5% of CMC-Na and 0.5% of Tween 80 solution and 20 mg/kgBW of lead acetate; **Treatment I**: Mice were administered 200 mg/kg bw of propolis ethanolic extract solution and 20 mg/kgBW of lead acetate; **Treatment II** : Mice were administered 400 mg/kgBW of propolis ethanolic extract solution and 20 mg/kg bw of lead acetate. **Treatment III**: Mice were administered 800 mg/kg bw of propolis extract and 20 mg/kg bw of lead acetate

2.4. Tissue Processing for Histology

Procedure to make histopathological slide are tissue fixation, dehydration, clearing, impregnation, embedding, microtome, histophatology anatomy staining and mounting.

2.5. Microscopic Examination

Preparats were examined using microscopic with 400 folds magnification and then counted the number of cell using NIS aplication.

3. Data Analysis

Histopathological data of testes was first tested using normality test and continued with ANOVA (Analysis of Variance) followed by Duncan test to compare the treatment effect of each group. SPSS 2.0 for windows software was used as statistical analysis program.

4. Result and Discussion

Research result for the number of spermatocyte, spermatid and sertoli cell of positive control showed lower amount compared to negative control (Table 1). This result proves that lead exposure can cause cell damage due to neither free radical rising

Table 1: The effect of propolis on lead acetate-induced changes on number of spermatocyte, spermatid and sertoli cell in Seminiferous Tubules of Mice Testes.

Groups*	Mean ± SD		
	Spermatocyte	Spermatid	Sertoli Cell
Negative Control	$42.40^{a} \pm 4.43$	$102.44^{a} \pm 17.04$	$6.04^{a} \pm 0.74$
Positive Control	$29.20^{b} \pm 3.69$	$52.68^{b} \pm 10.13$	$3.4^{b} \pm 0.24$
Propolis 200mg/kg	$44.88^{a} \pm 7.94$	$103.08^{a} \pm 17.97$	$4.88^{c} \pm 0.99$
Propolis 400mg/kg	$44.60^{a} \pm 3.18$	$107.60^{a} \pm 18.21$	$5.08^{ac} \pm 0.70$
Propolis 800mg/kg	$61.32^{c} \pm 15.27$	$126.68^{a} \pm 26.42$	$6.28^{ad} \pm 1.02$

Different superscript in the same column indicate significant differences ($p<0.05$).

nor lead hormonal mechanism that decrease the level of FSH and LH. The number of spermatocyte and spermatid are slightly the same.

In the number of spermatocyte and spermatid, group of propolis 200mg/kg and propolis 400mg/kg compared to negative control has non significantly different with the negative control, then followed by the the increasing of propolis 400mg/kg. This result is different with sertoli cell graphic that showed lower level of T1 and T2 group compared to negative control. This condition can be explained by the mechanism of cell to do a compensation. Sertoli cell in amount of that in Propolis 200mg/kg and Propolis 400mg/kg group still can compensate the requirement of spermatogenic cell nutrition. The arousing of the number of spermatocyte and spermatid in group Propolis 800mg/kg can not be compensate by sertoli cell in that amount anymore, need more sertoli cell to provide nutrition due to the spermatogenic arousing number in Propolis 800mg/kg result. Therefore in the number of sertoli cell of Propolis 800mg/kg showed an increasing upto the normal amount (Negative Control). Analyzing the increasing of number of spermatocyte, spermatid and sertoli cell in treatment groups we can conclude that there is positive correlation with the protective effect of propolis as antioxidant induced by lead acetate.

