The effect of cyanide exposure to larvae weight for post mortem interval estimation

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Abstract

Flies are one type of insect, which decompose the organic component in animals, and human cadavers. Therefore, the insects can be used to help the investigators related to human law. One of the methods to determine Post Mortem Interval [PMI] was by identifying the insects that come in the decay process. Some chemicals can affect the development of the insect and its life cycle. Cyanide is widely used to commit murder in Indonesia. The effect of cyanide on the development and life cycle of insects is a very interesting field to study. The purpose of this study was to determine the effect of cyanide exposure to larvae weight for PMI estimation. This research was an experimental research that used larvae of Wistar rat carcass. The control group was killed by neck dislocation, while experimental group were killed by oral lethal doses of cyanide. Data were obtained from the measurement and examination of larvae, started from the 1st day until 4th day after death. *Chrysomya*, *Sarcophaga*, and *Lucilia* larvae were identified from this study. *Sarcophaga* larvae first appeared and next day followed by *Chrysomya* and *Lucilia.* There were significant differences in *Chrysomya* larvae weight. There were *Chrysomya* third instar larvae on the 4th day on cyanide group, with 56.4±16.8 mg of weight. There were Chrysomya third instar larvae on the 4th day on control group, with 30.4±5.5 mg of weight. The differences of larvae's weight, among *Sarcophaga* and *Lucilia* larvae, could not be determined because there were not enough samples. *Chrysomya* third instar larvae of cyanide group significantly heavier than control group on 4th day. The differences of larvae weight, among *Sarcophaga* and *Lucilia* larvae, could not be determined because there were not enough samples.

Keywords: cyanide, larvae stage, larvae weight

Introduction

Forensic entomology is one branch of forensic science, which insects are used for the investigation of human or animal case (1),(2). Insects are group of animals that have a wide spread in all habitats, with a wide range of environmental conditions (3). Insects are able to survive by consuming a variety of foods such as carrion (*necrophagous*). Flies are one example of necrophagous insects that feed on carrion, especially corpses (cadaver), so that the animal

can be used as a guide to determine the PMI estimation (4).

The species of *Diptera*/flies (e.g., *Calliphoridae, Muscidae, Sarcophagidae, Stratiomyidae*) and *Coleoptera*/beetles (e.g., *Dermestidae, Cleridae, Silphidae*) have been associated with a criminal investigation that were used for PMI estimation (5) Other studies described types of necrophagous insect frequently encountered, such as *Diptera*/flies (*Calliphoridae, Muscidae, Fanniidae, Sarcophagidae, Piophilidae, Sepsidae, Phoridae, Sphaeroceridae, Heleomyzidae, Stratiomyidae, Drosophilidae, Ephydridae, Trichoceridae* family); *Coleoptera*/beetles (*Staphylinidae, Histeridae, Silphidae, Cleridae, Trogidae, Dermestidae, Scarabaeidae, Geotrupidae, Nitidulidae* family); *Lepidoptera*/butterfly; *Tineidae/*clothes moth; *Hymenoptera*/bees (*Ichneumonidae, Pteromalidae)*; mites; and ants (6), (7), (8).

Flies Activitiies are influenced by many factors, both internal and external. The internal factor is the character of fly species itself. The ambient temperature, humidity, rainfall, food, geography, contaminants (toxins) are the examples of external factors. Some chemicals such as triazolam, oxazepam, alimemazin, chloripiramin, phenobarbital, mercury, malathion, amitriptyline, nortriptyline, cocaine, heroin, morphine, and phenicyclidin could affect larvae life cycle (8),(9). Some research was conducted in Wistar rats, used amitriptyline and morphine. Amitriptyline might block the growth of flies larvae (8),(10). Morphine caused larvae growth significantly longer and heavier (on Wistar rat carcass) (11).

The effects of cyanide are very fast and can lead to death within a few minutes. According to the American Association of Poison Control Center Toxic Exposure Surveillance System, 5 of 242 cases in 2007 and 3 of 238 cases in 2008 were deadly exposure. Cyanide come into the body via parenteral, inhalation, oral, or dermal absorption (12). There was no clear relationship between the effects of these substances with the average weight of larvae. Wistar rats were used as a model for estimating PMI for human cyanide poisoning death case. This study was aimed to determine the effect of cyanide exposure to larvae weight for PMI estimation.

Materials and methods

This research was an experimental research, used larvae of Wistar rat carcass to determine the effect of cyanide exposure to larvae weight for PMI estimation. The samples of this study were 12 healthy male Wistar rats. The average rats age were about three to four months, the weight were about 150 grams, which were obtained from the Pharmacology and Toxicology Laboratory of Medicine Faculty UGM. The data were collected from direct measurement and inspection of larvae, starting from 1^{st} to 4^{th} days after death. In this study, control group were killed by neck dislocation, while experimental group were killed by lethal doses cyanide orally, LD100 (6 mg/kg). After rats were killed, they were placed in Biology Forest to be rotted naturally. Larvae measurement and posterior spiracles examination were conducted at Parasitology Laboratory. The clearance from Research Ethic Committee was obtained for this study.

