ORIGINAL ARTICLE:

Effect of per oral sipermetrin exposure on serum 17β estradiol and uterine malondialdehyde (MDA) levels in female Wistar strain rats (*Rattus norvegicus*)

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ABSTRACT

Objective: This study aimed to verify the effect of oral sipermetrin exposure to decrease serum estradiol 17β levels and increased malondialdehyde (MDA) in the uterus level of female Wistar strain rats (*Rattus norvegicus*).
Materials and Methods: The method of this study was true experimental post test only control group in vivo using 24 female rats, divided into 3 groups treated by administering a dose of 5, 10 and 20 mg/kg sipermetrin for 28 days and one control group. Then blood samples were taken from the heart for measurement of serum estradiol 17β levels by ELISA and uterine organs were taken for measurement of Malondialdehyde (MDA) with spectrophotometry method.
Results: The results of the measurement of serum estradiol 17β and uterus malondialdehyde (MDA) levels of female Wistar strain rats (*Rattus norvegicus*) showed an opposite pattern, where there was a decline in serum estradiol 17β levels and an increase in uterus malondialdehyde (MDA) level. There was a significant difference (p=0.000<alpha) in 17β estradiol serum and uterus Malondialdehyde (MDA) levels of female rats between control group and group exposed to sipermetrin treatment for 28 days.
Conclusions: Oral sipermetrin exposure can decrease serum levels of estradiol 17β and increase uterine levels of malondialdehyde (MDA) of female Wistar strain rats (*Rattus norvegicus*).

Keywords: Sipermetrin; 17β estradiol serum; malondialdehyde (MDA).

ABSTRAK

Tujuan: Membuktikan pengaruh paparan sipermetrin per oral terhadap penurunan kadar 17β estradiol serum dan peningkatan kadar Malondialdehyde (MDA) uterus tikus betina galur wistar (*Rattus norvegicus*).
Bahan dan Metode: True Experimental Post-Test Only Control Group design secara in vivo menggunakan 24 ekor tikus yang dibagi menjadi 3 kelompok perlakuan dengan pemberian sipermetrin dosis 5, 10 dan 20 mg/kg BB selama 28 hari dan 1 kelompok kontrol. Kemudian dilakukan pengambilan sampel dari darah jantung untuk pengukuran kadar 17β estradiol serum dengan metode ELISA dan organ uterus diambil untuk pengukuran kadar Malondialdehyde (MDA) dengan metode spektrofotometri.
Hasil: Kadar 17β estradiol serum dan kadar malondialdehyde (MDA) uterus tikus betina galur wistar (*Rattus norvegicus*) menunjukkan pola yang berlawanan yaitu terjadi penurunan kadar 17β estradiol serum sedangkan kadar malondialdehyde (MDA) uterus terjadi peningkatan. Ada perbedaan yang bermakna (p= 0.000<α) pada kadar 17β estradiol serum dan kadar malondialdehyde (MDA) uterus tikus betina antara kelompok kontrol dengan kelompok perlakuan yang dipapar sipermetrin selama 28 hari.
Simpulan: Paparan sipermerin per oral dapat menurunkan kadar 17β estradiol serum dan meningkatkan kadar Malondialdehyde (MDA) uterus tikus betina galur wistar (*Rattus norvegicus*).

Kata kunci: Sipermetrin; 17β estradiol serum; malondialdehyde (MDA)

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pISSN:0854-0381 ● eISSN: 2598-1013 ● doi: http://dx.doi.org/10.20473/mog.V26I12018.20-25
● Maj Obs Gin. 2018;26:20-25 ● Received 10 Jan 2018 ● Accepted 7 Mar 2018

INTRODUCTION

Pesticides are chemicals used to eradicate pest organisms, which can also have a negative impact on human health.[1] Pesticides that can interfere with hormonal function are called endocrine disrupting chemicals (EDCs). EDCs can increase (agonist) or inhibit (antagonist) action of endogenous hormones. Some pesticides have potential as endocrine disruptors and the female reproductive system may be considered a sensitive target.[2] There are 3 million cases of poisoning per year due to exposure to pesticides. From these data there are 5,000 - 10,000 cases that are fatal because they cause cancer, develop malformations, infertility, and even 250,000 end in death.[3]

Pyrethroid is a synthetic insecticide with a chemical structure based on natural pyrethrins found in the cinnamon flower of cineraraefolum and is widely used in the market because it has very low toxic properties against mammals compared to other types of pesticides.[4] In some studies, pyrethroid is suspected to be endocrine disruptors and many endocrine disruptors known to be estrogenic may affect reproductive function.[6,7] Sipermetrin is a member of the synthetic pyrethroid, belonging to the class II pyrethroid type, and is widely used in agriculture as well as other applications.[8] Sipermetrin exhibits an estrogenic effect in some in vitro experiments.[9] Estrogenic chemicals are chemicals responsible for directly activating or inhibiting estrogen action and indirectly modulating its action.

