

TOXIC EFFECTS OF CHLORPYRIFOS INSECTICIDE ON THE TESTIS OF PIGEON (COLUMBA LIVIA DOMESTICA)

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Abstract

The use of pesticides is increasing rapidly as a result of vector-borne diseases and modern agriculture, which harm human beings, the environment, and wildlife including birds. The diversity of birds' species to be a global decline. Pesticide contamination is a significant contributing factor to these declines, it is imperative to take this possibility into consideration. Birds are significant economically because they are the most valuable source of food and a vital component of the ecosystem. Therefore, the goal of the current study was to assess the testicular toxicity of organophosphate insecticide in pigeons. In order to assess the level of toxicity, eighty (80) healthy male Pigeons (*Columba Livia domestica*) were kept in neat and clean wooden cages. Throughout the experimental study a mixture containing 22% crude proteins and equal quantity of water were given to every pigeon. After acclimatization for fifteen (15) days pigeons were assigned in four equal groups (A, B, C, and D). For 84 consecutive days, each insecticide test group received oral doses of 1/25th, 1/20th, and 1/15th of the LD50 (1.3 mg, 1.6 mg, and 2.1 mg/kg body weight/day) of chlorpyrifos (CPF), while group (A) pigeons serving as the control. Birds from groups B, C, and D in this study exhibited significant clinical signs during the experimental study, including reduced food intake, tremors, salivation, open mouth breathing, ruffled feathers, lethargy, obvious depressive and dull symptoms, isolation, a decline in mating frequency, and watery diarrhea. The birds in the C and D groups shown a highly significant ($P<0.01$) decrease in body weights after exposure to CPF, while the B group observed with a significantly ($P<0.05$) decrease in body weight when compared to the birds in the control group. The testicular weights of the B, C, and D groups showed a highly significant ($P<0.01$) decrease due to the harmful effects of chlorpyrifos. At low dose of chlorpyrifos, the testes of group B pigeons showed condensed seminiferous tubules with empty lumens. The birds in the C and D groups showed hypertrophy/tumor between interstitial spaces, no spermatogonia, absence of Leydig's cells, and vacuolation in the tubules. The chlorpyrifos insecticide induced alterations in testicular histology of pigeons. The eco-toxicological risks of CPF insecticide to birds and other non-target organisms are evaluated, however, current research has shown that the chlorpyrifos insecticide has produced toxic effects on pigeons' male reproductive organs.

Keywords: Toxic Effect, Chlorpyrifos, Histological, Testis and Pigeon

INTRODUCTION

The widespread and indiscriminate use of pesticides to eradicate noxious insects and other pests in the agricultural sector is becoming more common, and its eco-toxicological effects are getting more attention. These chemical substances may increase yield

production, but the extensive use of pesticides is having a negative ecological impact. Pesticides are also used in the livestock and poultry industries to eradicate ticks, fleas, mosquitoes, and other pests. [1]. The undesirable effects of toxic chemicals cause various physical and biochemical abnormalities in both humans and animals [2]. In order to control and eradicate a wide variety of fruit fly, flea, tick, mite, and mosquito species, numerous broad spectrum pesticides are applied in homes and gardens [3]. Synthetic pesticides use is a significant source of environmental contamination and a serious health risks to the general public [4].

Organophosphates (OP) are commonly used in both developed and developing countries to eradicate or control noxious pests in the agricultural sector [5]. They cause notable physiological and cellular changes in exposed organisms by inhibiting the activity of the cholinesterase and acetylcholinesterase enzymes, [6]. Chronic exposure to organic pollutants (OP) can significantly impact exposed organisms, leading to severe health complications and ultimately reducing their lifespan [7]. Nonetheless, laws and enforcement pertaining to pesticides are absent from the majority of developing countries. They import and use a lot of organophosphate insecticides, which are illegal and unregulated [8]. Alzheimer's, asthma, cancer, and other diseases are associated to pesticide exposure that contains neurotoxins. Living beings can be frequently exposed to pesticides through a variety of channels, such as soil, contaminated food or water, or direct or indirect contact with the pesticides [9]. Organophosphate pesticides (OPs) significantly harm human health, particularly in diverse mammal and avian species [10], because the birds usually eat insects, grains, fruits, and fish contaminated with pesticides, the residue of these dangerous chemicals may seriously alter the organs those responsible for metabolism and reproduction [11].

