Original Research Article

Chronic toxicological effect of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on some Reproductive and Thyroid Hormones of New Zealand white Rabbits

ABSTRACT

Aim: To assess the chronic toxicological effect of 2, 2-dichlorovinyl dimethyl phosphate (sniper) on some reproductive and thyroid hormones of New Zealand white Rabbits
Study design: An experimental study.
Place and Duration of Study: Department of Biological Science, Rivers State University, Port Harcourt Animal House, Rivers State Teaching Hospital and Nigerian National Petroleum Corporation Hospital Laboratory, between January, 2020 and April 2020.
Methodology: Thirty-six (36) male New Zealand white rabbits weighing approximately 1.0mg/kg were used for the study. The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were divided into three (3) groups of four (4) rabbits each with four (4) matched control. For the chronic oral study, 10% of the LD50 (details not included) which is 0.005mg/kg dose of sniper, mixed with 1.0ml of distilled water was administered orally to the rabbits daily for the stipulated period of 0-30, 0-60 and 0-90 days. The matched control rabbits received only feed and water *ad libitum* during the study. Whilst, for the chronic inhalation study, 10% of the LD50 dose of sniper which is equivalent to 0.05mg/m^3 dose of sniper was mixed with 1.0ml of distilled water, sprayed in the closed cages. At day 30, 60 and 90, 4 rabbits were sacrificed each from the chronic oral and inhalation study groups and the matched control group. Blood specimens were collected at each stage, about 5.0mls of blood was collected into lithium heparin specimen container for the investigation of some reproductive and thyroid hormones, which were analyzed using the Enzyme linked immune-sorbent assay method (ELISA). Statistical Package for Social Sciences (SPSS) version 22.0 of windows statistical package was used to analyze the data generated and p values less than *.05* were considered significant.
Results: the results showed that chronic 2, 2-dichlorovinyl dimethyl phosphate exposure in rats caused a statistically significant decrease ($P<0.05$, $F=8.798$) in the levels of testosterone: control (3.70 ± 0.46), sniper treated group (1.48 ± 0.22). T4, FSH, T3 and LH also significantly decreased for both oral and inhalation routes of administration and as the duration of exposure increased. While the levels of prolactin: control (7.68 ± 1.19), sniper treated group (16.00 ± 1.12) significantly increased ($P<0.05$, $F=10.19$). TSH levels also increased significantly for both oral and inhalation routes of administration and as the duration of exposure increased.
Conclusion: chronic 2, 2-dichlorovinyl dimethyl phosphate exposure caused a decrease in the levels of testosterone, T4, T3, FSH, LH, while there were increases in the levels TSH and prolactin for both oral and inhalation routes, and across the duration of exposure.

Keywords: Chronic toxicological effects, 2, 2-dichlorovinyl dimethyl phosphate (Sniper), Reproductive, thyroid Hormones, New Zealand, white rabbits

1. INTRODUCTION

Dichlorvous (Sniper) is toxic to both humans and animals, yet it is indiscriminately used as a household insecticide and agricultural pesticide. The daily exposure limit set by the occupational safety for health administration (OSHA) which is 1mg/m^3 if exceeded could result to harmful health effects. WHO has rated dichlorvous as a highly hazardous chemical

[1]. An estimate of 3 million cases of dichlorvos poisoning occur every year worldwide resulting in an excess of 250,000 deaths [1]. The World Health Organization (WHO) has also estimated that the number of suicidal deaths will reach 1.5 million people in the year 2020 [1]. In dichlorvous poisoning, there are factors that can be applied to detect poisoning severity and death. Cholinesterase activity in blood is a prognostic factor of acute dichlorvous poisoning. Diagnosis of poisoning is essential both in living as well as in the dead for therapeutic and Medico-legal purposes. The annual dichlorvos release into the environment from 2010-2015 for agricultural purpose was about 1145 tones for African countries, 4342, tones for Caribbean and 10,013 for central American countries. Widespread use of organophosphate pesticides in general in agriculture, as well as in homes, park, schools, hospitals, aircraft and other public places has led to continuous human exposure which has caused various health hazards [2].

