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Effects of chlorpyrifos on testicular biochemistry and physiology of male sprague dawely rats

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Abstract

Pesticides and insecticides are essential piece of our organobusiness framework against the pests and insects to get better yields of crops. On the other hand, chemicals and reagents used in pestisides and insectisides are harmful and carcinogenic for other living organisms chlorpyrifos is broadly used organophosphate pesticide throughout the world but too much use of this organophosphate may have serious health issues and it is examined that it may cause infertility in the human beings. So it is important to control over and misuse of these pesticides. Therefore, this research was proposed to examine the hazardous effects of CPF on health of living organisms. Specially, male albino rats were used to check the degenerative effects of CPF on the testis. Different groups of male rates were exposed with CPF drug with different concentrations orally for 28 days of trial on consistent schedule. After completion of successful trial blood samples and testis sample were taken by disecting each rat according to their groups and hematological and biochemical analysis uncovered noteworthy damaging effects of CPF in the blood samples. Hematological and serum compound profiles of rats show increment in WBC's, PLT, LYM, Total Bilirubin, Glucose, ALP, ALT, and AST. Generous diminishing in the dimensions of HCT, MCV, MCH, MCHC, RBC's and HGB was noticed. Histology of testis of rats under microscopic observation shows the degenerative effects of CPF on the spermatogonial cell of testis as compare to the control group. So current investigation suggests that chlorpyrifos may cause histological, hematological and biochemical changes in the living organisms and should be banned over or misuse of CPF by world health organisations.

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Introduction

Pakistan is an agricultural country and the biggest user of pesticides in South Asia on the crucial demands of agriculture. Chemicals used in agricultural kill the harmful pests, insects and creepy crawlies that are harmful to the agricultural fields and can decrease the product. On the other hand, chemicals and reagents used in pesticides and insecticide are harmful and carcinogenic for a human being. It is a global survey that almost 200,000 die each year by organophosphorus pesticide poisoning in developing countries. Air and underground water contaminated with pesticides can cause serious health issues and can cause harmful effects on residential and occupational settings (Yadav *et al.*, 2015). There are many types of pesticides used in Pakistan majorly there are five kinds of pesticide used, these are wood preservative fungicides, herbicide, insect repellents, insect killer insecticides and rodenticide. Many other pest killers are used but the most commonly used pesticide chemical is chlorpyrifos.Chlorpyrifos is a colorless crystalline CPF 0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate (Muller *et al.*, 2000). Chlorpyrifos is very important for pesticide because it avoids the growth and development of harmful creepy crawlies that may cause diseases in crops. Scientists accept that by using this poisonous pesticide, the outcome of crops will be useful for the formers and play a great role in the availability of cheap and plenty of food worldwide (Eaton *et al.*, 2008).Chlorpyrifos comely present in different brands name in market Lorsban, Dursban, and Renobamn. It acts as prohibiting chemical by acting the sensory system of pests and insects by hindering acetylcholinesterase, and is an organophosphate pesticide (Rathod and Garg, 2017).

Organophosphate is the most widely used insecticide. Chlorpyrifos is an organophosphate pesticide, which has a major harmful effect on the health of humans. CPF also has a degenerative effect on the testicular structure of testes and severe exposure of CPF may cause infertility in humans. Exposure of CPF also disturbs the testosterone level in the blood and blood biochemistry. Chlorpyrifos also brought about a marked reduction in epididymal and testicular sperm counts in exposed males and a decrease in serum testosterone concentration. All these toxic effects are moderate at low doses and become severe at higher dose levels (Joshi *et al.*, 2007).

There are serious drawbacks to the environment by the overuse of pesticide chemicals for the killing of pests. Overuse of agricultural insecticides and pesticides have serious health issues (Chauhan and Singhal, 2006; Mahmood *et al.*, 2016). 95% of herbicide and over 98% of insecticide that are sprayed on crops reached on a destination other than their target species. These hazardous chemicals absorbed by soil and mixed with groundwater that is harmful and causing deadly diseases in humans and other living organisms. On the other hand, there are great chances when these chemicals are sprayed on crop containment the fresh air and these hazardous chemicals cause serious health issues (Hernández *et al.*, 2013). Also, overuse of these hazardous chemicals destroys the natural habitat of living, and cause the serious deficiency of nitrogen-fixing bacteria that plays an important role to fix nitrogen from the air from the soil for proper growth of plants to make organic compounds (Ergonen *et al.*, 2005; Hernández *et al.*, 2013). The high level of chlorpyrifos found in the urine sample of urban and rural residents of Lahore indicates that this chemical which is the main component of agricultural pesticides is mixed in the food and drinking water to the extremely dangerous level. Chlorpyrifos harm the environment and among the commonly utilized insecticide for self-harm (Eddleston, 2008; Lajmanovich., 2015; Zhou and Li, 2018;).

There are many studies on pesticides, chlorpyrifos is one of then that are linked with lungs cancer (Tanvir *et al.*, 2016). It may retard the mental development of a child when it is exposed to a mother during pregnancy because chlorpyrifos is commonly used pesticide for agriculture it was banned in 2001 US for residential use. Even mild exposure of CPF may cause headaches, nausea, sweating, increased saliva an eye-watering. Intermediate poisoning may cause impaired

vision vomiting diarrhea, muscle weakness and muscle spasms. Severe exposure of CPF symptoms includes paralysis, suffocation from lung failure, unconsciousness and seizure. On the exposure of CPF on children, they experience excessive saliva than sweating and tears, sleepiness or coma, and muscle weakness (Gibson *et al.*, 1998).

3,5,6-trichloro-2-pyridinol (TCP) is a primary metabolite of CPF, it is studied that over 82% of the population agricultural areas of Asia are affected by TCP which is indicated by the survey of urine tests (Ededdleston, 2005).