The chlorpyrifos (CPF) is a common organophosphate insecticide used to manage noxious pests in the fields of agriculture, livestock, poultry, and public health throughout the world. It is also used to control stored food and grains. Typically, the liver metabolizes CPF, and the kidneys remove its metabolites [12]. Animals' livers and kidneys may suffer from prolonged exposure to CPF, which can also lead to serious physiological problems [13]. Pesticides are poisonous substances that are easily absorbed by the environment. There, they react and quickly decompose into their metabolites, which build up in the ecosystem and affect all living being primarily predatory bird species. When an organophosphate pesticide gets into the food chain, it harms the exposed organism's nervous and reproductive systems. An (OP) insecticide (CPF) can find its way to enter in the food chain from insects to small birds, and from small birds to raptors like vultures, hawks, and eagles. As a result, pesticide accumulation in birds can harm their health and cause population declines. Consequently, the goal of the current study was to assess the intensity of histopathological effects of chlorpyrifos on the main reproductive organ of male birds.

MATERIALS AND METHODS

Experiment design and birds:

In the current study, 80 (eighty) male mature pigeons (*Columba liviademestica*) pigeons those in healthy conditioned were used to assess the testicular toxicity of the chlorpyrifos insecticide. Before the experiments began, all of the pigeons were vaccinated and acclimatized for fifteen (15) days in a standard and controlled laboratory conditions. They were kept in neat and clean wooden wire cages. The pigeons were given clean drinking water and normal feed (grains and seeds). After acclimatization divided into four equal groups (A, B, C and D) comprising of twenty pigeons each group and kept in separate cages. Prior to the trials, their body weights were recorded. Group a pigeons were assigned as the control birds and pigeons from group B, C, and D were designated as CPF insecticide treatment groups.

Preparations for insecticide and the study protocol

Using toxicology techniques, the (Chlorpyrifos 40EC) insecticide concentration was prepared on an LD50 basis [14]. The oral treatment was administered at 1/25th, 1/20th, and 1/15th of the LD50 (1.3 mg, 1.6 mg, and 2.1 mg/kg body weight/day) by adding one ml. of corn oil for consecutive period of 84 days, while birds in group A served as the control group. Throughout the study, all pigeons were fed a daily feed mixture consisting of 22% crude protein (grains and seeds) in their diet along with the same amount of water.

Observation of clinical signs, deed intake, body and weights and histological procedure:

Clinical and stress-related signs, symptoms, and behavioral changes in pigeons of all groups were observed twice a day through the experimental period. Food intake, body weights, and the histological procedures were observed. Eight randomly chosen pigeons from each group were sacrificed and dissected at the end of experimental study, with their body weights of each. Testicular absolute and relative weights were recorded. Testes were retained and preserved for 24 to 48 hours in Bouin's solution in order to evaluate histopathological changes. To dehydrate testicular tissues, a graded series of alcohol was employed, and tiny testes pieces were embedded in paraffin wax. By using of the Rotary microtome, thin tissue sections measuring 5 to 6 μm were cut and stained with Eosin (Putt, 1948) and Harris's hematoxylin (Gurr, 1956). Additionally, the Best Scope LCD digital biological LCD microscope BLM 260 was used for histological analyses and microphotography.

Statistics analysis:

The data was assessed by factorial one-way analysis of variance (ANOVA). Values defined as Mean \pm SD were considered significant at the ($P < 0.05$), ($P < 0.01$), and ($P < 0.001$) levels. The statistical software, Statistics 8.1, was used to analyze the mean difference between the groups using the least significant difference (LSD) test.

RESULTS AND DISCUSSIONS

The current study was aimed to evaluate correlations and deviations in the testicular tissues of pigeons exposed to the chlorpyrifos insecticide. Therefore, the goal of the current study was to assess the intensity to which CPF had histopathological effects on a vital reproductive organ in male pigeons. CPF is one of the widespread organophosphate (OP) insecticide used for killing a variety of noxious pests on residential gardens, agricultural crops, and inside homes. It exerts a direct effect on the nervous systems by inhibiting the acetylcholinesterase enzyme. The pigeons in the control group of the current study remained healthy and active throughout and showed no clinical symptoms. Nevertheless, pigeons receiving low doses of the CPF exhibited mild to moderate clinical signs, including tremors, diarrhea, dullness, and less frequent crowing, following exposure to chlorpyrifos insecticide. Hens exposed to chlorpyrifos also showed similar outcomes. [15]. These findings about symptoms and indicators that have previously been documented in living beings after exposure to organophosphate insecticides like CPF are linked to cholinesterase inhibition [16, 17].