Lead has been known as ubiquitous metal used in some materials but has toxic effect in human and animal body. Some studies have proved that no secure level for this metal. Acute toxicity is rarely found caused by lead, but during the prolonged time when the exposure of this metal comes to chronic, it will be accumulated in blood and cause some disorders (Flora *et al.*, 2012). Queiroz *et al* (2006) and Adikwu *et al* (2014) worked through the mechanism of lead toxicity that can be summarized as three pathways

First pathway is disrupting hypothalamic-pituitary testicular axis that cause lower level of LH caused by inhibition of GnRH, the second is arousing ROS, and the third

is declining antioxidant activity. Lead is declining antioxidant activity by inactivating glutathione by binding to its sulfihydryl moiety. Whereas glutathione is antioxidant present in liver working by quenching free radical, related to drug and toxin metabolism (Adikwu *et al.,* 2014).

Ahmed *et al* (2012) concluded that lead contribute to DNA defect by three mechanism; direct, oxidative, and indirect mechanism. Activation of caspases in the process of cell death is the example of indirect pathway. Koizumi and Li in Haouas *et al* (2015) stated that testes are highly composed of seminferous tubules, whereas seminiferous tubules are the place to produce spermatozoa. Spermatogenic cells that are not yet differentiate to become spermatozoa have also highly responsible to get damaged by the arousing of reactive oxygen species, considered that testes has high content of polyunsaturatesd fatty acids and low antioxidant capacity. This condition explains how could positive control group that only received lead at the dose of 20 mg/kgBW has showed a significant decrease in the number of spermatocyte, spermatid and sertoli cell.

De Queiroz *et al* (2006), Adikwu *et al* (2014), and Babu *et al* (2004) also explained the theory of how lead could reduce the number of spermatocyte and spermatid from the hormonal mechanism. Lead inhibits GnRH to produce LH and FSH, in which LH is functioned as inducer of leydig cell to produce testosterone. When leydig cell was inhibited, the production of this cell has also decreased. Considered to FSH function in male reproduction, it works in sertoli cell to stimulate spermatogenesis. Therefore the production of spermatocyte and spermatid in positive group was low. The same mechanism was also works on sertoli cell in positive group that showed a low level of sertoli cell. This conditioon was correlated to low level of FSH after the exposure of lead (Babu *et al*., 2004)

Inhibiting the ROS reaction and reducing ROS production are the ways of chelating ROS abundance effects, and these were the functions of antioxidant both derived from endogenous or exogenous. Another mechanism also provides by enzymatic pathway such as catalase and glutathione peroxidase that counteract O_2^- reactive radicals by catalyzing the formation of water from H_2O_2derived from O_2^-. In addition, non-enzymatic molecules are also supporting the defense mechanism against free radical by reacting to oxidants in the cell cytoplasm and blood plasm, also protecting cell membrane from lipid peroxidation (Aprioku, 2013).

Recent studies believed that propolis has potency on maintaining spermatogenic number against arousing of ROS by four mechanisms; decreasing ROS production by blocking the ROS production (Ibrahim, 2013), works as antioxidant (Margot, 2010),

protect genome DNA (El-Mazoudi *et al*., 2011), and increasing testosterone hormone degree (Salman *et al*., 2013). This comprehension has driven the research to prove the protective effect of propolis opposes lead exposure in a number of spermatogenic cells (spermatocyte spermatid), sertoli cell, and the thickness of seminiferous tubules epithelium of mice testes.

Spermatogenic cells observed here are spermatocyte and spermatid. These two kinds of cell are considered enough to represent the spermatogenic cell in seminiferous tubules.

A number of spermatocyte and spermatid of propolis treated groups showed an increase compare to group that only received lead acetate. The statement above was directed as the reason how propolis could maintain the number if spermatocyte and spermatid in propolis treated group. Propolis content, especially flavonoid group works as exogenous antioxidant. When there is increaing of ROS inside the cell caused by the induction of lead there will be imbalance between ROS and endogenous antioxidant. Therefore intake of propolis will help cells to maintain membrane integrity in lipid peroxidation by balancing the arousing of ROS with exogenous antioxidants derived from flavonoids.