Larvae weights were measured with scale (in mg). Larvae type was determined by observation of the larvae posterior spiracles. First instar larvae: there was 1 spiracle slit. Second instar larval: there were 2 spiracle slits. Third instar larvae: there were 3 spiracle slits. The 1^{st} day until the 4^{th} day after death (12.00 a.m.), the observed larvae were taken as many as 10% of the total existing larvae by using simple random sampling. The larvae were inserted in 70% alcohol containing tube and transported to Parasitology Laboratory for examination.

The temperature and humidity of the Biology Forest were recorded. Normality test of data used Kolmogorov-Smirnov test. The test results p value > 0.05, then data was normally distributed. Univariate analysis was used to analyze the descriptive data for larvae. Independent sample T-test was used to analyze the average differences between two groups.

Result and discussion

Decay process was occurred in Biology Forest. After the rats were killed, they were put in a cage, and laid on the ground. Temperature and humidity were measured every morning (04.30 a.m.) and noon (12.00 a.m.) (Table 1). Biology Forest was a miniature forest, with a variety of large trees and other plants. This research was done during transition season, from dry season to rainy season. *Chrysomya*, *Sarcophaga*, and *Lucilia* larvae were identified from this study. *Sarcophaga* third instar larvae first appeared (2^{nd} day on control group and 3^{rd} day on cyanide group), next day followed by *Chrysomya* and *Lucilia* (3^{rd} day on control group and 4^{th} day on cyanide group). *Sarcophaga* third instar larvae first appeared, because *Sarcophaga* is ovovivipar. Sarcophaga interested in carcasses or corpses in almost situations, either exposed or

sheltered from the sun, either wet or dry environment, and on the inside or outdoors. The eggs hatch in the uterus of female flies, and first instar larvae of *Sarcophaga* delivered if finding right environment (6).

Table 1: Temperature and humidity

Incubation Period	Temperature and Humidity			
	04.30 a.m.		12.00 a.m.	
	°C	%	°C	%
1	-	-	29	47
2	-	-	30	51
3	25	71	25	71
4	25	74	23	84

The average weight of *Chrysomya* third instar larvae on the control group tended to increase from 3th to 4th days. In LD100 cyanide group, there were no larvae in 1st to 3th days, after that the average weight of larva is 56,4±16,8 mg (Table 2).

Table 2: Average weight of *Chrysomya* third instar larvae on control and Cyanide Group

Incubation Period (Day)	Control	Cyanide LD100
	Weigth mg	Weigth mg
1	-	-
2	-	-
3	7,2±2,2	-
4	30,4±5,5	56,4±16,8

Cyanide is common used in insecticide. The heaviest *Chrysomya* larvae cyanide group was found on 4th day, because cyanide level in rats had already begun to fall, so the insects began to eat and to lay eggs. Study in rabbits was reported that cyanide level fallen down 20 minutes after death(13). Another study explained that high ambient temperature further accelerate the decline of cyanide level (14),(15).

The average length of *Chrysomya* adults flies were 6-14 mm, majority having metallic color ranging from green, blue, bronze or black. *Chrysomya* adults flies come to dead body, about 10 minutes after death (16). Length of Chrysomya egg was about 1 mm and 50 -200 eggs could be laid in one spawning. Female fly can lay up to thousands eggs, on the whole body of corpse (17). The eggs hatch approximately 18-24 hours at 25^{o}C and 10 hours at 37°C. First instar larvae became third instar larvae taken 5-6 days at 36^{o}C. The larvae will mature into pupae for 7-8 days and migrate from the body, to find the right place. Adult flies can lay eggs, after 6-7 days (18).

Based on the table 2, we found that average weight of *Chrysomya* third instar larvae on control group was lower than cyanide group. In the table 3, the average differences between two groups on 4th day was significant statistically (*p-value:* 0,002). It was because the rats on cyanide group relatively intact (because of delaying in hatching), compared with control group on the 3rd day. On cyanide group, third instar larvae began to emerge on 4th day. They had more food sources than control group. The number of these food sources caused the larvae ate more food, so they had higher number of weight. Besides, *Chrysomya* larvae have a longer life cycle than *Sarcophaga* and *Lucilia*. In control group, rats body had already decreased since the 3rd day, fewer food available for larvae and the other insect.

Sarcophagidae family (flesh flies) has more than 2000 species that can be found throughout the world, most species were found in tropical areas with warm temperatures. *Sarcophaga* adult flies have 2-14 mm in length, with black gray stripe color on the chest. This fly has three dark stripes on the chest. On the stomach has a complexion like a chessboard. Some species have bright red eye color (19).

