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Research Article

DUCK EGG WHITE POTENTIAL IN TREATING SUBACUTE LEAD POISONING

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ABSTRACT

Objectives: The aim of this research is to study duck eggwhite potential in treating subacute lead poisoning.

Methods: The potential of duck egg white was evaluated from the red blood cell profile and the clinical signs that emerged. The research used thirty male rats which were divided into 6 groups and 5 replications (i.e. rats as control, rats administrated only with lead, rats administrated with lead and antidote). Each of the four treatment groups were given one antidote (i.e. EDTA, 50% egg white, 75% egg white, and 100% egg white). Lead force feeding was conducted for 15 days, followed by the administration of the antidote for the same duration of 15 days, and concluded with blood sampling at the end of each treatment.

Results: There was no significant effect on haemoglobin but lead decreased total red blood cells ($p<0.05$) in subacute lead poisoning. Rats that were given 75% and 100% duck egg white as an antidote showed an increase in total red blood cell counts in addition to a faster recovery.

Conclusion: High concentration of duck egg white had shown positive results as an antidote for subacute lead poisoning.

Keywords: Duck egg white, Lead, Potential, Subacute.

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INTRODUCTION

Lead poisoning case has been frequently reported on human and animal alike [1]. Industrial manufacturing and motor vehicle exhaust turned lead into one of the largest environmental pollutions. Lead poisoning from work environment (work-related poisoning) may happen in battery industry, paint industry, printing, pottery, and lead smelting [2]. Lead exposure may happen during tank building process, pipe installment, and other activities with equipment harboring gas and liquid with corrosive superconductor properties, optical fiber technology, magnetic resonance imaging, and nuclear drugs. Lead can inadvertently contaminate body through polluted air, lead inhalation, skin contact, contaminated food and drinks, and swallowed lead-containing matter [3,4].

Lead accumulation within the body may cause acute and chronic poisoning, even death. The effect of acute and subacute lead poisoning is very particular in relation to the relatively high dosage exposure and relatively short exposure duration, whether it is counted in days or months. Acute lead poisoning effect may also occur dramatically in the form of sudden death, severe stomach cramp, anemia, behavioral change, and loss of appetite. In the event of lead poisoning, described effects may not all manifest and only some of them are clearly observable [5].

Chronic lead poisoning effects happen as a result of small amount of lead exposure for long period. It may happen in months or years. Chronic lead poisoning usually caused unspecific symptoms on almost every body system. Negative effects of lead poisoning in human based on a research by Pokras and Kneeland [1] consist of lowering libido and fertility (male and female), stillbirth and premature birth, intelligence impairment, hypertension, cardiovascular disease, aggression, and impaired renal function.

Generally, chelation therapy is a preferred medical treatment chosen to minimize toxic effect of heavy metal including lead [6]. Chelation agent can bind heavy metal ion within cell or outside cell, forming complex structure which is easily excreted outside the body [7]. Chelation agent can be administered intravenously, intramuscularly, through inhalation, or orally depending on the type of drugs. Several types of chelating agent had been proven effective. Calcium disodium ethylendiaminetetraacetic acid (EDTA), calcium trisodium diethylenetriaminepentaacetic acid, British anti-Lewisite, unithiol, EDTA, penicillamine, and succimer are several examples of chelating agent [7,8]. Main chelating agent most commonly used in hospital are usually administered parenterally (not orally) by trained medical staff. On the other hand, oral chelating agent can be used without special equipment and treatment, but its dosage must be a point of consideration.

Till date, chelating agents are generally expensive, cannot be obtained without prescription, not continually available, and available in restricted amount. With the aforementioned constraints, human and animal alike suffering from lead poisoning cannot be treated quickly and precisely. Delay of lead poisoning therapy may increase absorption and accumulation of lead within the body which can result in fatal consequence or even death [9].

The ability of protein to bond with heavy metal is the basis in finding alternative for effective heavy metal poisoning treatment which is safe, cheap, and easily obtainable. One of natural proteins potential to be used as heavy metal chelating agent is duck egg. Duck egg white is reported to have higher protein content compared to chicken egg [10]. In addition, duck egg is also commonly consumed and favored by people with relatively affordable price. Thus, research that study duck egg white potential in treating lead poisoning must be conducted. The aim of this research is to study duck egg white potential in treating subacute lead poisoning.

METHODS

Research was conducted in Laboratory Animal Management Unit, Pharmacology Laboratory, and Physiology Laboratory, Veterinary Medicine Faculty, Bogor Agricultural University. Animal used were 30 male rats (*Rattus novergicus*) of Sprague-Dawley strain. Materials used are duck egg white, lead acetate ($Pb(CH_3COO)_2$), Na-EDTA, aqua

distillate, and blood test reagent. Equipment used is maintenance cage, test tubes, 1 ml, 3 ml, and 5 ml syringes, bowls, plastic glass, napkins, gastric sonde, squeeze cages for rat, macro- and micro-scale, volumetric flask, stationery, ice box, counting chamber, microscope, capillary pipe, microcapillary hematocrit reader, and spectrophotometer.

