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Full Length Research

Sub-lethal Study of Organophosphorus (Chlorpyrifos) Toxicity on Reproductive Biomarkers in Female Wistar Rats

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Pesticide exposure may interrupt with the female hormonal function that may lead to adverse effects on the reproductive system which are attributed to menstrual cycle disturbances, prolonged time to pregnancy, prolonged estrous cycle, reduced fertility and others. Pesticides direct and indirect exposure is on the rise globally. The objective of this study was to evaluate the sub-lethal toxicity of chlorpyrifos on reproductive biomarkers in female Wistar rats. Female rats were randomly selected into four equal groups. Group-1 served as control and was not treated while groups-2, 3 and 4 treated with chlorpyrifos at 0.2%, 0.4% and 0.8% concentrations through feed. At the end of the treatment, blood samples were collected for reproductive biomarkers analysis. The results revealed that there was a dose dependent decrease in progesterone levels but found non-significant (*p>0.05*). Estrogen, FSH and LH were significant (*p<0.05)* in a dose dependent manner. This investigation revealed that sub-lethal concentrations of chlorpyrifos had the capacity to disrupt reproductive biomarkers balance and possible estrous cycle alteration in treated female rats. Thus, further studies of sub-lethal doses with chlorpyrifos are necessary in order to establish patterns of their toxicity as affecting the health of humans and animals.

Keywords: Chlorpyrifos, reproductive biomarkers, female Wistar rats.

INTRODUCTION

Environmental toxicity is a threat to life on earth; plants, animals and humans progressively faced with a large number of toxic chemicals. Organophosphorus (OPs) pesticides affect almost all body functions that may provide an insight to the population and ecological consequences of long-term exposure (Raley-Susman, 2014; Greaves and Letcher, 2017). OPs pesticides are widely used in agriculture, horticulture and veterinary medicine; they are also frequently used around household for public hygiene and control of disease vectors (Roberts and Aaron, 2007). The term pesticide covers a wide range of compounds including insecticide, rodenticide, fungicide, herbicide, plant growth regulators among others (Cope et al., 2004). According to the World Health Organization (WHO, 2004), 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (Yang and Deng, 2007).

Organophosphate pesticides are among the most widely used pesticides globally and leaders of the market for many years, account for 50% of all pesticides applied worldwide due to their rapid biodegradability and shorter persistence in the environment with chlorpyrifos, and their use is predicted to grow worldwide through 2022 (Grand View Research, 2014). The widespread use of pesticides in agriculture, medicine, industry, public health and household has reached the environment and has become a major global public health concern (Yurumez et al., 2007). Organophosphorus compounds exist in liquid and solid forms and are Phosphorodioates, Phorodithioates and Phosphates (Tripathi and Srivastav, 2010). Chlorpyrifos [0,0-diethyl-0-(3, 5, 6-trichloro-2-pyridyl)phosphor-dhioate] is a member of organophosphate class of pesticides exhibiting broad spectrum insecticidal activity against a number of important arthropod pests. Chlorpyrifos is initially activated to its active metabolite Chlorpyrifos-oxon by oxidative desulfuration which in turn is responsible for mammalian toxicity through inhibition of cholinesterase (Tongbai and Damrongphol, 2011). Once Cholinesterase is inactivated, acetylcholine accumulates throughout the nervous system (Latuszynska et al., 1999). Toxicity of chlorpyrifos induces adverse effect on many organs like Blood cell, Brain, Kidney and Liver (Bede and Panemanogalore, 2003). Chlorpyrifos is readily absorbed from gastrointestinal tract. The study of single dose oral administration of chlorpyrifos on human volunteers by Nolan et al., (1984) reported that chlorpyrifos was 70% absorbed from gastrointestinal tract and in rats chlorpyrifos absorption through gastrointestinal tract after single dose gavage study ranged from 84-90%. Chlorpyrifos is a non-synthetic pesticide designed to be effective by direct contact, ingestion and inhalation. The dynamics by which chlorpyrifos induce reproductive toxicity in animals are by altering the release of neurotransmitters, thus leading to impaired functions of Hypothalamo-Pituitary-Gonadal (HPG) regulatory axis and suppress steroidogenesis in the gonads (Senthilkumaran, 2015).