As a result, in the current study, metabolites of chlorpyrifos were connected to cholinergic toxicity, which showed up in birds in all exposed groups as clinical signs, symptoms, and behavioral changes. [18]. Chlorpyrifos poisoning can contemporary with a variety of clinical symptoms. Reported as decline in egg weight, eggshell thickness, hatchling weight, and body weights. [19]. The pigeons in groups B, C, and D displayed noticeable clinical symptoms during the experiment, such as ruffled feathers, lethargic, marked depression and dullness, open mouth breathing, salivation, decreased food intake, tremors, inactive and decreased motor activities, isolated and watery diarrhea, limb weakness, weight loss, loss of balance, and jerky movement, however the control group pigeons in present study were found in good health and active.

Pigeons in group B after being exposed to CPF insecticide, displayed mild to moderate clinical signs, while groups C and D displayed severe clinical signs and symptoms. During present study from third week to the eighth week of the treatment period, of toxicity-related mortality in eight (06) birds belonging to group B, eight (8) birds belonging to C, and ten (10) pigeons in group D were recorded. There was no mortality noted in the pigeons of the control group. After receiving CPF treatment, the pigeons in the treatment groups showed reluctance to intake of food and drink water (Fig.1). Birds of various species at different dosage levels exhibited diverse consequences in terms of feed intake and body weight [20, 21, 22]. Similar results to the current study have demonstrated that oxidative stress brought on by CPF with the enzymes required for a regular metabolic process led to a decrease in feed intake and body weight [23, 24]. Body weights of pigeons in group B, C and D showed significant ($P < 0.05$) and highly significant ($P < 0.01$) decrease in their body weight (Figures 2) when compared to the pigeons in the control group. The insecticide chlorpyrifos produced significant decline in Pigeons' body weights in a dose-dependent manner. Pigeons kept in the control group's body weight gain was highly significant with 23.98%. Among all the treated groups with chlorpyrifos, group D

experienced the greatest decrease in body weight (24.82%) due to the high dose (80 mg/kg body weight) (Figure 2 and Table 1). Following exposure to chlorpyrifos, birds in groups B, C, and D showed a decrease in their testicular relative weights. The effects of CPF treatment were evaluated in rats, found no correlation between the weight of the rats' organs and their body weight [25]. The decrease in the relative testicular weights in the current study may be related to the acute and sub-chronic effects of CPF exposures of the 84-days duration, as reported in another study [26]. The testes looked smaller than those of characteristic birds. When compared to all other groups, the D group's testicular weight showed numerous ($P < 0.01$) reductions during the chlorpyrifos treatment. (Table 2) as well as exhibited decrease ($P < 0.01$) in the absolute and relative testicular weights of the birds in groups B and C than the testicular weights of the control group. Histological examination reveals testicular congestion, mononuclear cell infiltration, pyknosis, cytoplasmic vacuolation, and nuclei degeneration in each of the treated groups. But the damage was more severe when the amount of CPF exposure was higher.

OP compounds, like CPF, are among the most widely used synthetic pesticides that are harmful to reproduction [27, 28, 29]. It has been demonstrated that alterations in testicular histology occurred on exposure to CPF that decrease the ability of the testes to produce testosterone [29]. In the current study, seminiferous tubular space reduced. The lumen of most tubules became empty. The CPF-treated pigeons in groups B and C exhibited hypertrophy vacuolation in their tubules. Seminiferous tubules were seen to be elongated and irregular, and the tubular spaces significantly shrank. The lumen of the majority of seminiferous tubules was empty, and vacuolization was visible. On the other hand, pigeons exposed to both high and low doses of CPF develop tumors or hypertrophy (Figure 5–6). Similarly, significant associations were found between testicular injury and CPF poisoning in male and female.

The testes may also display histological abnormalities at variable CPF dosage levels, ranging from mild to severe degenerative changes in the seminiferous tubules [30]. Significant histopathological changes were also noted in the testicular tissues of the group D pigeons that were exposed to higher concentrations of chlorpyrifos insecticide. The features of infertility were evident in the elongated form of seminiferous tubules with no Leydig's cells were present in the interstitial cells as well as the empty lumen and vacuolation in the tubules, interstitial spaces with congestion, hypertrophy, and tumor. Leydig's cells showed hypertrophy and seminiferous were seen raptured (Figure.7). The disorder of spermatogenic cells, which includes a decrease in Leydig's cells, a distortion in the lumen of the seminiferous tubules, and a reduction in the thickness of the seminiferous tubules epithelium, may be caus by the effects of OP pesticides. [31, 32, 33]. On the other hand, Leydig's cells were visible in the interstitial space in the control group. The bulk of the cells in the seminiferous tubules were spermatogonia, but spermatocytes were also occasionally seen with a normal articulation structure of the testes and seminiferous tubules. There were seminiferous tubules, which had a rounded and oval shape. The seminiferous tubules contained the Sertoli cells (Figs. 3 and 4).

