Indonesian Journal of Pharmacy
VOL 31 (2) 2020: 116–123 | RESEARCH ARTICLE

The Effect of Giving Deltamethrin and Cypermethrin Insecticides on the Level of Intratesticular Testosterone in Male Wistar Strain Rats

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Info Article

Submitted: 08-03-2019
Revised: 05-07-2020
Accepted: 29-07-2020

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ABSTRACT

The use of deltamethrin and cypermethrin insecticides to control pests has been implemented in Indonesia. Irregular and excessive use of insecticides can have toxic effects on the male reproductive system. Deltamethrin and cypermethrin insecticides can inhibit androgen biosynthesis and disrupt the growth of sperm. Therefore, this research was conducted to determine the effect of deltamethrin and cypermethrin insecticides toward the level of intratesticular testosterone in male Wistar strain rats. This research was conducted in vivo using male Wistar strain rats. Nine rats were divided into three groups of treatment, namely control group, deltamethrin group with 0.26mg/kg of weight, and cypermethrin group with 0.26mg/kg of weight. Treatment was conducted every day for 15 days orally, and then the rats were dissected to take their testicles. Testicles were chopped and their intratesticular homogenates were taken by adding the medium of DMEM and collagenase (0.25mg/mL). The measurement of the level of testosterone was conducted by using the Electrochemiluminescence immunoassay (ECLIA) method. Data on the change of the increase of bodyweight, organ weight, and the level of testosterone was analyzed statistically using the one-way ANOVA test. The research result shows that giving 0.26mg/kg of weight of deltamethrin and 0.26mg/kg of weight of cypermethrin does not affect the increase in bodyweight, relative weight of the reproductive organ, and the level of intratesticular testosterone of male Wistar strain rats.

Keywords: cypermethrin, deltamethrin, intratesticular testosterone

INTRODUCTION

In agriculture, the occurrence of insect pests is often a very detrimental problem (Boadu *et al.*, 2011). Therefore, it is necessary to control the pests using insecticides. Pyrethroid insecticide is one type of insecticide that is commonly used in agriculture, plantations, and households. The use of pyrethroid insecticide has been increasing since the prohibition of the use of organochlorine, organophosphate, and carbamate insecticides. A pyrethroid is included in synthetic analog pyrethrin insecticide, a natural compound found in the dried extract of white chrysanthemum flowers (*Chrysanthemum cinerariae* folium). This type of insecticide has better efficacy toward insects than other types of insecticides, so it is used more in various countries (Nollet and Rathore, 2010). The excessive use of insecticides can cause problems, both for agriculture and human health. Residues of pyrethroid have been identified in food (Markovic *et al.*, 2010) or even in breast milk (Sereda *et al.*, 2009) and urine (Xia *et al.*, 2008). It is often reported that pyrethroid insecticide has a toxicity effect on the human reproduction system and disrupts its endocrine system. Andersen *et al.* (2012) report that pyrethroid insecticide has

Indonesian J Pharm 31(2), 2020, 116-123 | DOI: 10.14499/indonesianjpharm31iss2pp116 indonesianjpharm.farmasi.ugm.ac.id

estrogenic, androgenic, and aromatase effects. Pyrethroid insecticide also tends to disrupt the process of steroidogenesis (Saravanan *et al.*, 2008; Ahmad *et al.*, 2012). Therefore, exposure to an amount of pyrethroid may interfere with male fertility (Oliva *et al.*, 2001). Pyrethroid has an effect as an antagonist of androgen receptor (Xu *et al.*, 2006; Sun *et al.*, 2007), and it is proven that it disrupts androgen biosynthesis (Zhang *et al.*, 2007; Wang *et al.*, 2010). Pyrethroid may also disrupt estrogen (Fei *et al.*, 2010) and progesterone biosynthesis (Chen *et al.*, 2005). Deltamethrin and cypermethrin are pyrethroid insecticides commonly used in Indonesia and worldwide (Mutiatikum *et al.*, 2009). Pyrethroid insecticide, including deltamethrin and cypermethrin, has antiandrogenic effects in vitro (Du *et al.*, 2010; Fang *et al.*, 2013). Other research reports that deltamethrin and cypermethrin insecticides have effects that disrupt spermatogenesis (Fang *et al.*, 2013; Orlu, 2014). The spermatogenesis process in the body needs testosterone hormone, while pyrethroid can cause antiandrogenic effects by interfering with the biosynthesis and secretion of androgens in the body, including testosterone (Fang *et al.*, 2013). There have not been many research works related to the effect of deltamethrin and cypermethrin insecticides toward the level of intratesticular testosterone. This research aims to find out the comparison between giving deltamethrin and cypermethrin insecticides (at a dose which farmers use to control pest) toward the production of intratesticular testosterone of male Wistar strain rats.