The widespread use of chlorpyrifos in agriculture and public sectors is alarming, and its toxicity requires investigation. The present study channeled made an investigation on the sub-lethal toxicity of chlorpyrifos on reproductive biomarkers in female Wistar rat.

MATERIALS AND METHODS

Animals

Thirty six (24 female and 12 male) mature healthy female and male Wistar rats (*Rattus norvergicus)*, weighing between 160-185 g were used in the present investigation. Animals were procured from the Animal House of the Department of Physiology, University of Port Harcourt, River State Nigeria. The protocols approved by institutional animal ethics committee and guide lines of National Research Council for care and maintenance of animals were followed (NRC, 2011).

Before the commencement of the treatment at normal room temperature female rats had been acclimatized for three weeks. The female rats were randomly assigned into four groups, group-1 served as control group while 2, 3 and 4 groups were the treatment groups, they were paired 2 female per 1 male for fertilization to occur, which was confirmed by a vagina smear test, being carried out each morning while confirmation of spermatozoa was considered day 1 of gestation.

They were housed in labeled plastic cages covered with wire gauze under standardized animal conditions, fed with pelleted food (Vita feeds) twice daily with each rat consuming estimated feed weight of 30 g per day and drinking water *ad libitum.*

Chlorpyrifos preparation

Chlorpyrifos (Rocket® 20% emulsifiable concentrate) of commercial grade purchased from agrochemical store located at Relief market Owerri, Imo State, Nigeria was used in this study. All the other chemicals were of analytical grade and obtained from commercial sources. Three concentrations 0.2%, 0.4% and 0.8% of solutions were prepared by diluting the commercially available chlorpyrifos liquid with distilled water (DW). Concentrations of chlorpyrifos given to the animals was based on the feed quantity and not animal's body weight.

From the stock (chlorpyrifos) solution, the following concentrations were taken and diluted with distilled water to make it up to 750 ml.

Concentration 0.2% was prepared by diluting 1.5 ml of chlorpyrifos stock solution with 748.5 ml distilled water.

Concentration 0.4% was prepared by diluting 3.0 ml chlorpyrifos stock solution with 747 ml distilled water. Concentration 0.8% was prepared by diluting 6.0 ml

Table 1. Serum levels of Progesterone, Estrogen, LH and FSH on female rats after treatment with different doses of chlorpyrifos.

Groups	Progesterone (ng/ml)	Estrogen(pg/ml)	LH (mIU/ml)	FSH (mIU/ml)
Control 1 (0.0%)	10.10 ± 0.34	62.3 ± 2.06	2.28 ± 0.386	2.89 ± 0.308
Treatment 2 (0.2%)	9.70 ± 0.20	60.6 ± 1.08	1.90 ± 0.200	2.10 ± 0.330
Treatment 3 (0.4%)	9.20 ± 0.30	59.8 ± 0.41	1.24 ± 0.210	1.90 ± 0.240
Treatment 4 (0.8%)	8.78 ± 0.40	58.5 ± 0.92	1.04 ± 0.190	1.73 ± 0.154

means ± SD; n = 6 for each treatment group.

chlorpyrifos stock solution with 744 ml of distilled water.

Chemical and treatment

The solutions were administered to the female Wistar rats only through feed by mixing 15 ml of different prepared concentration of chlorpyrifos, and were fed 15 g twice a day throughout the period of gestation and 21 days after parturition. The first day of administration was considered day 1 of treatment.

Blood sampling

At the end of treatment period, each animal was anesthetized by chloroform and blood sample collected through cardiac puncture. Heparinized bottles containing blood were then centrifuged at 3000 rpm during 20 min and obtained serum was conserved at -80^{0}C. The serum was subjected to hormonal assay by ELISA test kit method of progesterone, Estrogen, LH and FSH levels and were measured at 450 nm by spectrophotometry.

Statistical analysis

Data obtained were expressed as Mean ± Standard Deviation and analyzed using the SPSS package 20.0. One-way Analysis of Variance (ANOVA) was used. Values at $p<0.05$ was regarded as significant in comparison with appropriate controls.