Preparing 1000 ppm lead acetate

1000 ppm lead acetate solution was made by diluting 1 g lead acetate crystal within 1000 ml aqua distillate. Lead acetate crystal and aqua distillate were mixed inside volumetric flask and then stirred until they homogenized.

Rat preparation and group division

Thirty male rats were chosen and reared for 2 weeks for acclimatization. All rats have relatively similar body weight (BW) of 180-240 g. Rats were given standard feed and water given *ad libitum*. Rats were separated into six treatment groups with five rats for each treatment. Treatment 1 was control group, which are rats that were not exposed to lead and did not received duck egg whites or EDTA. Animal divided into six groups of five rats each. Group I: normal control rats were not exposed to lead and did not received duck egg whites or EDTA; Group II: Lead control rats given lead exposure; Group III: rats given lead exposure and administered EDTA; Group IV; rats given lead exposure and administered duck egg white (50%); Group V; rats given lead exposure and administered duck egg white (75%), Group VI; rats given lead exposure and administered duck egg white (100%).

Lead exposure

Exposure to lead was done by force feeding treatment rats by lead acetate (1000 ppm) for 15 days with 15 mg/100 g BW dosage. Dosage was calculated based on dosage conversion by Laurence and Bacharah [11]. After 15 days of lead exposure, the rats were given EDTA or duck egg white depending on their treatment group.

Red blood count and clinical symptoms observation

Blood count was conducted 3 times: Before treatment (day-0), after lead acetate administration (day 15 post administration), and after EDTA or duck egg white administration (day 15 post treatment). Blood count consists of red blood cell count, hematocrit, and hemoglobin (Hb) count. Clinical symptom observation of lead poisoning (lethargy and diarrhea) was done every week during the observation.

Blood collection was done through coccygeal lateralis vein by the tail from rats placed inside squeeze cages. Obtained blood samples were transferred into tubes containing EDTA anticoagulant. Afterward, method on erythrocyte count, Hb count, and hematocrit were conducted as follows.

Erythrocyte count

Erythrocyte count was done using hemocytometer (counting chamber). As much as 2.5 µl blood was homogenized with 0.5 ml Hayem's solution to reached 200 times dilution. Blood that has been diluted was dropped into hemocytometer and checked under microscope with 400 times magnification. The number of erythrocyte was counted from 5 erythrocyte box diagonally or all four sides and the middle box of counting chamber. Total erythrocyte count was obtained by multiplying the number of erythrocyte observed with 10,000/mm^3.

Hb count

Hb count was measured by cyanomethemoglobin method. As much as, 20 µl of blood was mixed into 5 ml of cyanomethemoglobin reagent and then homogenized. The absorbance value was read using spectrophotometer under 540 nm wavelength. Hb count was obtained by multiplying absorbance value with 36.8 g Hb/100 ml.

Hematocrit measurements

Hematocrit was measured using microcapillary method. Microcapillary pipette coated with heparin was dipped inside sample blood up to 2/3 of pipette were filled. The microcapillary pipette was centrifuged in 12,000 rpm for 5 minutes. Hematocrit was obtained from microcapillary hematocrit reader.

Observed variable

Variables observed in this research are specific clinical symptoms (lethargy and diarrhea) and red blood cell count (erythrocyte count, Hb count, and hematocrit).

Data analysis

Obtained data were analyzed by analysis of variance and followed by Duncan test. Data obtained were analyzed descriptively.

RESULTS

Anemia as one of the lead poisoning clinical symptoms can be recognized through blood count. Blood count was done to see red blood cell profile after lead acetate force feeding and after duck egg white administration. Red blood cell profile is presented on Table 1.

Post lead force feeding blood count showed a decrease of erythrocyte count ($p<0.05$). Rat Hb count in lead given rats is significantly different compared to other treatment group rats. There is no significantly different result in hematocrit between treatment groups. Post antidote administration, erythrocyte count was seen increasing except in rats given lead acetate and 50% duck egg white ($p<0.05$). The increase of erythrocyte in rats given 100% duck egg white is higher compared to rats given EDTA.

Hb count does not have good correlation with lead content in blood which cause anemia to not manifest in low lead exposure [12]. Mugahi *et al.* [13] explained that lead poisoning can cause decrease in erythrocyte and Hb count. Anemia that commonly happens is normocytic-normochromic anemia in subclinical phase, hypochromic anemia in acute phase, and macrocytic hypochromic anemia or normocytic-normochromic in chronic phase. Microcytic-hypochromic anemia happens in young with iron deficiency. Erythrocyte index of white rats in this research is presented in Table 2.