MATERIAL AND METHODS

Preparation of tested materials

The test preparations were obtained in the form of *Emulsifiable Concentrate* (EC) or solid-liquid, in which each of them contains 25g/L of deltamethrin and 30g/L of cypermethrin (PT. Sari Kresna Kimia). The test preparation was diluted using distilled water to obtain a test stock solution with a concentration of 0.052mg/mL.

Classification and treatment of tested animals

Tested animals used were male Wistar strain rats aged 2.5–3 months with an average weight of 227.1±9.29g. The tested animals were acclimatized for 5–7 days. The use of animals in the research was approved by the ethics commission to conduct a preclinical research at the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, with the certificate number 06.04/IV/UN1/LPPT/2017. The tested animals were divided randomly into three groups consisting of 3 rats per group. Dosage of treatment refers to a research conducted by Abu-Aita and Yassa (2008), namely 0.26mg/kg of weight and the doses which the farmer uses for pesticide control. Group I: Normal control (without treatment); Group II: Deltamethrin treatment of EC with a dosage of 0.26mg/kg of weight; Group III: Cypermethrin treatment of EC with a dosage of 0.26mg/kg of weight.

The tested animals were given solution orally once a day for 15 days in a row based on the recommendation of *The United States Environmental Protection Agency* (US EPA) to find out the potential of the androgenic effect and antiandrogenic effect. It is proven that this method can be used to find out a compound that has the potential of disrupting the endocrine system (O'Connor *et al.*, 2002). The weighing of the tested animals was conducted periodically on days 1, 3, 5, 8, 11, and 15. On the 15th day, the tested animals were sacrificed 2h after being given the test compound, before conducting surgery and retrieving of the reproductive organ.

Retrieval of intratesticular homogenate

The tested animals fast for 10h before their testicles were taken. The rats were sacrificed in cervical dislocation. Abdominal surgery was carried out to retrieve the rats' reproductive organs. The reproductive organs taken were testicles, seminal vesicles, prostate gland, and epididymis. The testicular organs were put in a tube containing a transport medium of 5mL of *Phosphate Buffer Saline* (PBS). The weight of testicles was calculated from the difference between the tube weight containing a medium only and the tube weight containing medium and testicles. Other reproductive organs were weighed using watch glass. The method of retrieving intratesticular homogenate was conducted based on a research by Paramita (2014). The intratesticular homogenate was obtained by chopping testicles. Testicles were put in a Petri dish to be encapsulated. After then, the testicular tissue was mechanically chopped using a blade scalpel or scissors until it becomes smooth. The chopped result was added with 7mL of media of *Dulbelcco's Modified Eagle Medium* (DMEM) and collagenase (0.25mg/mL), and then it was chopped again until it becomes smooth suspension. The mixture was transferred into a new tube and stirred using a shaker water bath at a rate of 100–120rpm and a

temperature of 3°C for 40min. After then, filtering was conducted using four layers of sterile gauze to separate the seminiferous tubules. A fraction of the filtering result is centrifuged at a rate of 2000rpm for 10min. The supernatant was taken as a sample to measure the level of intratesticular testosterone. The sample was stored in a deep freezer at a temperature of -80°C until the time of measuring the level of testosterone.

The measurement of the level of intratesticular testosterone

The measurement of the level of testosterone was carried out in the Clinical Pathology Laboratory of Dr. Sardjito General Hospital using ECLIA (Elecsys® Testosterone II) method. The step of measuring testosterone was started by thawing the frozen sample before diluting it using the media of DMEM for four-time dilution. The procedure of preparation and measurement of the level of testosterone was based on the procedure on *insert kit* Elecsys® Testosterone II. The level of intratesticular testosterone obtained was then multiplied with the factor of dilution to get the actual level of intratesticular testosterone.