It has been reported that target organs of dichlorvos toxicity include the central nervous system (CNS) and less in other organs of the body. This indirectly affects the levels of hormones affected by the hypothalamus. Presently, the increasing health challenges involving the CNS call for proper study on the chronic toxicological effects of dichlorvos using rabbit in order to carry out further studies in man. Animal studies have shown a decrease in spermatogenesis in relation to dichlorvous toxicity. Other organophosphates such as chloropyrifos, methyl parathion and parathion, can affect sperm levels by injuring the seminiferous epithelium through germ cell proliferation [3]. The physiological levels of reactive oxygen species (ROS) and nitric oxide (NO) are important for cell signaling processes and cell function in different types of cells such as sperm. While reactive oxygen species can promote the acrosome reaction, presence of superoxide dismutase and catalase inhibits this reaction. The pathway of inducing the acrosome reaction seems to be ROS – modulated tyrosine phosphorylation. Tyrosine phosphorylation promotes sperm membrane binding to the zona pellucid glycoprotein 3, which promotes sperm-oocyte fusion. It has also been revealed that Nitric oxide (NO) affects sperm motility, and acts as chemo attractant, and modulates the acrosomal reaction. Dichlorvous could pass the epididymal epithelium because of its lipophilic nature and can reach the stored spermatozoa. This could be the reason for its destructive effects on sperm structure and function [4]. Waheed et al. [5] revealed that exposure to pesticides is associated with high testosterone level in occupational and residential users of pesticides. Abolfazl et al. [6] in their study found that there was a significant decrease in the level of luteinizing hormone (LH), as the testosterone level increased. This is an indication that organophosphate insecticide can affect LH and Testosterone hormones by two completely different mechanisms. Pesticides such as Marathon and chlorpyrifos can affect testosterone metabolism by inhibiting CYP3A4, this result in an increase in serum testosterone and its perpetuity [7]. However, effect of dichlorvous on hypothalamic-pituitary endocrine functions could cause decrease in LH level. Dichlorvous can alter hypothalamic – pituitary endocrine functions by decreasing the AChE activity in the brain.

Changes in serum reproductive hormone, such as testosterone, have been observed in men that were exposed to dichlorvous, [8]. A positive relationship between dichlorvous and total testosterone level have been observed in cross-sectional study in male Thai [9]. A tendency for increased LH and follicle stimulating hormone (FSH) levels in pesticide sprayers has been reported. Such alterations could be associated with semen quality in infertile men. Dichlorvous causes changes in reproductive function through oxidant and antioxidant balance disruption in the brain, with resultant effect of impairing hypothalamic, pituitary endocrine functions, and gonadal processes [6]. Some epidemiological studies reveal that dichlorvous may be associated with infertility in male. It was reported that the occurrence of primary infertility was higher among farm workers than in the normal population, in the Kavar region of Fars province of Southern Iran [9].

A study on the effects of dichlorvos on fertility of male mice injected with dichlorvos intraperitoneally showed pronounced decrease in sperm number and increase in sperm

abnormalities [10]. Significant reduction in testosterone levels of adult male rats fed with water contaminated with dichlorvos has been reported. The study also revealed some degree of distortions in the seminiferous cells as well as hypertrophy of the spermatogonia cells. Recent experimental and epidemiological researches have revealed that pesticides have serious negative impact on overall human fertility. The mechanism of their effects is that they damage spermatozoa, alter Sertoli or Leydig cell function and this in return affects semen quality [11]. A number of other animal studies have reported reproductive effects such as premature ovulation, endocrine disruption and organophosphates estrogenic and antiandrogenic effects while other studies do not. In an inhalation exposure study dichlorvos in male volunteers, 0.42 mg/ml of di-chloroethanol a specific metabolite of dichlorvos, was detected in the first urine sample of a volunteer who was exposed to dichlorvos at an extremely high concentration of 38 mg/m^3 (4.2ppm) for 105 minutes [12]. Therefore, it was indirectly evidenced that dichlorvos was absorbed through an inhalation route. Again, dichlorvos has been implicated in several cases of infertilities, it is therefore necessary to evaluate the effect of dichlorvos on reproductive and thyroid hormones. The aim of this study was to assess the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (sniper) on some reproductive and thyroid hormones of New Zealand white Rabbits