Male reproductive function may be influenced by long-term exposure to CPF, which may lead to sperm destruction, decreased semen quality, and hormonal and reproductive system alterations [34]. The testicular tissues of pigeons exposed to chlorpyrifos insecticides exhibited severe effects, including vacuolation and an empty lumen. The interstitial space was narrow and elongated, and there was no space at all between the seminiferous tubules. The development of hypertrophy was also evident, clearly demonstrating the elimination of Leydig's. According to the current study's findings, male pigeons exposed to CPF for a sub chronic period could develop clinical symptoms and histological alterations in their male reproductive organs that indicated cellular and mutagenic toxicity.

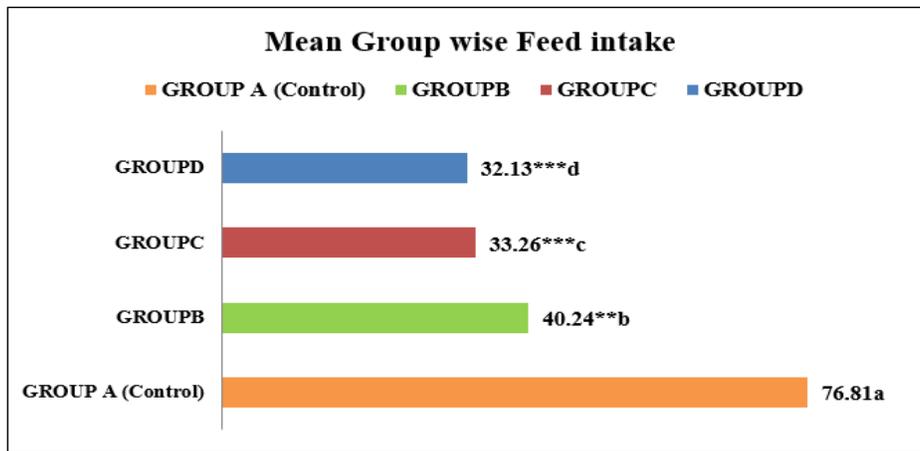


Fig 1: values not sharing same letters showing significant variance with each other when pigeons exposed to CPF

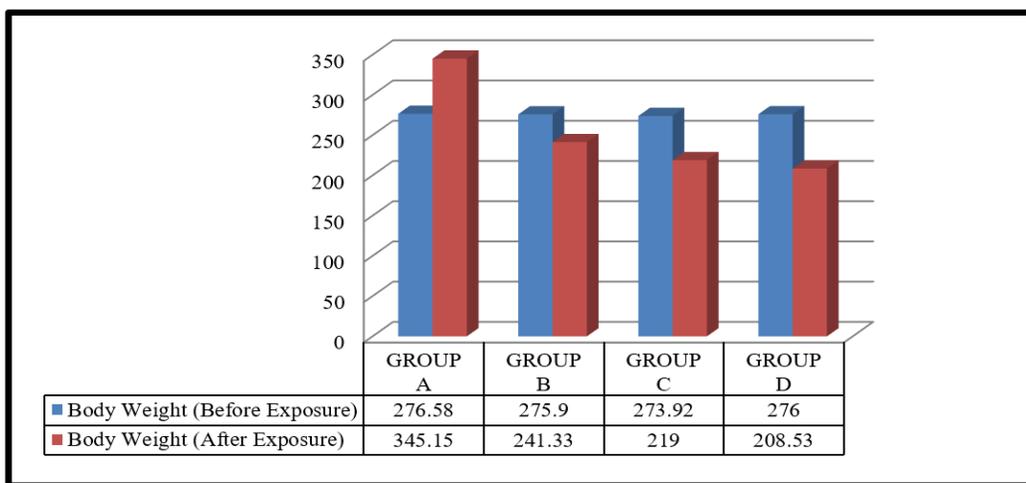


Fig 2: Pigeons of group B, C & D are indicating significant decline in their body weights after exposure to Chlorpyrifos insecticide when compared with before exposure

Table 1: Body weights on exposure to CPF in pigeons of groups B, C & D with significant decline ($P < 0.001$) * as compared to control group.**

Body Weight (BW)	GROUP A (Control)	GROUP B	GROUP C	GROUP D
BW (Grams)	↑ 66.57a	↓ 36.00**b	↓ 54.92**c	↓ 68.55**d
(BW) percentage	↑ 23.98%	↓ 13.02%	↓ 19.97%	↓ 24.82%