Estrogen is a hormone that plays a role in menstrual cycle and female reproduction. Hormone 17β estradiol (E2) is the most effective estrogen and has a function as a regulator of body homeostasis, both in reproductive and non-reproductive tissues.[10] Review of the journal by Bretveld et al. (2006) about exposure to pesticides explains that endocrine disruptors that mimic hormones 17β estradiol can bind and activate estrogen receptors. The possible effects are the decreased production of Gonadothropin Releasing Hormone (GnRH) by the hypothalamus (negative feedback system) and LH and FSH by the pituitary gland. As a result, LH and FSH levels will drop and eventually cause estradiol to decrease.[11]

Estrogen itself is an antioxidant and estrogen deficiency has been linked to oxidative stress.[12] Oxidative stress occurs when there is an imbalance of oxidant and antioxidant ratios.[13] Giray et al. (2000) suggest that oral exposure to sipermetrine causes significant oxidative stress in brain tissue and rat liver.[14] Sipermetrin may affect reproductive-related steroids, and the toxicity of cutaneous hemethrin can be partially mediated by oxidative stress.[15]

Sipermetrine and other pyrethroids that are metabolized in the liver produce reactive oxygen species (ROS) responsible for oxidative stress in mammals. ROS directly reacts with biomolecule cells, causing lipid peroxidation, protein damage and DNA8. Lipid peroxidation is derived from an unstable fatty acid and readily decomposes to form a series of complex compounds, including Malondialdehyde (MDA). MDA is a major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress.[16] This study aims to identify the effect of oral sipermetrin exposure on decreased levels of 17β serum estradiol and elevated levels of uterine Malondialdehyde (MDA) in female Wistar rats (*Rattus norvegicus*).

MATERIALS AND METHODS

The design used in this study was true experimental with post test only control group design. The study was conducted in Physiology Laboratory and Biomedical Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia, from June to July 2017. The experimental protocol was approved by the Ethics Committee, Faculty of Medicine, Brawijaya University of Malang.

This study used healthy and non-pregnant Wistar rats, ages 10-12 weeks, weight 100-150 gr. A total of 24 rats were divided into four groups: 1 control group and 3 treatment groups, each with 6 rats. Three treatment groups received sipermetrin diluted with distilled water at doses of 10, 15 and 20 mg/kgBW per day with sonde for 28 days, while the control group did not deceive sipermetrin. All experimental animals from all four groups were fed and drank ad libitum. On day 29 of the exposure, vaginal swab was performed to determine the estrus cycle. Rats in proestrus phase were operated, one by one until all rats were sacrificed. The animals were sacrificed by anesthesia using 1% ketamine intramuscularly (IM) on the thigh in a dose of 0.2 ml.

The measurement of serum 17β estradiol level was performed by using blood from the rats' heart which was taken as much as ± 3 cc, centrifugated, and the serum was obtained. The measurement of serum 17β estradiol was performed by ELISA method using a Cusabio kit with catalogue no. CSB-E05110r. The measurement of uterine MDA levels was performed using the uterine organ. The uterus weighing ± 100 mg was taken and ground. Uterine malondialdehyde (MDA) levels were measured using spectrophotometric method using a Northwest kit with catalogue no. NWK-MDA01. The results were expressed as mean and standard deviation (SD). Data were tested using One Way ANOVA test,

followed by LSD (Least Square Differences) post-hoc test and Path analysis using SPSS version 22.0 (SPSS Inc, IBM). Convidence interval was determined 95% and expressed significantly when the p-value <0.05.

RESULTS AND DISCUSSION

Serum 17β-estradiol level

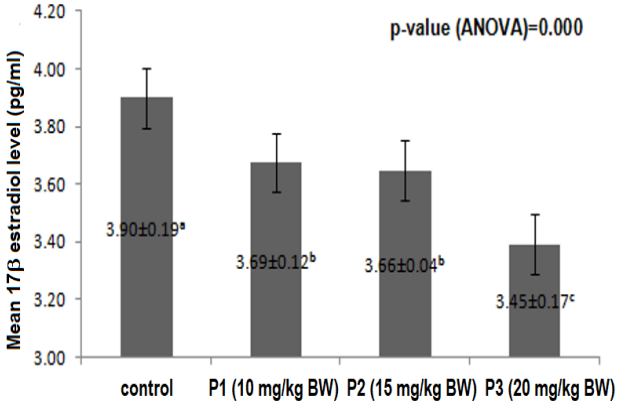

Figure 1. Serum 17β-estradiol levels after oral sipermetrin exposure at doses of 10, 15, 20 mg/kg BW for 28 days. Serum 17β-estradiol levels in treatment group were lower than that in control group, the difference was significant (p=0.000<β).