Data analysis

Quantitative data obtained was the level of intratesticular testosterone, the average weight increase per day and the average of the reproductive organ weight of each group. After then, data was tested statistically to determine whether there is a significant difference between the control group and the experimental group. The normal distribution test was conducted using *Shapiro-Wilk* and the test of variance homogeneity was carried out using *Levene.* Data has a normal distribution and homogeneity if $p>0.05$ and has abnormal distribution and inhomogeneity if $p\leq0.05$. Normal and homogeneous data was then analyzed using One-way ANOVA, continued by *Post-Hoc Test*, to determine the difference between the average values of the non-identical sample. Value $p>0.05$ shows that there was no significant difference in the average number of population (Sari, 2005; Dahlan, 2008).

RESULTS AND DISCUSSION

Research result

Parameter of the increase of bodyweight

The measurement of the increase of bodyweight was conducted to evaluate the health condition of the tested animals after being given the test material. The weighing of bodyweight was needed to calculate the percentage of the organ weight toward the bodyweight of the rats. The average increase in bodyweight per day for 15 days was obtained by calculating the difference between the bodyweight of the tested animals on the 15th day and on the 1st day, then by dividing the result by the number of treatments (15 days) (Table I). Increase in bodyweight resulting from giving deltamethrin and cypermethrin insecticides tends to be lower than in the control group (Table I). The result of the One-Way ANOVA test to the data of the increase of bodyweight shows a significant value of 0.646 ($p>0.05$). The result shows that there was no significant difference in the value of the increase in bodyweight between experimental groups. Therefore, it can be concluded that giving 0.26 mg/kg of weight of deltamethrin and 0.26mg/kg of weight of cypermethrin does not produce a significant effect on the increase of bodyweight of rats after 15 days.

Table I. The Average of the Increase of Bodyweight Per Day of Male Wistar Rats in Control Groups, 0.26mg/kg of Weight of Deltamethrin and 0.26mg/kg of Weight of Cypermethrin after a 15-day Treatment

Group	n	PKBP ± SEM (g/day)
Control	3	1.74±0.20
Deltamethrin 0.26 mg/kg of weight	3	1.59±0.50
Cypermethrin 0.26 mg/kg of weight	3	1.28±0.26

Information: There was no significant difference between experimental groups ($p>0.05$)

The reproductive organs whose weight was measured were testis, epididymis, seminal vesicles, and prostate gland. Seminiferous tubules inside the testicular organ can determine the increase in the testis' weight. The increase of the weight can be influenced by the content of spermatogenic cells inside the seminiferous tubules (Lea *et al.*, 2004). The weight of organs measured was the relative weight on the rats' weight to minimize the significant difference of various weights of absolute organs caused by several possibilities such as the swelling of organs.

The relative weight of the organs was measured by dividing the weight of organs by the weight of the rats on the 15th day, and it was written in percentage. The result of the One-way ANOVA test shows significant value obtained, that is,

Table II. The Average Value of the Relative Weight of Male Rats' Organ on the Control Groups, Experimental Group Using 0.26Mg/Kg of Deltamethrin and Experimental Group Using 0.26 Mg/Kg of Cypermethrin after a 15-Day Treatment

Group	n	The average value of the relative weight ± SEM (%)			
		Testis	Prostate Gland	Epididymis	Seminal Vesicle
Control	3	1.01±0.04	0.28±0.03	0.69±0.06	0.48±0.08
Deltamethrin 0.26 mg/kgBW	3	0.99±0.10	0.25±0.01	0.57±0.03	0.67±0.05
Cypermethrin 0.26 mg/kgBW	3	1.11±0.03	0.27±0.05	0.72±0.06	0.49±0.04

Information: There was no significant difference among experimental groups (p>0.05)

Table III. The average level of intratesticular testosterone of male Wistar rats after 15-day treatment

Group	The average level of intratesticular testosterone ± SEM (ng/mL)
Control (n=3)	38.61 ± 5.11
Deltamethrin 0.26 mg/kgBW (n=3)	46.35 ± 4.12
Cypermethrin 0.26 mg/kgBW (n=3)	27.88 ± 8.92

Information: there was no significant difference among experimental groups (p>0.05)

testicular organ (p=0.421), prostate gland (p=0.849), epididymis (p=0.177), and seminal vesicles (p=0.126). The value shows that the average value of the relative weight of organ for testicular organ, prostate gland, epididymis, and seminal vesicles does not have a significant value among the control groups, the experimental group using 0.26mg/kg of deltamethrin, and experimental group using 0.26mg/kg of cypermethrin.