Table 2: Testicular weights (Absolute) and relative gonado-somatic index on CPF chlorpyrifos exposures in pigeons of group B, C and D with significant decrease ($P < 0.01$) ** and * as compared to control**

Parameters	Mean Wt. of testis (relative and Absolute Weights) (Mean ± SD)			
	Group (A) Control	Group B	Group C	Group D
Weight of Testes	3.10±0.09 ^a	1.95±0.06 ^{***b}	1.65±0.03 ^{***c}	1.50±0.03 ^{***d}
Relative Testicular Weight	0.90±0.05 ^a	0.81±0.05 ^{**b}	0.75±0.03 ^{***c}	0.72±0.03 ^{***cd}

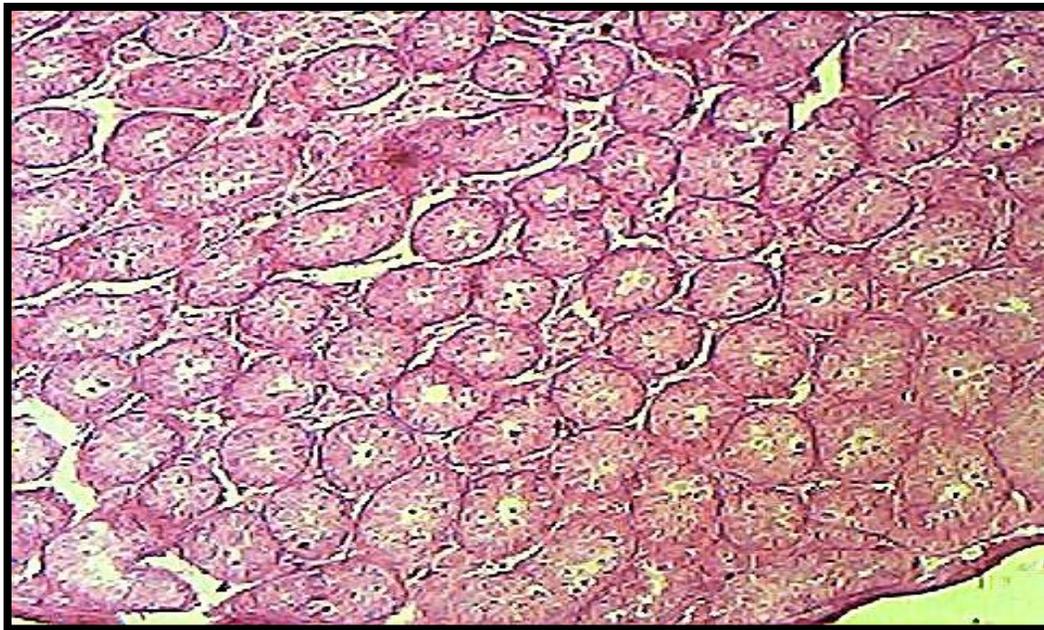
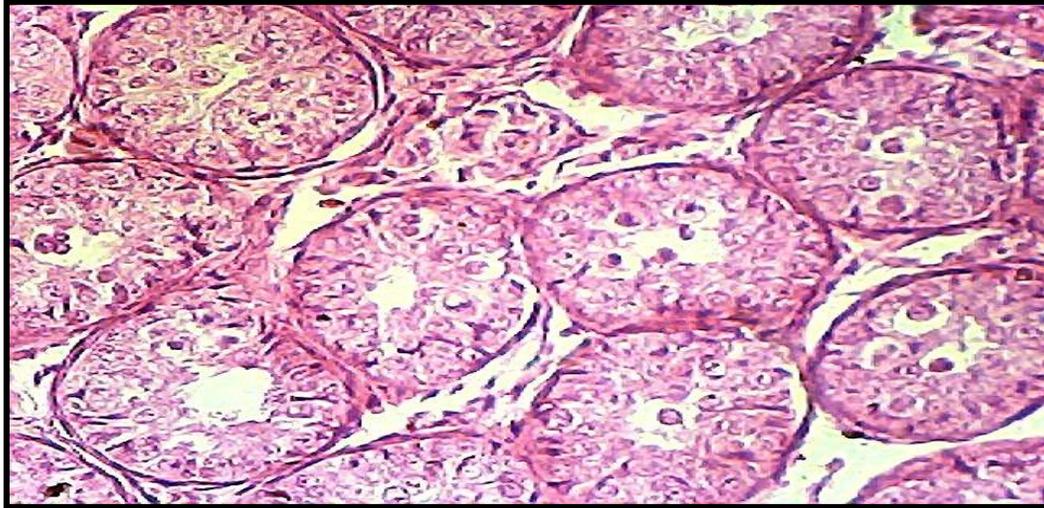
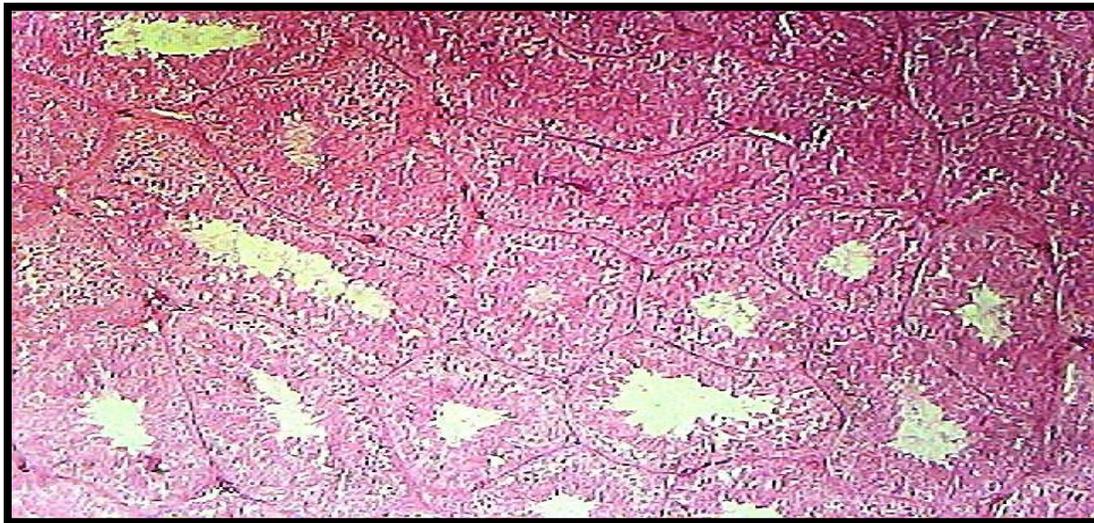