A study of Al-Moudy (2016) was also revealed the correlation between propolis and the increasing of testosteron and LH level. This is directly proportional with this research result that propolis can increase the number of spermatocyte and spermatid. High production of spermatogenic cell is inseparable with the function of sertoli cell as nursing cell. Sertoli cell provides support, protection and nutrition until the spermatids are differentiated into mature spermatozoa during spermatogenesis. Sertoli cells play a role in controlling the entry and exit of nutrients and hormones. During stage of spermiogenesis, sertoli cells phagocyte the unneeded portions of the spermatozoa (Sharma and Garu, 2011). The increasing of sertoli cell in propolis treated groups was also directly proportional to the increasing of spermatogenic cells due to its function. Different with the other paramaters, thickness of seminiferous tubules has different result. There is no significant difference between all of the groups, this condition maybe due to the different density of seminiferous tubules.

Thickness of seminiferous tubules epithelium is measured start from basal membrane to the outer spermatogenic cell, near the lumen of seminiferous tubules. Seminiferous tubules density is not the same in all groups, even if the thickness is slightly similar. This condition can explain why seminiferous tubules have the similar lenght of seminferous tubules epithelium but different in the number of spermatogenic cells.

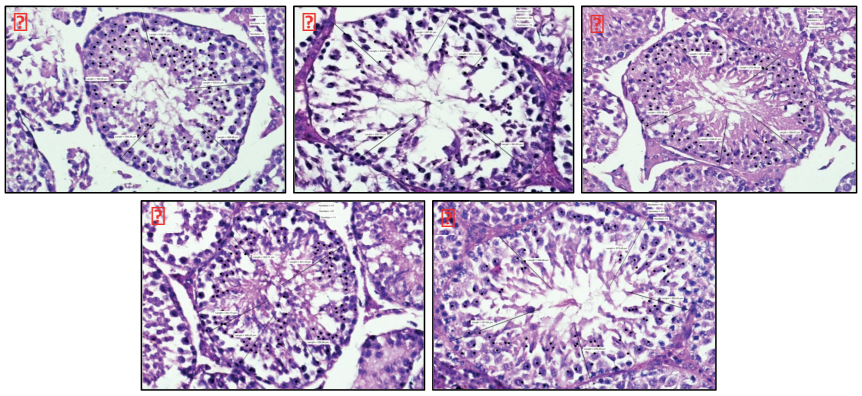

Figure 1: Microscopic Views of thickness of Seminiferous Tubules of Mice Testes. Negative control group (A); Positive control group (B). Rats treated with propolis 200 mg/kg BW (C); 400 mg/kg BW (D) and 800 mg/kg (E), using haematoxyline and eosin stain technique (x400).

Table 2: The effect of propolis on lead acetate-induced changes on thickness of seminiferous tubules epithelium.

Groups	Mean ± SD
Negative Control	$290.66^{a} \pm 43.06$
Positive Control	$305.40^{a} \pm 28.18$
Propolis 200mg/kg	$284.25^{a} \pm 62.22$
Propolis 400mg/kg	$334.09^{a} \pm 43.88$
Propolis 800mg/kg	$306.02^{a} \pm 19.09$

Different superscript in the same column indicate significant differences ($p<0.05$).

Microscopic view of positive control showed a loose spermatogenic cell up to the adluminal area, in the contrary the picture of negative control and propolis treated group showed a dense spermatogenic cells. This condition should be certainly impact on this non correlated result with the other parameters (Figure 1).

In spite of non correlated result of the thickness of seminiferous tubules epithelium, all aspects are well achieved by propolis against lead acetate in testes especially for T3 group that received 800 mg/kgBW of propolis ethanolic extract (Table 2).

5. Conclusion

Based on the research result, it can be concluded that propolis can maintain the number of spermatocyte, spermatid and sertoli cell of mice testes from cell damage induced by lead acetate, but propolis can not maintain the thickness of seminiferous tubules epithelium of mice testes that is induced by lead acetate.

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