The level of intratesticular testosterone

Testosterone is one of the most important androgens in male organs. Testosterone is generated by the Leydig cell inside the testis and it has several functions, namely having a role in sexual development and spermatogenesis process, generating anabolic proteins that are important for the growth process, and supporting the mechanism of feedback of LH pituitary (Guyton and Hall, 2006; Ye *et al.*, 2011; Kaiin *et al.*, 2013). LH located inside the Leydig cell will be related to the receptor of LH (LHR) and stimulate testosterone biosynthesis (Sharpe, 1984; Heffner and Schust, 2008). Testosterone and FSH will stimulate the Sertoli cell to support the spermatogenesis process (Sadler, 2012). Abnormality of testosterone biosynthesis can disrupt the spermatogenesis process.

Intratesticular testosterone was chosen due to its higher concentration than testosterone inside serum (about 30 times bigger) (Turner *et al.*, 1984). Furthermore, the level of intratesticular testosterone is also closely related to the function of the Leydig cell which is responsible for maintaining the biosynthesis process of sexual hormones inside the testis (Guyton & Hall, 2006). Thus, the measurement of the level of intratesticular testosterone may directly describe the influence of the exposure of test material toward the production of endogen testosterone.

Table III shows the result of measurement of the level of intratesticular testosterone of male Wistar rats that have been given 0.26mg/kg of the weight of deltamethrin and 0.26mg/kg of the weight of cypermethrin orally for 15 days. Based on the result, cypermethrin insecticide has a lower level of intratesticular testosterone compared to deltamethrin insecticide. Based on the statistical calculation, data of the level of intratesticular testosterone was distributed normally with a value of 0.738 and a homogeneity of p=0.352. The result of One-way ANOVA test shows that the data of the level of intratesticular testosterone has significant value as much as 0.203 (p>0.05). The value shows that the level of intratesticular testosterone of male Wistar rats between the control group and the experimental group using deltamethrin and cypermethrin was not significantly different. Deltamethrin and cypermethrin insecticides are included in pyrethroid, which is commonly used in Indonesia and around the world (Mutiatikum *et al.*, 2002).

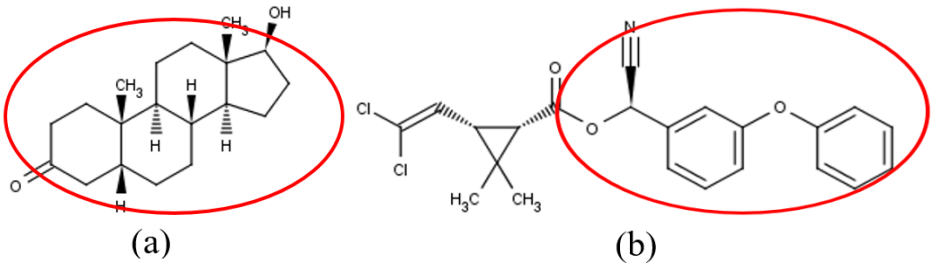

Figure 1. Structure of steroid hormone (a) and structure of pyrethroid (b) (Ujiantari *et al.*, 2016) Information: red circle (⬭) shows structure similarity in the part of pyrethroid alcohol with reproductive hormone (steroid)

Based on the previous research, pyrethroid insecticides, including deltamethrin and cypermethrin, have a toxic effect on the male and female reproductive systems. On males, a high dosage of deltamethrin and cypermethrin insecticides have activity inhibiting the spermatogenesis process (Fang *et al.*, 2013; Orlu, 2014). Pyrethroid compound, including deltamethrin and cypermethrin, has an antiandrogenic effect that can result in the inhibition of the spermatogenesis process. Pyrethroid can cause an antiandrogenic effect by disrupting biosynthesis and secretion of androgen inside the body (Du *et al.*, 2010; Fang *et al.*, 2013).

