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THE ORGANOPHOSPHATE – PIRIMIPHOS-METHYL ALTERS ESTROUS CYCLE AND HORMONE CONCENTRATIONS IN FEMALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Aim: Pirimiphos methyl is an organophosphate (OP) which has widespread agricultural and domestic use against pests of stored grain and various insects. Apart from their intended effects, pesticides have been linked with adverse effects on the environment and non-target organism. The aim of this study was to evaluate the effect of exposure of sublethal doses of pirimiphos methyl (PM) on estrous cycle and hormonal changes in female rats. **Methods:** Cycling rats were divided into 4 groups. Group A received distilled water (control), groups B-D received PM at a dose of 10mg/kg, 60mg/kg and 120mg/kg respectively for 21days. The pattern of estrous cycle during the administration was studied. Blood samples collected at the various phases of estrous cycle were assayed using enzyme-linked-immunosorbent serologic assay (ELISA) techniques. The fimbriated part of the oviduct was dissected out from the rats in estrus, suspended in normal saline and placed on a microscope slide with a cover slip to count the number of ova in the oviduct under a dissecting microscope. **Results:** Rats treated with PM exhibited an extended estrous cycle with a significant decrease ($P<0.05$) in the proestrus and estrus and a significant increase in metestrus phases of the cycle. There was a disruption in the level of secretion of LH, FSH, Estradiol and Progesterone during the different phases of the estrous cycle in female rats treated with PM. **Conclusion:** These results indicate that PM has induced disturbances in reproductive homeostasis as indicated by altered serum hormone levels and ovarian cycle irregularities.

KEYWORDS: Pirimiphos-methyl, ovulation, estrous cycle, pesticide, rat.

INTRODUCTION

There has been global concern over the years about potential deleterious effects that may result from exposure to chemicals that have the potential of interfering with the endocrine system. Pesticides are among these class of chemicals.

Pesticide exposure is a global public health issue. Pesticides have played vital role in controlling agricultural, industrial, home and public health pest worldwide.[1] However, the use of pesticides poses animal and human health concerns because of their toxicity, widespread use and release into the environment.[2]

Various pesticides have been considered endocrine disruptors because they have the ability to block or activate hormone receptors as well as affect sex hormone levels.[3]

Pirimiphos-methyl (O,2, diethylamino-6-methylpirimidin-4-yl O,O-dimethyl phosphorothioate) is a broad spectrum, non-cumulative organophosphate pesticide which is active against ants, aphids, beetles, caterpillars, cockroaches, fleas, flies (including houseflies, Drosophila, and biting flies), mites, mosquitoes, moths, thrips, whiteflies and scales.[4] Pirimiphos-methyl, a cheap pesticides widely used to protect food against pests as well as in houseold applications. The use of pesticides could affect non-target organisms and wildlife in the ecosystem and ultimately human health leading to various effects like immunotoxicity, carcinogenesis, endocrine and developmental toxicity.

The female reproductive cycle in rats known as estrous cycle lasts 4 or 5 days and is divided into four stages (proestrus, estrus, metestrus and diestrus) which is characterized by changes in vaginal cytology and hormone levels.[5] Interference of pesticides with the reproductive function of the female rat is commonly expressed as a disturbance in the duration of particular phases of the estrous cycle[6] which is under the

hormonal control through the hypothalamic - pituitary-gonadal axis that regulates its various functions.

Though very effective as an insecticide, this compound has the probability to affect non-target organisms and wildlife and ultimately human health. Previous report has shown that PM by oral route disrupted spermatogenesis and reduced male fertility but there is a dearth of information on studies of the female reproductive functions in PM exposed rats.[7] Hence, this study was undertaken to assess the changes in the estrous cycle and hormonal concentrations of female rats exposed to sublethal doses of pirimiphos-methyl.