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of thirty-six (36), two-month-old New Zealand white rabbits (*Oryctolagus cuniculus*) that weighed averagely 1.0kg were used for this study. The rabbits were purchased from Department of Biological Science, Rivers State University, Port Harcourt animal house. They were used for oral and inhalation chronic studies. The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum* from the animal house, department of animal and environmental science, Rivers State University, Port Harcourt. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.2 Procurement and administration of Sniper

1 litre of concentrated solution of sniper (DDVP) insecticide 1000EC (which contains 1000mg of 2-2 dichloro vinyl dimethyl phosphate compound was purchased in Nigeria from Swiss–Nigeria chemical company which is the sole marketing company for sniper in Nigeria). For the chronic oral study, 10% of the LD50 dose which is 0.005mg/kg dose of sniper, mixed with 1.0ml of distilled water was administered orally to the rabbits daily for the stipulated period of 0-30, 0-60 and 0-90 days. The matched control rabbits received only fed and water *ad libitum* during the study. Whilst, for the chronic inhalation study, 10% of the LD50 dose of sniper which is equivalent to 0.05mg/m^3 dose of sniper was mixed with 1.0ml of distilled water, sprayed in the closed cages. The rabbits were transferred into the closed cages that have been flirted with sniper to spend 4 hours daily before returning them back to their normal cages.

2.3 Experimental Design

The rabbits were divided into three (3) groups of four (4) rabbits each with four (4) matched controls. A total of 20 cages were used for this experiment as shown below:

Duration	Chronic oral study	Chronic inhalation study	Matched control
0-30 days	4	4	4
0-60 days	4	4	4
0-90 days	4	4	4

Table 1 : Chronic inhalation study

2.4 Sample Collection, Storage and Analysis

2.4.1 Sample collection

At day 30, 4 rabbits were sacrificed each from the chronic oral study group, chronic inhalation study group and from the matched control group. Blood specimens were collected at each stage, about 5.0mls of blood was collected into lithium heparin specimen container for estimation of reproductive and thyroid hormones.

2.4.2 Laboratory Investigation of Parameters

2.4.2.1 Quantitative Determination of Testosterone

Method: Enzyme Immunoassay. ELISA technique as described by the manufacturer. Catalog Number 10007.

2.4.2.2 Quantitative Determination of Follicle Stimulating Hormone (FSH)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number E-EL-R0391

2.4.2.3 Quantitative Determination of Rat Luteinizing Hormone (LH)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number: E-EL-R0026

2.4.2.2.4 Quantitative Detection of Rat Prolactin (PRL)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number: E-EL-R0052

2.4.2.5 Quantitative Determination of Free Triiodothyronine (fT3) Concentration

Method: Accu-Bind Microplate competitive Enzyme Immunoassay, (ELISA technique as described by the manufacturer. Product Code: 1325-300.

Principle

2.4.2.6 Quantitative Determination of the Free Thyroxine (fT4] Concentration

Method: Accu-Bind Microplate Enzyme Competitive Immunoassay (ELISA technique as described by the manufacturer. Product Code: 1225-300

2.4.2.7 Quantitative Determination of Rat Thyroid Stimulating Hormone (TSH)

Method: Sandwich-ELISA as described by the manufacturer. Catalog Number: E-EL-R0976

2.5 Statistical Analysis

SPSS version 22.0 of windows statistical package was used to analyze the data generated. The mean ± standard deviation was determined. One-way analysis of variance (ANOVA) with Tukey's Post Hoc test, bar charts were also done using the same statistical package. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than *.05* was considered statistically significant.