The process of sperm formation inside the body needs testosterone. Thus, if biosynthesis and secretion of testosterone are inhibited, the spermatogenesis process will be inhibited too. A pyrethroid compound can result in an antiandrogenic effect due to the structural similarity to the reproductive hormone (Figure 1), so pyrethroid can fill the binding site on the receptor of reproduction such as androgen receptor. It enables pyrethroid, which has an affinity to bind with the androgen receptor. The stricture similarity is located in the part of alcohol of pyrethroid compound, especially diphenyl ether cluster, cyano cluster, and carbonyl cluster. Those clusters, in silico, can bind the residue of amino acid, which is important for binding reproductive hormones in the receptor of reproduction (Ujiantari *et al.*, 2016).

Average increase in bodyweight per day and the organ weight in this research shows insignificant result between the experimental group and the control group. The result was in line with a research by Fang *et al.* (2013) stating that giving pyrethroid insecticide (cypermethrin) with a dosage of 7.5, 15, 30, and 60 mg.kg of weight for 15 days does not give effect to the increase of bodyweight and reproductive organ weight. However, Fang *et al.* (2013) continued the research by examining the histopathology of the reproductive organ, and it was found that there were atrophy and distortion on the seminiferous tubular organ in an experimental group of 30 and 60 mg/kg of weight. Furthermore, there was also damage and a decrease in the number of Sertoli and Leydig cells. Thus, the histopathology examination on the organ can show the effect of giving a pyrethroid compound on the reproductive organ more clearly.

The insignificant result of bodyweight per day and reproductive organ weight in this research was caused by several possibilities such as the short duration of exposure, low amount of dosage, and variation of the tested animals. It can be proven by comparing the result of this research to other research. A research conducted by Orlu (2014) states that giving deltamethrin at the dosage of 25mg/kg of weight for 35 days can decrease the rats' bodyweight and reproductive organ weight (testis, prostate gland, epididymis, and seminal vesicle) significantly compared to the control groups. Orlu (2014) continues the research through a histological examination of the testis. In the examination, giving deltamethrin at the dosage of 25mg/kg of weight can lose primary spermatocyte on seminiferous tubules and spermatozoa in the lumen. Other research states that giving deltamethrin formulated by EC at the dosage of 0.6mg/kg of weight for 60 days to Wistar rats can decrease the testis weight and epididymis. Histopathologically, giving deltamethrin can result in the damage of seminiferous tubules, a decrease in the number of spermatozoa in epididymis lumen, and the occurrence of congestion and edema on the prostate gland (Oda and El-Maddawy, 2012).

Giving cypermethrin at the dosage of 18.93 and 39.66 mg/rat/day for 12 weeks can decrease the rats' bodyweight significantly, while the weight of testis and seminal vesicle becomes higher than that of the control group (Elbetieha *et al.*, 2001). In this research, the relative weight of the seminal vesicle organ was higher in the experimental group compared to the control group. According to Elbetieha *et al.* (2001), the increase in the production of androgen can result in an increase in the relative weight of seminal vesicle organ. It can be correlated with the result of the level of intratesticular testosterone. The experimental group of deltamethrin has higher levels of intratesticular testosterone and relative weight of seminal vesicle organ compared to other experimental groups despite of insignificant difference.

Table III shows that the testosterone level resulting from giving cypermethrin tends to be lower compared to that of the control groups. Meanwhile, the result of giving deltamethrin was higher in the control group. Based on the result, compared to deltamethrin, cypermethrin seemingly affects the decrease of the production of intratesticular testosterone in rats. This result was not in line with a research by Du *et al.* (2010) stating that pyrethroid compound with the side group in the form of bromine has an affinity toward androgen receptor higher than pyrethroid insecticide compound with the side group of chlorine. Deltamethrin structure has a bromine cluster, while cypermethrin has chlorine structure, so the antiandrogenic effect of deltamethrin is higher than cypermethrin. The antiandrogenic effect of the pyrethroid compound can occur indicated by the disruption of biosynthesis and androgen secretion in the testis. However, the result of an in-vitro test was not necessarily the same as the result of an in-vivo test because there are other factors on the in vivo test that can evaluate the effect of a compound inside the body, such as metabolism and compound distribution inside the body (Fang *et al.,* 2013).