MATERIALS AND METHODS

Animals

Sexually matured female rats (160–200 g) of the Sprague–Dawley strain were obtained from the Animal House of the College of Medicine University of Lagos. The rats were acclimatized for 2 weeks under standard conditions of temperature and illumination (12 hours dark: 12 hours light) cycle. They were fed commercially available rat's pellets (Ladokun feeds, Ibadan) and had access to drinking water *ad libitum.* The rats were divided into four (4) treatment groups as described below:

Group A - Distilled water (control),
Group B - 10mg/kg of pirimiphos-methyl, (~0.8% LD50)
Group C - 60mg/kg of pirimiphos-methyl, (~5% LD50)
Group D - 20mg/kg of pirimiphos-methyl, (~10% LD50).

Chemical

An OP pesticide, pirimiphos-methyl (Emulsifiable concentrate, 250 g/L, ACTELLIC, Batch no: CHL5G11-06, Syngenta, UK) with the chemical name, O-2-dimethylamino-6-methyl-4-pyrmidinyl O-O-dimethylphosphorothioate, C11H20N3O3PS, was utilized for the study. PM was administered orally for 21days.

Estrous monitoring and ova count

Female rats that have undergone three successive four-day cycle were used for the study. Daily vaginal smears were performed every morning to monitor the pattern and duration of the estrous cycle.[8] Rats on estrus sacrificed by cervical dislocation and ventral laparotomy was done to dissect the oviduct. The fimbriated part of the oviduct was dissected out from the rats, suspended in normal saline and placed on a microscope slide with a cover slip to count the number of ova in the oviduct under a dissecting microscope.[9,10]

Serum LH, FSH, Estradiol and Progesterone

At the end of the treatment period, blood samples were collected at the various phases of oestrous cycle and were assayed using Enzyme-linked immunosorbent serologic assay (ELISA) techniques. Assay Kit obtained from Elabscience, Wuhan, China were used for the assays. The samples were centrifuged for 15 min at 3000 rpm using a bench top centrifuge and the serum was stored at -20º C.

Statistical analysis

All the values are expressed as mean ± standard error of mean (SEM). The values were analysed by one-way ANOVA followed by Student's Newman–Keuls Multiple comparison test using the Graph Pad software. Differences were considered significant when $p< 0.05$ vs control.

The research was approved by Research asnd Experimentation Ethics Committee.

RESULTS

Effect of PM on phases of estrous cycle and ovulation

Effect of PM on percentage occurrence of phases of estrous cycle in animals is shown in Figures 1. Percentage occurrence of proestrus and estrus phases was significantly ($P<0.05$) reduced in animals exposed to PM at various doses when compared with control. Percentage of occurrence of metestrus phase was significantly ($P<0.05$) increased in animals exposed to PM at various doses when compared with control.

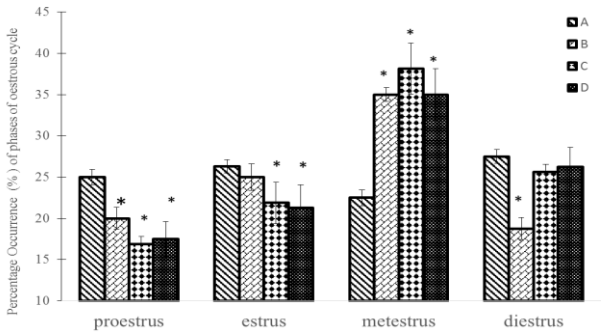

Fig 1: Effect of PM on phases of estrous cycle in female rats. Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

The number of ova shed during estrus following exposure to PM is shown in Figure 2. There was a significant ($P<0.05$) and progressive dose-dependent reduction in the number of ova released in the treated groups compared with control.

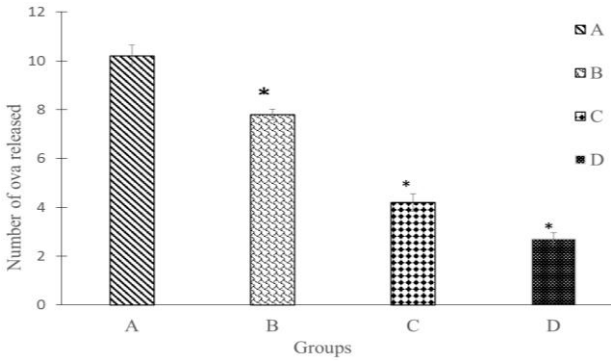

Fig 2: Effect of PM on the ova released at estrus in female rats. . Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