RESULTS

Reproductive hormones

The treatment of different concentrations of chlorpyrifos to female Wistar rats from gestation day 1 through to weaning (Table 1) was found to decrease progesterone serum levels in a dose dependent manner but non-significant ($p>0.05$) as represented in Figure 1. Estrogen levels significantly decreased ($p<0.05$) in a dose dependent manner (Figure 2). FSH and LH levels showed a significant dose dependent decrease ($p<0.05$) in chlorpyrifos treated rats when compared with that of untreated (control) group as shown in Figures 3 - 4.

DISCUSSION

Because of toxicity and widespread use of pesticides, chlorpyrifos (Organophosphorus) pesticide is recognized as important pollutant that can interrupt hormonal balance. Numerous studies indicate associations with pesticide exposure and their consequential health implications. Organophosphorus pesticide is an endocrine disruptor that directly affects reproductive processes thereby interacting with steroid hormone receptors (McKinlay et al., 2008; Fei et al., 2010; Liu et al., 2010).

A non-significant decrease was observed at progesterone levels in a dose dependent manner among treated groups. There was a significant dose dependent decrease in estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in treated rats compared to control. Progesterone levels decline observed in this study may be the disruption of hormone balance necessary for normal reproductive physiology and it is in agreement with Yu et al., (2011) and Astiz et al., (2014). Estrogen levels showed a significant dose dependent decrease in treated groups compared to control. A significant decrease in serum estrogen levels after treatment with chlopyrifos from gestation day 1 to weaning suggests that this chemical exposure can

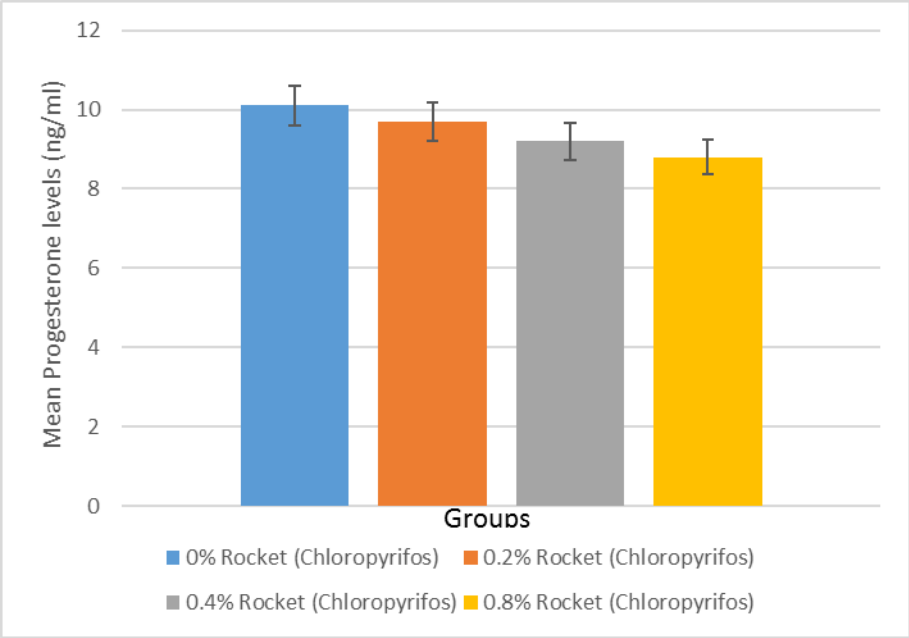

Figure 1. Effects of chlorpyrifos on progesterone.