Fig 3: Testis of the Control Group Pigeon X10

Oval/round shaped seminiferous tubules comprising numerous types of spermatogenic cells, spermatogonia and spermatocytes. Interstitial space (lst.) containing Leydig's cells



**Fig 4: Section of testis of the control subgroup Pigeon X 20
Leydig's cells (Ldg.) in the interstitial space, Spermatogonia(SSg.)
Spermatocytes scatteredly present in throughout the seminiferous
tubules**



**Fig 5: Histological section of testis of the treated Pigeons in group B on low
dose days X 10. Space reduced between seminiferous tubules, lumen of
tubules is empty of spermatids and spermatozoa. Vacuolation was also
distinguished throughout the seminiferous tubules**

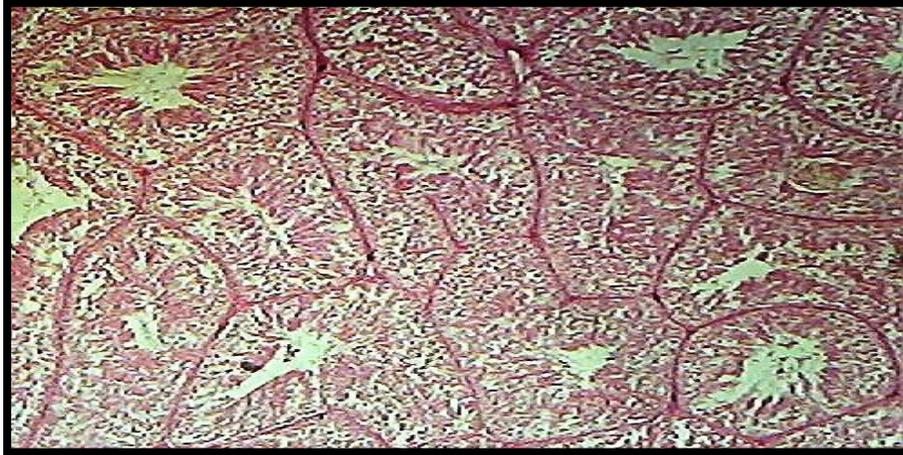


Fig 6: Histological section of testis in the Pigeon of group C exposed to Chlorpyrifos X 10.