Table 3: Average differences between control and Cyanide group

Incubation Period (Day)	Control	Cyanide LD100		
	Chrysomya Weigth mg	Weigth mg	*Chrysomya p-value*	*95% CI*
1	-	-	-	-
2	-	-	-	-
3	7,2±2,2	-	-	-
4	30,4±5,5	56,4±16,8	**0,002**	**(-40 - -11,9)**

The average weight of *Sarcophaga* third instar larvae on the control group tended to increase from 2nd to 3rd days. In LD100 cyanide group, there were no larvae in 1st to 3th days, after that the average weight of larva is 54, 1±1, 3 mg (Table 4).

Table 4: Average weigth of *Sarcophaga* third instar larvae on control and Cyanide group

Incubation Period (Day)	Control	Cyanide LD100
	Weigth mg	Weigth mg
1	-	-
2	5,7±5,6	-
3	53,5±34,7	-
4	-	54,1±1,3

Sarcophaga is ovovivipar, so the eggs hatch in the uterus of female flies, and first instar larvae of *Sarcophaga* delivered if finding right environment. The female lays 20-40 first instar larvae directly in the right place. Only within 3-4 days, the larvae were already in the final stages of larva and ready to become pupae. *Sarcophaga* pupa size were 5-10 mm. Female adult flies can reproduce again after 10-14 days (20).

Table 5: Average differences between control and Cyanide group

Incubation Period (Day)	Control	Cyanide LD100		
	Sarcophaga Weigth mg	Weigth mg	*Sarcophaga p-value*	*95% CI*
1	-	-	-	-
2	5,7±5,6	-	-	-
3	53,5±34,7	-	-	-
4	-	54,1±1,3	-	-

Based on the figure table 5, average differences in larvae weight on cyanide and control group, could not be found. It was because there were no larva from both groups on the same day. However, there was a delay in first instar larva laying on cyanide group. *Sarcophaga* fly delayed to lay its first instar, until the cyanide level falled down. Rats body on cyanide group were relatively intact compared with control group on 2nd day. However, there were many *Chrysomya* third instar larvae which were longer than *Sarcophaga* larvae. There was competition between *Chrysomya* and *Sarcophaga*, in obtaining food. As a result, the food sources for *Sarcophaga* larvae were similar to the control group. Besides, first Instar *Sarcophaga* (3-4 day) faster become pupa than *Chrysomya* (5-6 day) (6).

The average weight of *Lucilia* third instar larvae on the control group tended to increase from 3rd to 4th days. In LD100 cyanide group, there were no larvae in 1st to 4th (Table 6).

Tabel 6: Average weight of L*ucilia* third instar larvae on control and Cyanide group

Incubation Period (Day)	Control	Cyanide LD100
	Weigth mg	Weigth mg
1	-	-
2	-	-
3	17,6±8,4	-
4	29,9±8,9	-

The *Lucilia* adult flies have 8 mm body length, metallic green body color, black colored legs, and 7.5 mm wing venation. *Lucilia* adult flies not only useful in investigation, but also played role in larvae therapy. These larvae could eat dead tissue and bacteria naturally, as well as released antiseptic substances (21). *Lucilia sericata* larvae were placed on necrotic tissue, then wrapped so that the larvae could work. Larval activity could stimulate growth of healthy tissue (22). *Lucilia* adult flies could lay up to 200 eggs in carrion. At 27° C, the eggs required 18 hours to hatch into larvae, while at 21°C taken 21 hours. First *Lucilia* instar larvae taken 3 days at 27 ° C became pupae, whereas at 20 ° C taken 4 days (23).

Table 7: Average differences between control and Cyanide group

Incubation Period (Day)	Control		Cyanide LD100	
	Lucilia Weigth mg	Weigth mg	*Lucilia* *p-value*	*95% CI*
1	-	-	-	-
2	-	-	-	-
3	17,6±8,4	-	-	-
4	29,9±8,9	-	-	-

Based on the figure table 7, average differences in larvae weight on cyanide and control group, could not be found. It was because there were no larva from both groups on the same day.

PMI estimation could be summarized form significant results from Independent Sapmle T-tests (Table 3). There were *Chrysomya* third instar larvae on the 4th day on cyanide group, with 56,4±16,8 mg of weight. There were *Chrysomya* third instar larvae on the 4th day on control group, with 30,4±5,5 mg of weight.

Conclusion

Chrysomya third instar larvae of cyanide group significantly heavier than control group on 4th day. The differences of larvae weight, among *Sarcophaga* and *Lucilia* larvae, could not be determined because there were no enough samples.

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Conflict of Interest

None declared

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