Figure 1 shows that the mean value of 17β-estradiol levels in all four groups of samples have a significant difference (p=0.000<α). In addition, mean levels of 17β-estradiol decreased in the treatment group, respectively, along with the increased dose of sipermetrin administered.

Uterine malondialdehyde (MDA) levels

The effect of sipermetrin on malondialdehyde (MDA) level of female rats in the treatment group receiving the doses of 10, 15, 20 mg/kgBW can be seen in Figure 2. The results showed that the mean in the treatment group was significantly higher than those in control group (p-value = 0.000<α). Figure 2 also shows increased MDA levels along with the addition of sipermetrin dose.

Effect of 17β-estradiol level on uterine MDA level

The results of path analysis on the effect of 17-estradiol levels on female Wistar strain rats (*Rattus norvegicus*) uterus receiving sipermetrin were described and presented briefly in Table 1.

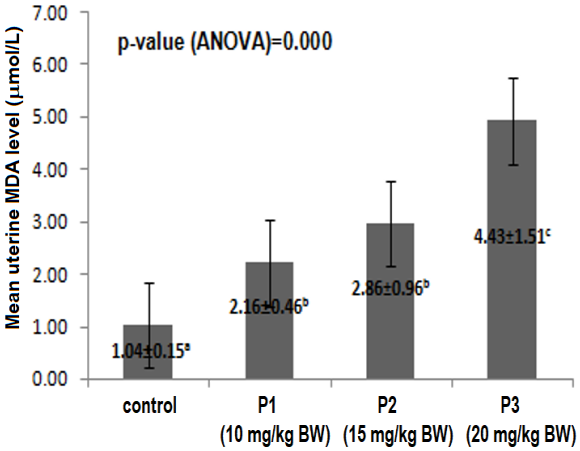

Figure 2. Uterine malondialdehyde (MDA) level after oral sipermetrin exposure in doses of 10, 15, 20 mg/kgBW for 28 days. Uterine MDA levels in the treatment group were higher than that in control group, significantly different (p=0.000<α).

Table 1. Path analysis results of the effect of 17β - estradiol on uterine MDA levels

Regression equation model	p-value
$\hat{y}$= - 0.922 x (p=0.000) (p=0.000)	0.000 <α

Table 1 shows that the effect of 17-estradiol levels on uterine MDA level was statistically significant (p-value=0.000<α) after sipermetrin administration to the female rats. The negative value on the coefficient of influence (-0.922) indicated opposite effect. Decreased levels of 17β-estradiol resulted in elevated uterine MDA levels.

This study proved the presence of sipermetrin exposure to decreased 17β-estradiol level and increased uterine MDA levels of female Wistar strain rats. Sipermetrin is one of the pyrethroids that have similar metabolic pathways in mammals and produces metabolite chemistry similar to 17-estradiol.[17] The decrease in 17β-estradiol level in the treatment group along with the addition of sipermetrin dose is because sipermetrin is able to bind to estrogen receptors that affect the hypothalamus in hormone synthesis.

This is in accordance with a study by Saravanan et al. (2016) which states that exposure to sipermetrin lowers 17β-estradiol level in the brain of the fish E. danricus. The brain plays an important role in the initiation and coordination of reproductive function. The hypothalamus is a direct target tissue for estradiol (E2). Decreased 17β-estradiol level may be because sipermetrin acts directly through the neural pathways to reduce neurosteroids or acts through reproductive interactions.[18] This also corresponds to a study by Singh et al. (2008) who concluded that sipermetrin causes endocrine system disruption by affecting steroidogenesis via the hypothalamus-pituitary-gonadal axis.[19]

The classical role of estrogen in mammalian brain is to provide positive and negative feedback action on hypothalamic-pituitary axis to regulate female repro-ductive cycle. At the hypothalamic level, estrogen acts on a gonadothropin-releasing hormone (GnRH) cell that includes GnRH neuron output and presinaptic neurons (eg, GABA, glutamate, β-endorphin neurons). Estrogen acts as a homeostatic feedback between the gonads and the hypothalamus that dictate the biosynthesis and secretion of GnRH neuronal activity. In some of the ovarian cycles, estrogen retains the secretion of luteinizing hormone (LH) through a negative feedback action. This occurs partly through the inhibition of GnRH and E2 secretions whose effects are mediated directly on GnRH neurons and indirectly through GABA and interneurone opioids.[20]