3. RESULTS AND DISCUSSION

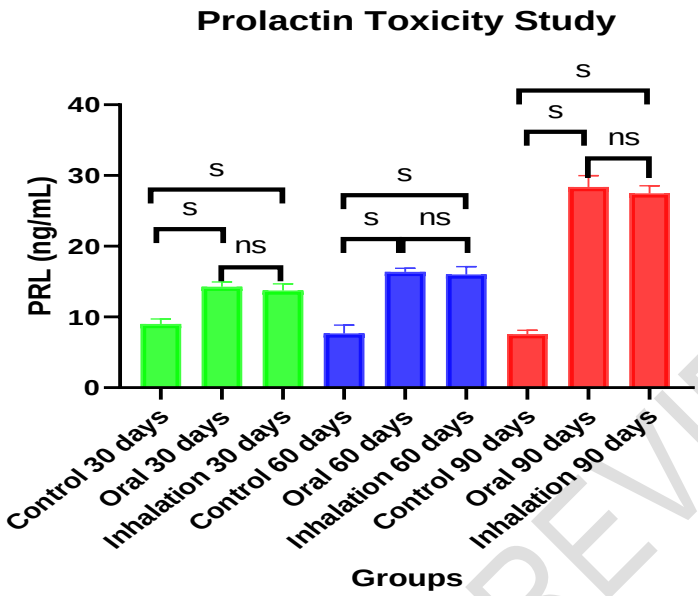

Fig. 1: Prolactin levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

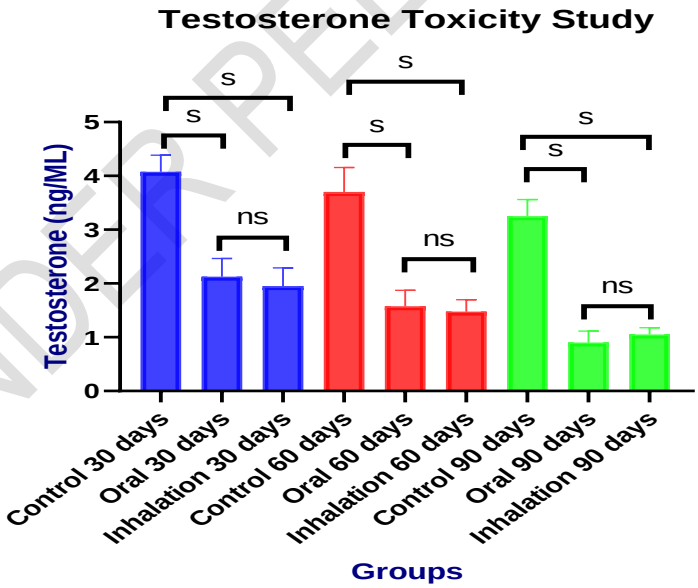

Fig. 2: Testosterone levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

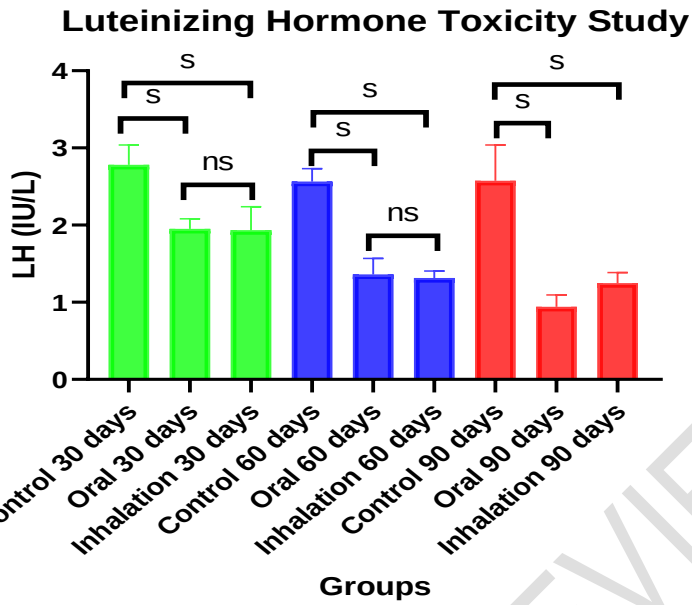

Fig. 3: Luteinizing Hormone levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