Hormone levels during the estrous cycle

The effect of PM on serum levels of oestradiol, progesterone, FSH and LH are shown in figure 3 – figure 6. Oestradiol was significantly (P<0.05) reduced in treated groups at proestrus phase in all doses when compared with control. However, it was significantly increased at both metestrus and diestrus phases when compared with control in all doses used. Increase was only significant (P<0.05) at estrus in the 120mg/kg group when compared with control (Fig. 3). Progesterone was significantly (P<0.05) increased at proestrus (60 and 120mg/kg) and at estrus (120mg/kg) but was decreased significantly (P<0.05) at estrus (10 and 60mg/kg), metestrus (10mg/kg) and at diestrus in all doses (Fig. 4). LH was increased significantly at proestrus (120mg/kg), at metestrus (60 and 120 mg/kg) but decreased significantly (P<0.05) at estrus and diestrus (60 and 120 mg/kg) when compared with control (Fig. 5). FSH was significantly (P<0.05) increased at proestrus and estrus in all doses used when compared with control but was reduced significantly (P<0.05) at metestrus in all doses when compared with control. At diestrus, a significant (P<0.05) increase was seen only in 10mgh.kg while 60 and 120 mg/kg exhibited a significant (P<0.05) decrease (Fig. 6).

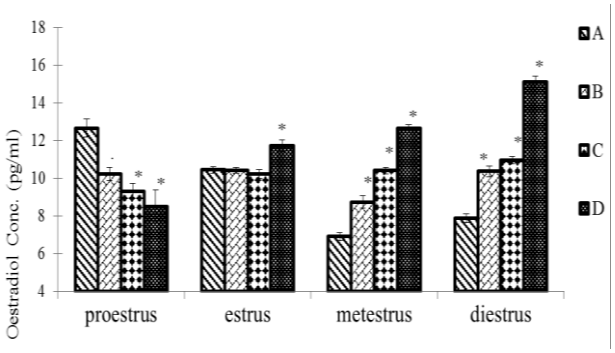

Fig 3: Oestradiol Conc. in female rats administered PM during estrous cycle. Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

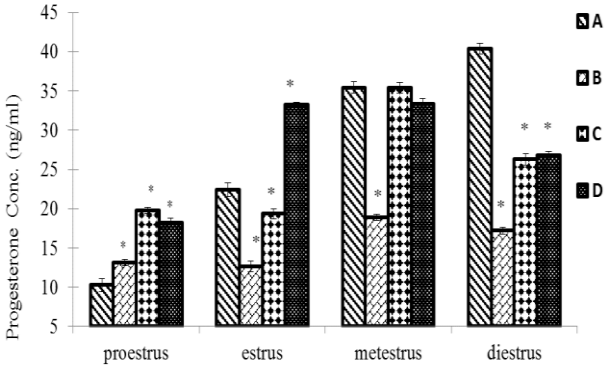

Fig 4: Progesterone Conc. in female rats administered PM during estrous cycle. Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

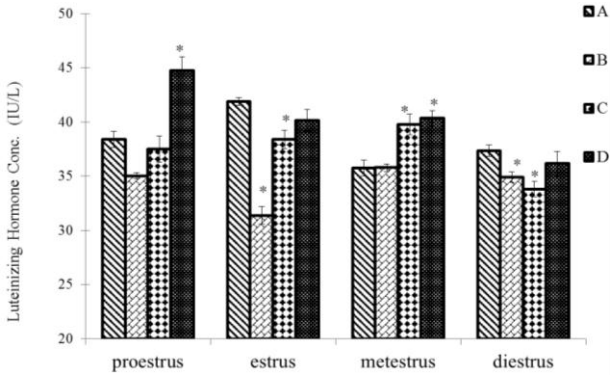

Fig 5: Luteinizing Hormone Conc. in female rats administered PM during estrous cycle. Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