Sipermetrin can effectively suppress the opening of voltage-gated chloride channels (VGCC) and inhibit GABA. GABA neurotransmitter is one of the most dominating neurotransmitters that regulate chloride channels in the brain.[21] This study proved that sipermetrin was able to act as an endocrine disruptor mediated by neurotoxicity and interaction with estrogen receptors so as to influence the reproductive hormone by decreasing serum 17β-estradiol level in female rats (*Rattus novergicus*).

Increased levels of malondialdehyde (MDA) after exposure of sipermetrin for 28 days could be due to excessive ROS formation that resulted in oxidative stress. This is in accordance with a study by Muthuviveganandavel et al (2008). Male rats exposed to sipermetrin in different doses have increased malondialdehyde (MDA) content in the brain, heart, liver, kidneys and testes.[22] A study by Banke et al (2014) showed that MDA concentrations in the brain increased after exposure to a mixture of sipermethrin and clorfirifos for 12 weeks.[23]

Malondialdehyde (MDA) is a lipid peroxidation product resulting from the reaction of oxygen radicals with polyunsaturated fatty acid residues in phospholipid membrane, and shows the presence of protein and DNA damage.[23] The main source of MDA in biological samples is the peroxidation of polyunsaturated fatty acids with two or more methylene double binds. MDA is produced by lipid peroxidation and is considered an indicator of oxidative stress.[24]

Several studies have shown that sipermetrin damages the brain, liver, kidneys, and erythrocytes by causing oxidative stress.[8] During metabolism, sipermetrin forms cyanohydrine, further decomposing it into cyanides and aldehydes, substances that can induce the production of reactive oxygen species (ROS).[25] If ROS accumulates and oxidative stress occurs, proteins, lipids, and DNA can be damaged.[26]

This study showed that the administration of oral sipermetrin at doses of 10 mg/kg BW, 15 mg kg BW and 20 mg/kg BW appeared to increase uterine MDA levels of wistar female rats (*Rattus novergicus*) as dose increased. It can be inferred that sipermetrin acts as a xenobiotic or foreign substance that enters the body and created an abundance of ROS and eventually oxidative stress that can be seen from elevated levels of MDA. This study also proved the presence of opposite effects of 17β-estradiol levels against MDA levels of the uterus. A decrease 17β-estradiol level results in elevated uterine MDA levels.

This is in line with the theory of Nazrun et al (2008) that estrogen is an antioxidant with radical scavenging properties and its deficiency has been associated with oxidative stress.[12] Stirone et al (2005) also mentions that estradiol (E2) modulates mitochondrial function in blood vessels. Mitochondria are the main source of ROS in cells.[27] Excessive ROS causes interaction with lipids, proteins and nucleic acids, resulting in loss of membrane integrity, structural or functional changes in protein, and damage to nucleic acids.[28]

DNA damage in oxidative stress process is also caused by the destruction of the unsaturated fatty acids of the membrane, leading to the formation of lipid hydroperoxides. The existence of certain metals will stimulate the production chain with more free radicals and causes a phenomenon known as lipoperoxidation. One way to determine the membrane lipid peroxidation index is by measuring the peroxidation end product, such as malondialdehyde (MDA).[29]

CONCLUSION

Oral sipermetrin exposure significantly decreased the levels of 17β-estradiol and increased uterine MDA levels in female wistar strain rats. There is an opposite effect between the levels of 17β-estradiol and uterine MDA levels.