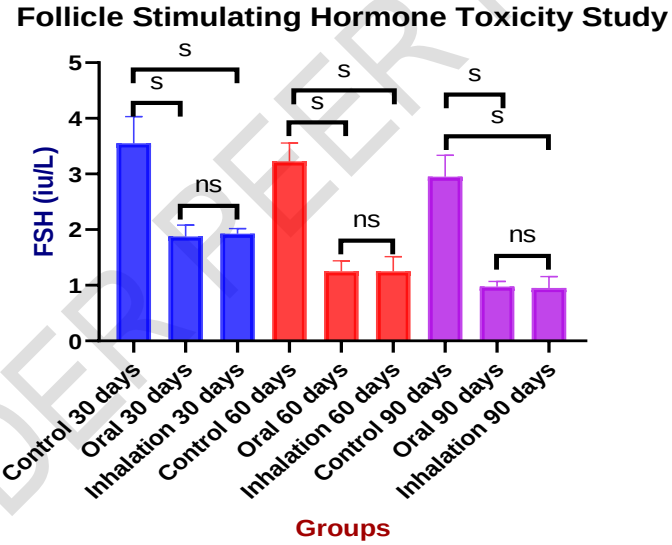

Fig. 4: Follicle Stimulating Hormone levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

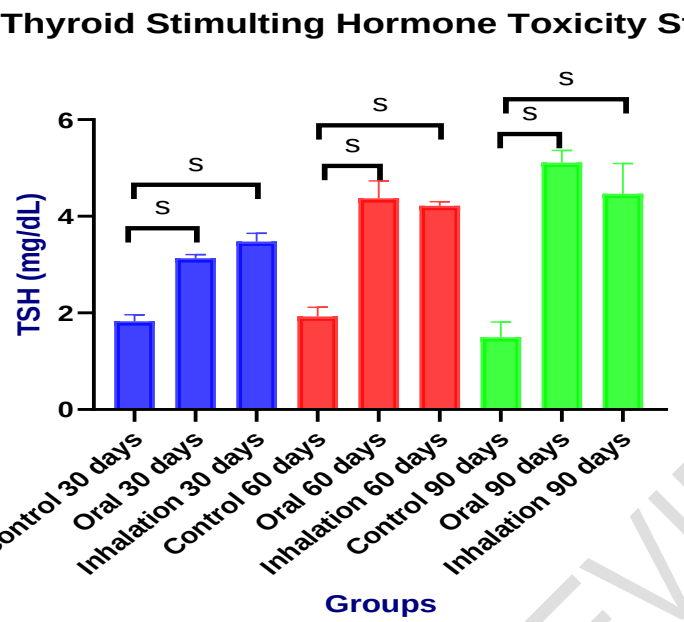

Fig. 5: Thyroid Stimulating Hormone levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