Vacuolation, no leydig's cells were observed, no spermatogonia were present in the vicinity of the seminiferous tubules. Hypertrophy between interstitial spaces were seen

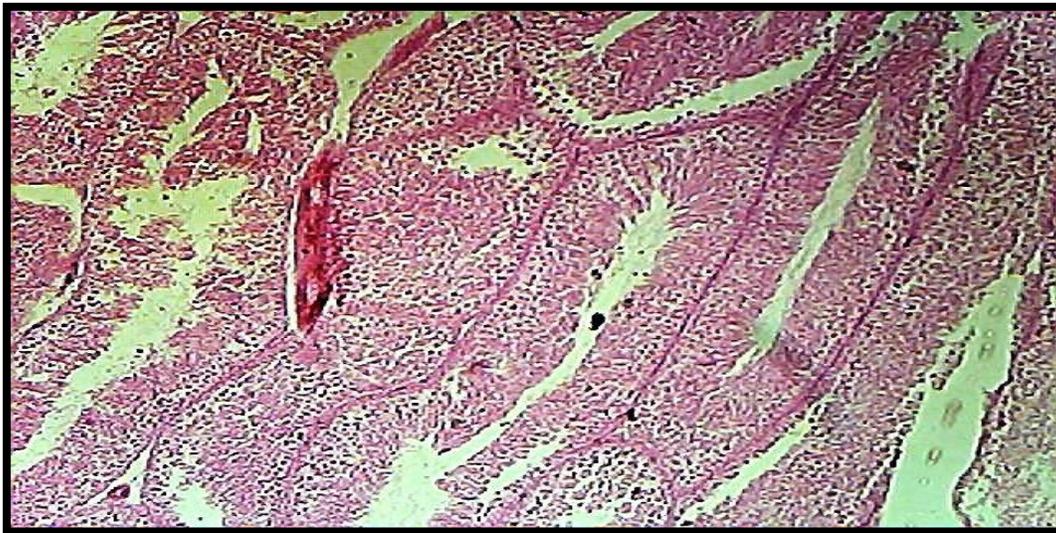


Fig 7: Histological section of testis of the Pigeon D exposed to Chlorpyrifos X 10. Space between seminiferous tubules reduced, elongated and irregular seminiferous tubules seen, empty lumen in majority of seminiferous tubules with vacuolization. Hypertrophy formation was observed with no Leydig's cells

References

- 1) Zhang W, Jiang F, Ou J (2011) Global pesticide consumption and pollution: with China as a focus. *ProclntAcadEcol Environ Sci* 1(2):125
- 2) Daley, H., Doyen, J.T., Purcell, A.H. III., 1998. Introduction to insect biology and diversity, 2nd edition. Oxford University Press. New York, New York. Chapter 14, pp 279 - 300.
- 3) Khan, M.A., Iqbal, M., Ahmad, I., Soomro, M.H., Chaudhary, M.A., 2002. Economic evaluation of pesticide use externalities in the cotton zones of punjab, Pakistan [with Comments]. *The Pakistan development review*, 683-698.
- 4) Akhtar, W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology* 2, 1-12.
- 5) Food and Agriculture Organization – FAO. (2019). Pesticide use. Retrieved from <http://www.fao.org/faostat/en/#data/RP>
- 6) Khan, M. I., Shoukat, M. A., Cheema, S. A., Arif, H. N., Niazi, N. K., Azam, M., Bashir, S., Ashraf, I., & Qadri, R. (2020). Use, contamination and exposure of pesticides in Pakistan: a review. *Pakistan Journal of Agricultural Sciences*, 57(1). <http://dx.doi.org/10.21162/PAKJAS/20.7437>
- 7) Waheed, S., Halsall, C., Sweetman, A. J., Jones, K. C., & Malik, R. N. (2017). Pesticides contaminated dust exposure, risk diagnosis and exposure markers in occupational and residential settings of Lahore, Pakistan. *Environmental Toxicology and Pharmacology*, 56, 375-382
- 8) Roberts, T.J. *The Birds of Pakistan*. Vol. I. Non-Passeriformes. Oxford University Press. Karachi, 1991, pp 598.
- 9) John E Cooper, The role of birds in sustainable food production. *Journal of Biodiversity Conservation*, 1995, vol. 4, issue 3, pp 266-280.
- 10) International Ornithological Committee, <http://www.worldbirdnames.org/> 2014
- 11) John E Cooper, The role of birds in sustainable food production. *Journal of Biodiversity Conservation*, 1995, vol. 4, issue 3, pp 266-280.
- 12) Roger Ledere, Medicinal uses of birds <http://ornithology.com/medicinaluses-of-birds>. 2015.
- 13) Gregory, R.D., Noble, D.G. & Custance, J. (2004) the state of play of farmland birds: population trends and conservation status of lowland farmland birds in the United Kingdom. *Ibis*, 146, 1–13
- 14) Kati, V and C. H., Sekercioglu (2006) Diversity, ecological structure, and conservation of the landbird community of Dardia reserve, Greece , *Diversity and Distributions* 12, 620–629
- 15) Slotkin, T.A., Seidler, F.J. and Fumagalli, F. (2007). Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. *Environmental Health Perspectives*, 115(6): 909-916.
- 16) Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., Mc. Khann, G., Mobley, W.C., Nadel, L., Neubert, D., Hermann, R.S. and Spencer, P.S. (2008). Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Critical Reviews in Toxicology*, S2: 1-125.
- 17) Suliman., Khan, A., Shah, SA., Gulfam, N., Khisroon, M and Zahoor, M. (2020) Toxicity evaluation of pesticide chlorpyrifos in male Japanese quails (*Coturnix japonica*) *Environmental Science and Pollution Research* 27: 25353–25362
- 18) Gile JD, Meyers SM. 1986. Effect of adult mallard age on avian reproductive tests. *Arch Environ. Contam. Toxicol.* 15, 751-756.