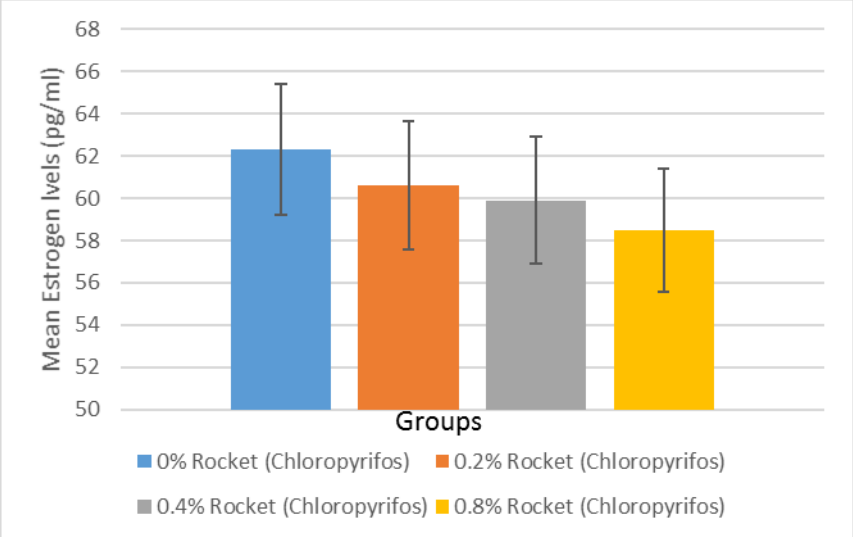

Figure 2. Effects of chlorpyrifos on estrogen.

disrupt estrous cycles and increase the duration of diestrus phase which might reduce the reproductive life span of the rats. In the present study, the decrease in estrogen level due to treatment with chlopyrifos might be attributed to direct toxicity of chlopyrifos on ovarian steroidogenesis. Estrogen synthesis is essential for normal folliculogenesis and is crucial for the survival of ovarian follicles and its decline is related to inhibition of granulosa-cell proliferation (Krishna and Abhilasha, 2000). Conformity with the present study it is the study by Oluwatoyin et al., (2017) study on chronic exposure of male Wistar rats with fumes of different pesticides on reproductive hormones; it is reported that prolong

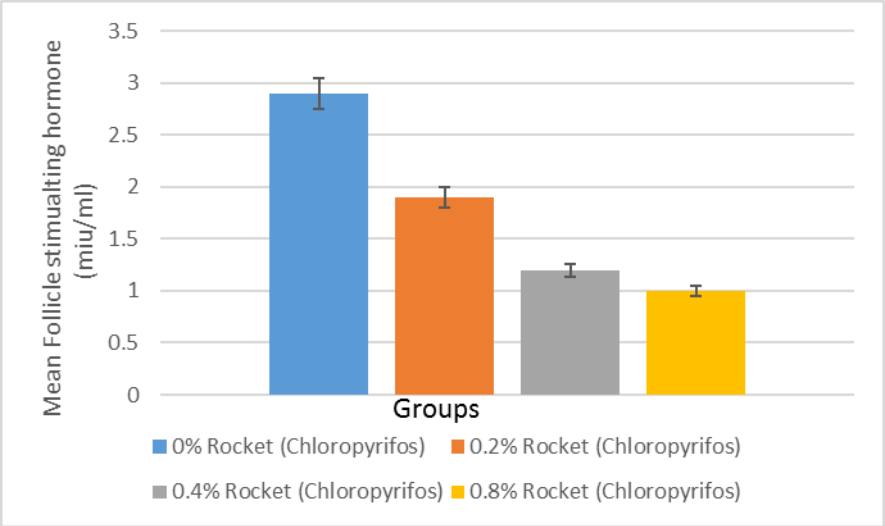

Figure 3. Effects of chlorpyrifos on follicle stimulating hormone.