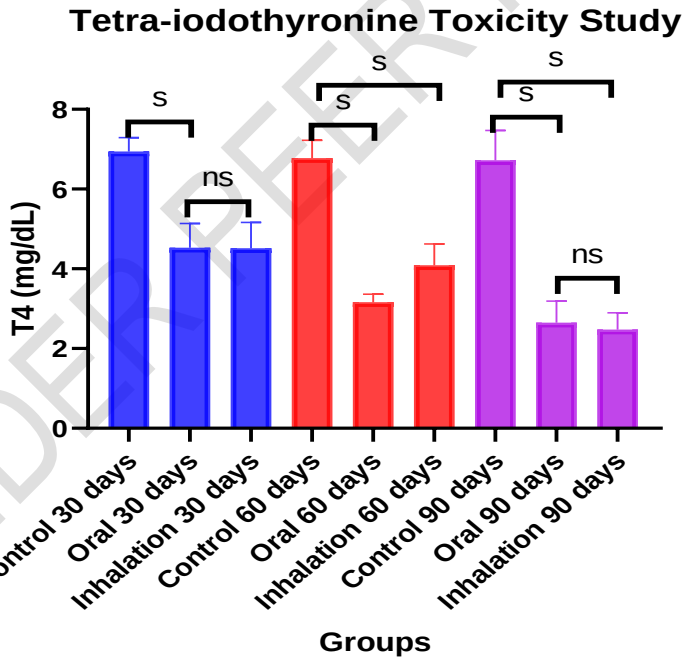

Fig. 6: T4 levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

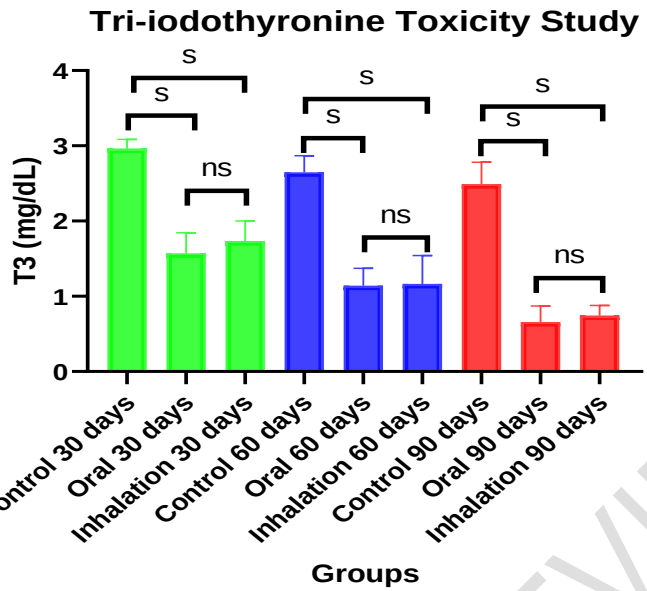

Fig. 7: T3 levels in Control and treated rabbits (s- significant, ns – not significant at p<0.05)

The analytes used in the assessment of the effect of dichlorvos on the reproductive and thyroid hormones of the male rabbits were prolactin, testosterone, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone. These hormones play a crucial role in the pathogenesis of infertility.

The result of this study revealed that exposure of the rabbits to dichlorvos (sniper) through the oral and inhalation routes caused a remarkable increase in the level of prolactin. The levels increased significantly as the duration of dichlorvos treatment increased from day 30-day 90 at (*P<.05*) (Fig. 1). This showed that dichlorvos (Sniper) has the ability to affect the dopaminergic systems play active roles in the physiologic regulation of prolactin. Previous studies have revealed that dichlorvos affects the hypothalamic pituitary axis. hyperprolactinaemia has been involved in several cases of infertility. It is regarded as one of the very common endocrine disorders that lead to increase secretion on the hypothalamic pituitary axis. This condition occurs more in young women and can result in several abnormalities, one of which includes infertility. Prolactin is crucial in breast development and lactogensis. hyperprolactinaemia tends to suppress the ovulatory cycle by inhibiting the secretion of follicle stimulating hormone (FSH) and gonadotropic – releasing hormones that are important for ovulation.

In males, hyperprolactinaemia may cause erectile dysfunction, decreased libido, infertility, gynecomastia, impotence and increased bone mass. In women, hyperprolactinaemia may cause decreased libido, infertility, oligomenorrhea/ amenorrhea and galactorrhea.

Significant decrease was observed in FSH (Fig. 4) with the administration of dichlorvos by oral and inhalation routes from day 30 to day 90 when the results obtained from the experimental rabbits were compared with the matched control values at (*P<.05*), again significant difference was observed between day 30, 60 and 90 at *P<.05*. The significant decrease in the level of FSH shows that dichlorvos (Sniper) can affect spermatogenesis in the male rabbits. FSH in association with high intratesticular testosterone concentrations enhances spermatogenesis. FSH stimulates testicular growth and assist in the protein by the Sertoli cells, these are components of the testicular tubule necessary for sustaining the maturing sperm cell. This is an important factor in the development of normal

spermatogenesis. When Sertoli cells are influenced by androgens, they secrete inhibin, which is a polypeptide that may help to locally regulate spermatogenesis. Maturation of spermatozoa therefore requires FSH and LH.

The menstrual cycle is determined mainly by ovary (Follicular growth and maturation) pituitary (LH and FSH) and hypothalamus (gonadotropins). Development of the ovarian follicle is influenced by FSH. Again, estrogen secretion from this follicle depends on LH and FSH. FSH reduction by dichlorvos my affect folliculogenesis and delay maturation of the follicle in the pre-ovulatory [13]. FSH secretion is controlled by the gonadotropic releasing hormone secreted by the hypothalamus; therefore, dichlorvos may have exerted its effect on the anterior pituitary or the hypothalamus. Low FSH level may have an effect on female conception. FSH is the central hormone of reproduction in mammals. It is important for gonadal development and maturation at puberty. It is also important for gamete production during fertile phase of life. The growth of maturation of ovarian follicles is stimulated by FSH, by acting directly on the receptors located on the granulose cells.