- 19) Kamrin, M.A. (1997). Organophosphates in pesticide profile: Toxicity environmental impact and fate, CRC Lewis Press, Michigan. 135240.
- 20) Malik G., Dahiya J.P., Gera S., 2004. Biochemical studies on chlorpyrifos toxicity in broiler chickens. *Indian J. Anim. Sci.*; 74: 473-476.
- 21) Joshi SC, R Mathur and N Gulati, 2007. Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat. *Toxicol Ind Health*, 23: 439-444.
- 22) Meggs WJ and KL Brewer, 2007. Weight gain associated with chronic exposure to chlorpyrifos in rats. *J Med Toxicol*, 3: 89-93.
- 23) Ambali SF, DO Akanbi, M Shittu, AG Giwa, OO Oladipo, and JO Ayoa, 2010. Chlorpyrifos-Induced clinical, hematological and biochemical changes in swiss albino mice- mitigating effect by co-administration of vitamins C and E. *Life Sci J*, 7: 37-44
- 24) Ambali SF, DO Akanbi, OO Oladipo, LS Yaqu and MU Kawu, 2011. Sub-Chronic chlorpyrifos induced clinical hematological and biochemical changes in swiss albino mice: Protective effect of vitamin E. *Int J Bio Med Res*, 2: 497-503.
- 25) El-Desoky G, A Mohammed, ALO Abdulaziz, ALO Zeid, M Mohamed and Y Kareen, 2012. Potential hepatoprotective effects of vitamin E and selenium on hepatotoxicity induced by Malathion in rats. *Afr J Pharm Pharmacol*, 6: 806-813.
- 26) El-Deep AEA, IMA El-Aleem and SG Sherin, 2007. Harmful effect of some insecticides on vital parameters of albino rats. *J Egypt Soc Toxicol*, 36: 53-60.
- 27) Kammon AM, RS Brar, S Sodhi, HS Banga and S Sodhi, 2010. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. *Vet Arhiv*, 80: 663-672.
- 28) Kammon AM, RS Brar, S Sodhi, HS Banga, J Singh and NS Nagra, 2011. Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. *Open Vet J*, 1:21-27
- 29) Shittu, M., Olatunji, A.O., Ambali, S.F., Oyedepo, I.T., Kawu, M.U., Yusuf, P.O., Kobo, P.I., and Isa, H.I. (2014). Amelorative effect of hibiscus sabdariffa linn on subchronic chlorpyrifos induced alterations in sex and thyroid hormone in male Wistar rats. *American Journal of Pharmacology and Toxicology*, 9(1): 96 – 106.
- 30) Akhtar N, MK Srivastava and RB Raizada, 2009. Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*. *J Environ Biol*, 30: 1047-1053.
- 31) Sikka SC., 2009. Relative impact of oxidative stress on male reproductive function. *Curr Med Chem* 2001; 8: 851-862. 40. Uzun FG, Kalender S, Durak D, et al. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food Chem Toxicol.*; 47: 1903-1908.
- 32) Ferdinand N, Pierre W, Augustave K, 2014. Effect of Dimethoate (an organophosphate insecticide) on the reproductive system and fertility of adult male rat. *Am J Pharmacol Toxicol*; 9: 75-83.
- 33) Farag AT, Ahmed F, Aswad E, 2007. Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. *Reprod Toxicol.*; 23: 232-238
- 34) Miranda-Contreras L, Gomez-Perez R, Rojas G, Cruz I, Berrueta L, Salmen S, Colmenares M, Barreto S, Balza A, Zavala L, Morales Y, Molina Y, Valeri L, Contreras CA, Osuna JA, 2013. Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among Venezuelan farm workers. *Journal of Occupational Health* ;55(3):195–203