Identification of subacute lead poisoning cannot be easily identified from blood count profile alone since anemia was not clearly observable. Lead poisoning diagnosis cannot be upheld without other supporting evidence, for example lead content in blood and protophorphyrin enzyme. Manifestation of clinical symptoms is a sign of lead accumulation of at least 200 mg/kg KB in rats [13]. Clinical symptoms' observation was conducted based on treatment group and not individually. Clinical symptoms' observation during research result is presented in Table 3.

Table 3 showed that control rat did not present lead clinical symptoms. All rats that were given lead for 15 days showed lethargy and diarrhea. Rats with lethargy appeared to be more apathetic and weaker. Before the symptoms, rats were very active, ran around, and struggled during handling. Diarrhea was seen from watery excrement left around anus or tail. Watery excrement also mixed with woodchip bedding, which piled with one another. Clinical symptoms did not appear after 7 days of 75% and 100% duck egg white administration. Lethargy still appeared on rats only given lead, rats given lead and EDTA, and rats given lead and 50% duck egg white. Diarrhea only appeared in rats given only lead.

DISCUSSION

The decrease of erythrocyte count presumably happened as a result of lead in the bloodstream that disrupted hematopoiesis enzyme. Increase of reactive oxidative species perpetrated by free radical lead ion can cause red blood cell damage as explain by Weiss and Wardrop [14,15]. Hb tends not to be affected in subacute lead poisoning since there was enough time for body to regenerate. In this research, there is no decrease Hb in rats forcefed by lead. The form of anemia observed also varied; however, none exhibit anemia caused by lead poisoning. Rats given only lead suffered from macrocytic-hypochromic anemia while rats given lead and antidote

Table 1: Erythrocyte count, Hb count, and hematocrit profile

Parameter	Control	Lead	Lead treatment+				p
			EDTA	Egg 50%	Egg 75%	Egg 100%	
Before treatment							
Erythrocyte (juta/mm^3)	8.2±1.99^a	8.3±0.62^a	7.4±1.09^a	6.9±0.47^a	7.7±0.65^a	8.2±1.11^a	0.327
Hb (g%)	14.4±2.67^a	14.7±0.40^a	15.1±1.01^a	14.1±1.37^a	14.4±0.74^a	14.4±0.47^a	0.893
Hematocrit (%)	39.8±2.08^a	39.3±3.45^a	38.9±3.88^a	41.4±1.39^a	39.6±2.13^a	37.6±4.55^a	0.583
After lead force feeding							
Erythrocyte (juta/mm^3)	8.9±0.79^a	8.8±1.62^a	5.9±1.35^b	7.4±1.31^{ab}	6.6±1.61^b	7.8±0.71^{ab}	0.008
Hb (g%)	14.1±1.02^{ab}	12.9±2.38^{ab}	15.7±1.74^a	15.2±0.81^a	15.6±1.21^a	14.7±0.72^{ab}	0.048
Hematocrit (%)	40.9±1.49^a	41.0±4.94^a	40.8±3.16^a	38.1±4.15^a	39.2±2.62^a	42.3±1.96^a	0.429
After duck egg white administration							
Erythrocyte (juta/mm^3)	7.4±0.14^{cd}	8.9±0.67^a	8.0±0.37^{bc}	7.1±1.01^d	7.7±0.39^{bcd}	8.2±0.40^{ab}	0.001
Hb (g%)	13.5±1.17^a	14.8±0.47^a	14.7±0.49^a	13.5±0.42^a	13.9±2.32^a	13.7±0.39^a	0.277
Hematocrit (%)	40.8±1.95^b	43.5±1.62^a	42.3±0.74^{ab}	41.1±2.42^{ab}	43.1±0.74^{ab}	38.5±1.97^c	0.001

Different superscripted letter on the same row showed significantly different result ($p<0.05$). Hb: Hemoglobin, EDTA: Ethylendiaminetetraacetic acid