In this research, deltamethrin and cypermethrin treatments at the dosage of 0.26mg/kg of weight for 15 days (a dose which the farmers use to control pest) was not enough to decrease the level of intratesticular testosterone level as what has been reported by the previous research. The insignificant result of intratesticular testosterone levels can be caused by several possibilities such as the duration of exposure and the dosage used. It is supported by other researches; one of them was conducted by Issam *et al.* (2009) stating that the effect of deltamethrin insecticide toward the reproduction system depends on the dosage used and the duration of exposure. Andrade *et al.* (2002) state that giving deltamethrin either in the pure form or formulation of emulsifiable concentrate at the dosage of 2 and 4 mg/kg of weight for three days (Hershberger method) does not show antiandrogenic activity in male rats. Other research shows that giving another pyrethroid, lambda-cyhalothrin, at the dosage of 63 and 100mg/kg of weight orally for seven days does not have any effect on the fertility of male mice (Ratnasooriya *et al.*, 2002). The result can be different compared to other research using a higher dosage and a longer duration of exposure.

A research conducted by Issam *et al.* (2009) clarifies that giving deltamethrin subcutaneously at the multiple dosages (2ppm for 30days; 20ppm for 45days; and 200ppm for 60days) shows that there was a decrease of testosterone level in the plasma after giving deltamethrin at the dosage of 200 ppm for 60 days. Giving deltamethrin orally at the higher dosage (0.6mg/kg of weight) and longer duration of exposure (60 days) can decrease the testosterone level in the serum (Oda and El-Maddawy, 2012). Other research states that giving cypermethrin orally at the dosage of 25mg/kg of weight for 35 days can decrease the level of serum testosterone and intratesticular testosterone in male mice (Wang *et al.*, 2009).

Another possibility that can influence the production of testosterone is the metabolism compound factor given. There is a long process of metabolism in the test compound given orally, starting from the digestive tract until the systemic tract, so the concentration of the compound decreases inside the body, and its metabolites will change. Therefore, the effect will possibly decrease before reaching the target. Deltamethrin compound inside the rats' body will experience metabolism to become its metabolites, namely 3-(2.2-*dibromoethenyl*)-2.2-*dimethylcyclopropane carboxylic acid*, and 3-*phenoxybenzoic acid* (El-Maghraby, 2007). Meanwhile, the cypermethrin compound also experiences to become its metabolites, namely 3-(2.2-*dichlorovinyl*)-2.2-*dimethylcyclopropanecarboxylic acid* (DCVA), 3-*phenoxybenzoic acid* (3PBA), and 3-(4'-*hydroxyphenoxy*) *benzoic acid* (Woollen *et al.*, 1992). The compound of 3-*phenoxybenzoic acid* is a general metabolite of pyrethroid insecticide that has an antagonistic effect on the androgen receptor. However, the effect is still lower than its active

compounds (deltamethrin and cypermethrin) (Du *et al.*, 2010), which can cause an insignificant decrease in the production of testosterone.

Giving pyrethroid insecticides at a higher dosage and longer duration of exposure will possibly decrease the production of testosterone inside the body significantly due to the accumulation of pyrethroid compounds and metabolites. A research by Meeker *et al.* (2008) explains that there was an increase of metabolite compound of 3-*phenoxybenzoic acid* on the patient's urine with low sperm concentration. Thus, the accumulation of pyrethroid compounds and metabolites inside the body will cause a toxic effect in the reproductive system. Moreover, various results caused by the dosage and the duration of exposure in several research works of deltamethrin and cypermethrin. In this research, deltamethrin and cypermethrin treatments at the dosage of 0.26mg/kg of weight for 15 days (a dose which the farmers use to control pest) was not enough to decrease the level of intratesticular testosterone level.

CONCLUSION

Giving cypermethrin and deltamethrin at the dosage of 0.26mg/kg of weight for 15 days was not enough to decrease the level of intratesticular testosterone significantly compared to that in the control group.

ACKNOWLEDGEMENT

We thank to Physiology Laboratory Faculty of Medicine, Public Health and Nursing UGM and Clinical Pathology Laboratory of Dr. Sardjito General Hospital.

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