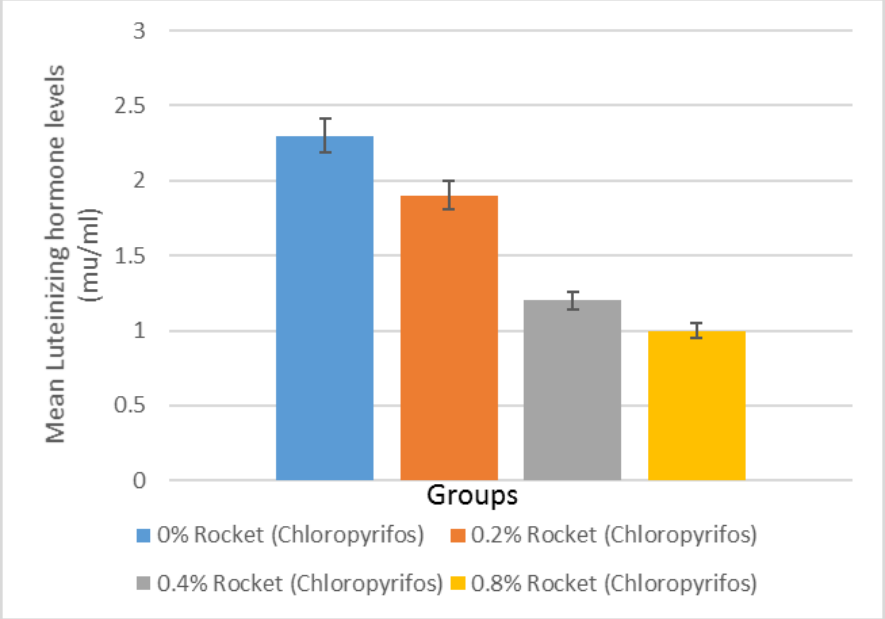

Figure 4. Effects of chlorpyrifos on luteinizing hormone.

exposure of toxicants may lead to reproductive toxicity and possible interference with fertility profile (decreased FSH and Testosterone). Similar to the Present study corroborates Cooper et al., (1993) reported that estrous periodicity is regulated by cyclic secretion of pituitary and ovarian hormones which includes estrogen and progesterone. Deviation from normal cyclic secretion of ovarian hormones can interrupt this delicate balance. Therefore, any deviation in estrous cyclicity points to hormonal imbalance and altered function of hypothalamus-pituitary-ovarian axis. Consistent with the present study, Sangha and Kaur (2011) reported that treatment of rats with high dose of cypermethrin 50

mg/kg decreased serum estrogen levels which affected the granulosa cells in the ovary thereby disrupted the estrous cycle due to the increase in diestrus.

Organophosphorus pesticides may be responsible for male and female reproductive hormones alteration by decreasing FSH, LH levels. In agreement with the present study, Elbetieha and Da'as (2003) reported significant decrease in FSH and LH and testosterone levels when following pesticide exposure. In line with the present study, Gore (2001) and Flehi-Slim et al., (2016) reported a significant decrease in FSH and LH following treatment with Malathion pesticide, they attributed the decrease to the direct toxicity of the pesticide on hypothalamic Gonadotrophic Releasing Hormone (GnRH) gene expression. Consistent with the present study, Kumar, (2004) and Sharma et al., (2014) reported pesticides toxicity in women as premature birth, spontaneous abortions, low birth weight, developmental abnormalities, ovarian disorders and disruption of hormonal function leading to decreased fertility. The significant decrease in reproductive biomarkers may be due to ovarian follicles alteration that may lead to disruption of estrous cyclicity in the rats thereby undermining their reproductive life span.

CONCLUSION

Chlorpyrifos has been increasingly adopted for agricultural, industrial and domestic uses because of their less persistence in nature, but a number of articles in international literature demonstrates that neither they are not safe for not only non-target organisms including humans nor when they are exposed to very low concentrations for long period. Indiscriminate and excessive use of chlorpyrifos may exert deleterious effect on reproductive system by acting on the endocrine system. Chlorpyrifos antagonistic effects may be due to their potent anti-acetylcholine esterase actions in the cholinergic nervous system that may lead to impaired functions at any level of the hypothalamo-pituitary-gonadal axis on the ovary. A new concept in agriculture and pests control is urgently needed because of the toxicity associated with pesticides (chlorpyrifos) exposure. This study confirmed that chlorpyrifos has toxic effects on reproductive biomarkers in female rats in dose-dependent manner. Future studies on chlorpyrifos are necessary to investigate their reproductive biomarkers potential toxicity in humans when there is accidental and professional exposure.

RECOMMENDATION

Since dietary exposure with chlorpyrifos altered reproductive biomarkers balance in a dose dependent manner in treated female rats in the present study, showing that chlorpyrifos dietary exposure had toxic effects, it is therefore recommended that green pesticide concept be adopted by farmers and the general public in the control of pest, thereby prevent humans, animals and environment from chlorpyrifos hazards. Also, further studies on chlorpyrifos reproductive biomarkers toxicity in humans (farmers, workers in pesticide manufacturing companies and fumigators) are needed.

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