REFERENCES

1. Suhartono. Dampak Pestisida Terhadap Ke-sehatan. Proceeding. Seminar Nasional Pertanian Organik. 2014.
2. Sangha GK, Kaur K, Khera KS 2013. Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats. Journal of Environmental Biology. 2013.
3. Halim. Bahaya pestisida terhadap kesuburan. [cited 2017 Jan 24]. Available from: http://harian.analisadaily.com/kesehatan/news/bahaya-pestisida-terhadap-kesuburan/ 300134/2017/01/16
4. Saillenfait AM, Ndiaye D, Sabaté JP. Pyrethroids: Exposure and health effects. An update. International Journal of Hygiene and Environmental Health. 2015;218:281–92
5. Purba IG. Analisis Faktor-Faktor Yang Berhubungan dengan Kadar Kolinesterase pada Perempuan Usia Subur di Daerah Pertanian. Thesis. Semarang: Diponegoro University; 2009.
6. Sun H, Chen W, Xuc X et al. Pyrethroid and their metabolite, 3-phenoxybenzoicacid showed similar (anti) estrogenic activity inhuman and rat estrogen receptor β-mediated reporter gene assays. Environmental Toxicology and Pharmacology; 37:371–377
7. Porte C, Janer G, Lorusso LC, et al. Endocrine disruptors in marine organisms: Approaches and perspectives. Comparative Biochemistry and Physiology Part C. 2006;143:303–15.
8. Sankar P, Telang AG, Manimaran A. Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Experimental and Toxicologic Pathology. 2012;64:487–93.
9. Guo D, Wang Y, Qian Y, et al. Joint acute and endocrine disruptive toxicities of malathion, cypermethrin and prochloraz to embryo-larval zebrafish, Chemosphere. 2016; 166:63-71
10. Kiyama R and Wada-Kiyama Y. Estrogenic endocrine disruptors: Molecular mechanisms of action. Environment International. 2015;83:11–40
11. Bretveld RW, Thomas CMG, Scheepers PTJ et al. Pesticide exposure: the hormonal function of the female reproductive system disrupted? Reproductive Biology and Endocrinology. 2006; 4:30
12. Nazrun AS, Khairunnur A, Norliza M, et al. Effects of palm tocotrienols on oxidative stress and bone strength in ovariectomised rats. Med & Health. 2008;3(2):247-55.
13. Khazri A, Sellami B, Dellali M et al. 2015. Acute toxicity of cypermethrin on the fresh water mussel Unio gibbu. Ecotoxicology and Environmental Safety. 2015;115:62–6.
14. Giray B, Gurbay A, Hincal F. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by Vitamin E or allopurinol. Toxicology Letters. 2001;118:139–46.
15. Khatab AE., Hashem NM, El-Kodary LM et al. Evaluation of the effects of cypermethrin on female reproductive function by using rabbit model and of the protective role of chinese propolis. Biomedical Environmental Science. 2016;29(10):762-66.
16. Singh Z, Karthigesu IP, Singh PKR. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: A review. Iranian J Publ Health. 2014;43(Suppl. 3): 7-16.
17. McCarthy ARM, Thomson B, Shaw IC, Abella AD. Estrogenicity of pyrethroid insecticide metabolites. Journal of Environmental Monitoring. 2005.
18. Saravanan N, Uma T, Narendiran NJ. Effect of cypermethrin on estradiol and vitellogenin in the tissues of Esomus danricus. Journal of Entomology and Zoology Studies. 2016;4(5):524-28.
19. Singh PB and Singh V. Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17b and 11-ketotestosterone, and sperm motility in Heteropneustes fossilis (Bloch). Chemosphere. 2008. 72:422–31.
20. Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen signaling in the hypothalamus, vitamins and hormones. Vitam Horm. 2005;71:123-45.
21. Singh AK, Nath T, Manindra OP, Singh MP. 2012. A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. Current Neuropharmacology. 2012;10:64-71.
22. Muthuviveganandavel V, Muthuraman P, Muthu S, Srikumar K. 2008. A study on low dose cypermethrin induced histopathology, lipid peroxidation and marker enzyme changes in male rat. Pesticide Biochemistry and Physiology. 2008; 91:12–6.
23. Banke IS, Folorunsho AS, Mohammed B, et al. Effects of melatonin on changes in cognitive performances and brain malondialdehyde concentration induced by sub-chronic coadministration of chlorpyrifos and cypermethrin in male Wister rats. Asian Pac J Trop Biomed 2014;4(4):318-23.
24. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005;15(4):316-28.
25. Idris SB, Ambali SF, Ayo JO. Cytotoxicity of chlopyrifos and cypermethrin: The ameliorative

effects of antioxidants. African Journal of Biotechnology. 2012;11(99):16461-7.
26. Yuan L, Dietrich AK, Nardulli AM. 17b-Estradiol alters oxidative stress response protein expression and oxidative damage in the uterus. Molecular and Cellular Endocrinology. 2014;382(1):218-26.
27. Stirone C, Duckles SP, Krause DN, Procaccio V. Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. Molecular Pharmacology. 2005;68(4).
28. Tamura H, Takasaki A, Taketani T, et al. 2012. The role of melatonin as an antioxidant in the follicle. J Ovarian Res. 2012;26(5):5.
29. Gomez-Zubeldia M, Hinchado AG, Arbue´s JJ, et al. Influence of estradiol on oxidative stress in the castrated rat uterus. Gynecologic Oncology. 2001; 80:227–32.