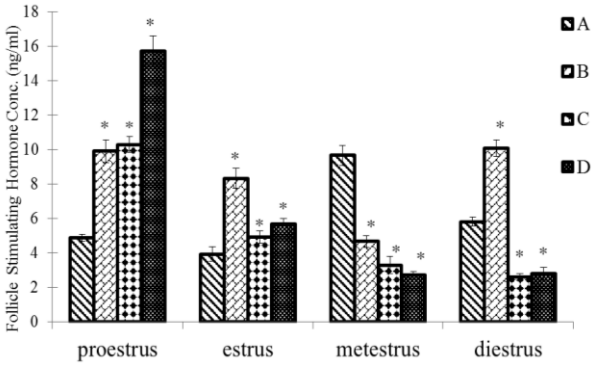

Fig 6: Follicle Stimulating Hormone Conc. in female rats administered PM during estrous cycle. Group A- rats in the control group exposed to distilled water only; group B- rats exposed at a dose of 10mg/kg of pirimiphos-methyl; group C- rats exposed at a dose of 60mg/kg of pirimiphos-methyl,; group D- rats exposed at a dose of 120mg/kg of pirimiphos-methyl. *shows significant difference from control (P<0.05).

DISCUSSION

The duration of the various phases of the estrous cycle was disrupted by the administration of PM. An index of ovarian and uterine activities includes cyclic changes in the estrous cycle. The cycle was prolonged due to the increase in duration of the metestrus phase. The significant reduction in the occurrence of the proestrus and estrus phases is an indication of poor sexual receptivity in the female rat as reported previously.[11] The proestrus phase marks the beginning of sexual receptivity as well as a phase of follicular development [11]. A significant reduction in the proestrus and estrus phases which make up the follicular phase is an indication of reduced fertility. This could also lead to the reduced number of healthy follicles. The metestrus phase marks the beginning of the luteal phase. During the metestrus phase, the rise in FSH level triggers the growth of follicles. The reduced level of FSH during metestrus phase in PM treated rats could be a factor for the increased duration of the metestrus phase. Altered cyclicity was observed after the administration of PM which is in line with observations with methyl parathion (an organophosphate) treatment as well as in chlorpyrifos-treated rats as previously.[12,13]

Exposure to PM caused a significant reduction in the number of ova released at estrus. Ovulation occurs during the follicular phase which was significantly reduced in PM treated rats which may account for the reduced number of ova released in the treated groups. A reduction in the duration in the follicular phase means an insufficient time for the follicles to develop which is similar to previous report.[14]

Exposure to PM resulted in the distortion of the hormone milieu controlling reproduction. The hypothalamic-pituitary-gonadal axis (HPG) controls estrous cycle, ovulation and sexual behavior as well as the growth, development and function of the female reproductive tissues.

Significant reduction in oestradiol concentration during proestrus phase is as a result of reduced folliculogenesis. When the circulating concentration of oestradiol reaches a critical level, the negative feedback onto the hypothalamus and anterior pituitary leading to an inhibition of FSH is observed. A positive feedback then takes over which stimulates the release of LH leading to ovulation.[14,15] In this study, there was a significant decrease in oestradiol secretion in PM treated groups, leading to an increase in FSH during proestrus. This indicates that the expected negative feedback on LH/FSH was removed thereby leading to an increase in LH and FSH. A low oestradiol secretion with an increase in progesterone during proestrus suppresses ovulation as seen in this study which supports previous finding.[16]

A postovulatory rise in FSH levels which is seen at the time of ovulation or shortly after is required so as to trigger the growth of the next set of follicles.[17,18] In PM treated rats, this postovulatory rise of FSH was reduced in the metestrus phase which could account for the increase in duration of the metestrus phase.

Luteinizing hormone secretion was significantly affected by the 120mg/kg body weight dose during proestrus. It was in this group of treated rats that ovulation was greatly inhibited. The proestrus surge of LH triggers ovulation but as the number of ova released during the ovulation was decreased, it may have caused the continuous secretion of LH.

Low oestradiol concentration with an increase in progesterone during proestrus suppresses ovulation was observed in this study which is in support of previous study.[16] This could be one of the reasons for the reduction in ovulation seen in the PM treated rats.

During proestrus phase in the PM treated rats, decreased folliculogenesis as well as increased follicular atresia was observed which led to the reduced number of ova shed at estrus. There was a dose-dependent reduction in the number of growing follicles in PM treated rats due to reduction in FSH concentration during metestrus phase leading to an increase in the duration of the phase.