Dichlorvos has been observed to have multiple alterative action on reproductive hormone sites, with the greatest effects being on the hypothalamic pituitary axis. Signals within and between the hypothalamus and anterior pituitary appears to be disrupted, which is dependent on the duration of dichlorvos exposure on the rabbits. The significantly lowered mean value of FSH in dichlorvos treated rabbits in this study is in consonant with previous studies in which dichlorvos significantly reduced FSH level in albino rats and rabbits.

Significant decrease was found in the value of LH (Fig. 3) of the male rabbits that were treated with dichlorvos in the oral and inhalation routes of exposure. More marked reduction was observed as the duration of dichlorvos exposure increased. The mean values of the oral and inhalation routes when compared with the matched control were significantly difference at ($P<.05$). This result reveals the devastating effect of dichlorvos on the hypothalamic pituitary gonadal axis.

Luteinizing hormone plays a central role in follicle development and spermatogenesis while stimulating the production of steroid hormone and mediating proliferative signals. When LH is elevated, it stimulates ovulation and development of the corpus luteum. Gonadotrophin releasing hormone triggers the LH surge that precedes ovulation. Low LH could cause anovulation in the females. Testosterone secretion by the Leydig cells is stimulated by LH. This implies that a low LH level will seriously affect testosterone levels. Again, testicular function is under the control of gonadotropins. The reduction in the LH is an indication of enzyme inhibition in steriodogenetic pathway. Disruption of hypothalamic secretion of hormones and spermatogenesis could be associated with dichlorvos toxicity. The pronounced decrease in the LH of the dichlorvos treated rabbits indicated impairment of the Leydig cell function.

The findings in this study show that the administration of dichlorvos to the rabbits by oral and inhalation routes produced a decrease in the value of their testosterone (Fig. 2). Testosterone is a male hormone that plays a significant role in spermatogenesis. The Leydig cells of the testis, the adrenals and the ovaries all secrete testosterone. Testosterone is the most essential androgen that is secreted into the blood. If the total sperm count is low, it may imply a problem with testosterone levels. In females, testosterone is implicated in cases of polycystic ovary syndrome, in that case, it is slightly raised. It appears that the Leydig cells could be the target of dichlorvos exposure. There could be a likelihood that dichlorvos accumulates in the epididymis and other accessory glands. Leydig cells appear to be the primary target. Interstitial cells and Leydig cells are the cells that secrete testosterone. Dichlorvos has been observed to cause hypospermia, low testosterone levels and testicular atrophy in males.

The significant decrease in the mean values of the serum testosterone implies decreased steroidogenesis.

Thyroids hormones are useful in the assessment of thyroid function in humans and experimental animals. Thyroid stimulating hormone (TSH), Triodothyoxine (T3) and Thyroxine (T4) are the hormones that make up the thyroid hormones. The T3 and T4 formation are influenced and controlled by the pituitary.

In this investigation, the mean values obtained following the administration of dichlorvos through the oral and inhalation routes showed significant decrease in the level of TSH (Fig. 5) of the rabbits that were chronically exposed to subtle dose of dichlorvos. Significant decrease was observed at ($P<.05$) when the values were compared with the matched control values. The decrease became more pronounced as the duration of exposure increased in the both routes of administration. This result is in agreement with who in their study found significant decrease in the TSH value of rats that were treated with dichlorvos.

TSH is considered as the more precise marker for identifying thyroid malfunctioning; for this reason, TSH level determination is majorly used for the diagnosis of thyroid disorders.

Reduction in the levels of T3, T4 (Figs. 6 and 7) and TSH might be as a result of structural damage of thyroid follicular cells due to accumulation of dichlorvos in the thyroid gland and its effects on the regulatory enzymes associated with hypothalamic pituitary thyroid (HPT) axis (Singh & Parwan, 2009).

4. CONCLUSION

Chronic 2, 2-dichlorovinyl dimethyl phosphate exposure caused a decrease in the levels of testosterone, T4, T3, FSH, LH, while there were increases in the levels TSH and prolactin for both oral and inhalation routes, and across the duration of exposure

ETHICAL APPROVAL

Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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