Table 2: Rat erythrocyte index

Parameter	Control	Lead	Lead treatment+				p
			EDTA	Egg 50%	Egg 75%	Egg 100%	
Before treatment							
MCV (fl)	50.5±10.04^a	47.8±5.27^a	53.3±8.32^a	60.3±4.77^a	51.7±6.59^a	46.1±7.09^a	0.071
MCH (pg)	18.1±3.34^a	17.8±1.15^a	20.7±2.88^a	20.5±2.32^a	18.7±1.80^a	17.7±2.50^a	0.208
MCHC (%)	36.4±6.94^a	37.6±4.00^a	39.3±5.82^a	34.2±3.70^a	36.3±1.58^a	38.6±4.32^a	0.586
After lead forcefeeding							
MCV (fl)	46.3±4.75^b	47.3±4.63^b	40.8±22.26^a	52.2±7.21^b	60.9±9.65^{ab}	54.9±5.13^b	0.010
MCH (pg)	15.9±1.80^{cd}	14.9±2.55^d	27.5±7.12^a	20.8±2.86^{bc}	24.4±5.18^{ab}	19.0±1.96^{bcd}	0.000
MCHC (%)	34.5±3.63^{ab}	31.7±4.67^b	38.8±5.06^a	40.0±3.61^a	39.9±4.26^a	34.6±1.28^{ab}	0.011
After duck egg white administration							
MCV (fl)	55.2±2.56^a	48.9±2.81^b	52.8±3.38^{ab}	58.5±8.44^a	56.5±2.91^a	46.9±3.63^b	0.003
MCH (pg)	18.3±1.31^a	16.7±1.04^a	18.3±1.40^a	19.3±2.95^a	18.2±3.41^a	16.7±1.16^a	0.362
MCHC (%)	33.1±1.95^a	34.1±1.50^a	34.6±0.72^a	32.9±1.86^a	32.2±5.26^a	35.7±1.04^a	0.304

Different superscripted letter in the same row showed significantly different result ($p<0.05$). MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, EDTA: Ethylendiaminetetraacetic acid

Table 3: Clinical symptoms observed in treatment group during research

Clinical symptoms	Treatment					
	Control	Lead (%)	Lead+EDTA (%)	Egg 50% (%)	Egg 75% (%)	Egg 100% (%)
Day 0						
Lethargy*	-	-	-	-	-	-
Diarrhea*	-	-	-	-	-	-
Day 7 post lead force feeding						
Lethargy	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-
Day 15 post lead force feeding (Day 0 of duck egg white administration)						
Lethargy	-	+(100)	+(100)	+(100)	+(100)	+(100)
Diarrhea	-	+(80)	+(60)	+(60)	+(80)	+(60)
Day 7 post duck egg white administration						
Lethargy	-	+(40)	+(20)	+(40)	-	-
Diarrhea	-	+(20)	-	-	-	-
Day 15 post duck egg white administration						
Lethargy	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-

*Showed that change was not caused by treatment. EDTA: Ethylendiaminetetraacetic acid

suffered from macrocytic-hyperchromic anemia. This difference of anemia type was caused by different blood count parameter value.

Lead can replace calcium and disrupt calcium and calcium ion canal on cell membrane which cause intestine cells function impairment [16]. Lead in body can disrupt cell normal function, especially smooth muscle contraction and neurotransmitter release. Diarrhea happens because decreasing water absorption in colon caused by colon motility impairment. This resulted in higher water content in the feces. Intestine motility impairment lowered nutrition absorption into cell. This causes energy to no longer able to support rat activity. Prolonged diarrhea increased water loss and caused dehydration and lethargy. Lethargy is also caused by muscle cell contraction impairment because lowered cell calcium.

From the result of this research, 75% and 100% duck egg white antidote administration able to eliminate clinical symptoms in rats. Protein in duck egg white is presumed to catch lead ion remaining at the surface

of intestines and prevent it from being absorb into bloodstream. Fat and protein contained in duck egg white presumably help in supplying energy to rats, which prevent lethargy.

Other apparent lead poisoning clinical symptoms is severe colic; however, colic is not generally seen in adult [17]. Egg proteins especially lysozyme, ovalbumin, and ovotransferrin are the best heavy metal chelator contained in egg. Through sulfhydryl bond, protein will trap metal ion in gastrointestinal tract [18]. Other than protein, fat is also abundant in duck egg white. Fat and protein content in duck egg white is higher than chicken egg and low in cholesterol [10]. The lack of protein intake reported to cause diarrhea; thus, exogenous protein intake is hoped to fix the condition [19].

In this research, colic symptom was unobservable because the rats used were reaching adulthood. Adult rat only absorbs 10% of lead consumed and the rest will be excreted through urine and feces [20,21]. Lead administration in this research used 15 mg/100 g BW for 15 days, or subacute period, while colic usually happened in acute lead poisoning case in young animal. Subacute lead poisoning did not present specific clinical symptoms which broadened differential diagnosis.

CONCLUSION

75% and 100% duck egg white have the potential as antidote for subacute lead poisoning. Duck egg white can able to increase erythrocyte count in rat force-fed by lead ($p<0.05$). Duck egg white contains protein that can bind and prevent lead metal ion absorption and thus prevent further blood destruction. Fat and protein contained in duck egg white are presumed to be high energy supply which can treat clinical symptoms in subacute lead poisoning.

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