CONCLUSION

During the estrous cycle, the maturation and ovulation of pre-ovulatory follicles takes place under the combined and balanced influence of ovarian and pituitary hormones. Imbalance in these hormones leads to irregularity in ovarian function and changes in the duration of estrous cycle. Treatment with sublethal doses of pirimiphos methyl was responsible for interference of the estrous cycle in the rats. This interference involved a reduction in the number of ova released at estrus as well as hormonal changes, which has a negative impact on female reproduction.

REFERENCES

1. Bjørling- Poulsen M, Andersen HR, Grandjean P. Potential developmental neurotoxicity of pesticides used in Europe. *Env. Health*, 2008; 7(50): 1-23.
2. Calvert GM, Karnik J, Mehler L et al. Acute pesticide poisoning among agricultural workers in the United States, 1998- 2005. *Am. J. Indus. Med.*, 2008; 51: 883-898.
3. Vinggaard AM, Hnida C, Breinholt V, Larsen JC. Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. *Toxicol In Vitro*, 2000; 14: 227-234.
4. Tamura H, Maness SC, Reishman K, Dorman DC, Gray LE, Gaido KW. Androgen Receptor antagonism by the organophosphate insecticide fenitrothion. *Toxicological Sciences*, 2001; 60: 56-62.
5. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: Some helpful considerations. *Brazilian J. Biol*, 2002; 62: 609-614.
6. Yuan Y Foley GL. Female reproductive system. In Handbook of Toxicologic Pathology 2nd ed., Vol 2. (W. M. Haschek, C. G. Rousseaux, M.S. Wallig eds) pp 847 -94 Academic Press, London.
7. Ngoula F, Watcho P, Dongmo MC. Kenfack A, Kamtchouing P Tchoumboue J. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. *Afr Health* Sci, 2007; 7(1): 3–9.
8. Goldman JM Murr AS Cooper RL. The Rodent Estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res B Dev Reprod Toxicol*, 2007; 80(2): 84-97.
9. Iranloye B Owokunle B. Effect of *Garcinia kola* Seed Extract on Female Reproductive Functions in Rats. *International Journal of Pharmacology*, 2008; 4(4): 276-281.
10. Noah OT, Gbotolorun SC, Oremosu AA. Fertility enhancing potential of Mucuna pruriens seeds in female Sprague-Dawley Rats. *British Journal of Medicine and Medical Research*, 2014; 4(16): 3148-3157.
11. Paccola CC, Resende CG, Stumpp T, Miraglia SM, Cipriano I. The rat estrous cycle revisited: a quantitative and qualitative analysis. *Anim. Reprod*, 2013; 10(4): 677-683.
12. Sortur SM Kaliwal BB. Effects of methyl parathion formulation on estrous cycle and reproductive performances in albino rats. *Indian J. Exper. Biol*, 1999; 37(2): 176-178.
13. Nishi K. Hundal SS. Chlorpyrifos induced toxicity in reproductive organs of female Wistar rats. *Food Chem Toxicol*, 2013; 62: 732-8.
14. Gomes CM, Raineki C, Ramos de Paula P, et al. Neonatal handling and reproductive function in female rats. *Journal of Endocrinology*, 2005; 184: 435-445.
15. Micevych, P. Sinchak, K. Synthesis and function of hypothalamic neuroprogesterone in reproduction. *Endocrinology*, 2008; 149: 2739-2742.
16. Ogilvie KM. Rivier C. Effect of alcohol on the proestrous surge of luteinizing hormone (lh) and the activation of lh-releasing hormone (lhrh) neurons in the female rat. *The Journal of Neuroscience*, 1997; 17(7): 2595–2604.
17. Duran-Pasten, ML. Fiordelisio T. GnRH-induced Ca^{2+} signaling patterns and gonadotropin secretion in pituitary gonadotrophs. Functional adaptation to both ordinary and extraordinary physiological demand *Frontiers in Endocrinology* 2013; doi: 10.3389/fendo.2013.00127.
18. Young JM. MeNeilly AS. Theca: the forgotten cell of the ovarian follicle *Reproduction*, 2010; 140(4): 489-504.