CPF exposed on a person to a low dose for long term or higher concentration of CPF is exposed may lead to the acute toxicity and cause serious health issues. On child or fetuses, a very low concentration may be more hazardous (Landrigan *et al.*, 1999). Coronary heart diseases and atherosclerosis is caused by the disturbance in the serum and lipid profile (Gofman *et al.*, 2007). Exposure of CPF chronically and acutely causes damage in the DNA of the brain and liver cells. By this study, it is confirmed that CPF has a genotoxic effect in vivo. Organophosphate is 1/3 of commonly used pesticides in rural areas of Asia (Eddleston *et al.*, 2005).

WHO classify the different types of pesticide based on their harmful effect on the environment and living organism. CPF classifies as moderately hazardous of class 2nd. There is a different concentration of CPF dose for complete infection in the body for different types of living organisms or experimental animal models based on their body weight. LD_{50} dose of CPFfor experimental animals ranges from 32 to 1000 mg/Kg. when rabbits are used as an experimental animal then LD_{50} will be 1000 to 2000mg/Kg. for rats, it will be 2000mg/Kg (Zhao *et al.*, 2006). Excessive use of pesticide or agrochemicals affect the environment and increase pollutants in the fresh air. Over 95% of agrochemicals do not approach their target insects these excessive chemicals induce carcinogenic and mutagenic on other microorganisms like nitrogen-fixing bacteria and other beneficial bacteria in the soil. So, these genotoxic effects in microorganisms may cause disturbing effects in the environment caused by agrochemicals (Pimental, 1971).

Chlorpyrifos is an agrochemical that is not degraded by routine processes in the environment. Degradation of agrochemicals is very crucial for being not a part of the food chain. If pesticides like CPF are not degradable, underground water is contaminated with hazardous chemicals which may cause serious health issues on drinking contaminated water. On the other hand, the pest may produce resistance against agrochemicals (Mandour, 2012).

The objective of this study minimizes the deadly effects of chlorpyrifos and other agrochemicals having a serious hazardous and dangerous effect on the testicular physiology and biochemistry of Sprague dawely rats and to produce awareness among farmers against the excessive use of these chemicals affect the natural environment of living which is also harmful to the crops and their products.

Materials and methods

Pesticide and animal model

Chlorpyrifos was purchased from the market, which was present in different brands names in the market. Like Renbmn, durshban and lorsban with molecular formula of 0, 0-diethyl-0-3, 5, 6-trichloro-2-pyridyl phosphorothioate. One twenty healthy male Sprague Dawely rats of the same weight and same age were taken from animal House of the University of Agriculture Faisalabad. These male rats were kept in clean and sterilized condition according to the biosafety rules in the Animal House of Government College University Faisalabad. Temperature is maintained at 25°C in animal house. The animals were acclimatized for two weeks before treatment.

Experimental design

LD_{50} for CPF for Sprague dawely rats is 140 mg/Kg. these are divided into eight group T0, T1, T2, T3, T4, T5, T6 and T7each containing five male rats for elaborated study of the biochemical and physiological

changes occurring in the body of experimental animals due to chlorpyrifos. One of these groups is the control group To treated with only pure water and proper feed daily. Other groups T1, T2, T3, T4, T5, T6 and T7 are treated with 1/3 LD_{50}, 1/4 LD_{50}, 1/5 LD_{50}, 1/6 LD_{50}, 1/ 7 LD_{50}, 1/8 LD_{50} and 1/10 LD_{50} lethal dose of chlorpyrifos was given orally on daily basis according to their body weight for four weeks and then biochemical and physiological changes are checked after 4 weeks. After four weeks of a consecutive dose of CPF with different dose concentration according to the protocol designed based on rat's body weight and good feed, and pure water, each group of rats is dissected and blood samples and testes samples of each group were taken and preserved for further biochemical and physiological checkups.

Histological changes

To check the effect of CPF on testes of different experimental and control group rats divided according to the different concentrations of CPF dose, histopathological tests are done under microscopic observation to check the damaging effect of chlorpyrifos on testicular cellular morphology and cellular structure **(**Sayım, 2007).

Preparation of tissue microscopy

After dissection of male rats, testes were taken into 10% formalin (100ml) and normal saline 900ml in 25 ml falcon tubes labeled according to the groups. Testes samples preserved in 10% formalin solution for 48-72 hours (Drury and Wallington, 1967).

Preparation of tissue for segment cutting

This was finished by the paraffin wax installing technique. Segment cutting is done by the paraffin wax installing technique. After preserving samples in 10% formalin samples were taken in ethyl alcohol for washing and cleaning purposes. Paraffin wax was electrically warmed and tissue samples were saturated into liquid paraffin to fix in the wax. Cooling of paraffin to fix tissues require 2-4 hours. Casting (block making) was finished by taking Lockhart's L-formed molds of required size segments of the required size which is 5-7 µ thickness were cut with revolving microtome. The segment of tissue fixed on a slide by putting a drop of Mayer's egg and fixed the slid in position and place the slide to dry.

Steps of staining the dissection

Staining of tissue slide was done by Harris Hematoxylin and Eosin stain. Slides were placed on a hot plate for melting paraffin then slide treated with xylene for 3-5 minutes then xylene was removed by absolute alcohol 1/2-1 minutes then rinse the slid with running tap water. Slides were treated with Harris Hematoxylin solution for 5-7 minutes. After rinsing with tap water slides were treated with 1% acid alcohol solution for 1/2-2 minutes. Color of slides changes from blue to red by the action of acid. Rinse the slide with water then treated with 1% aqueous-eosin for 1-2 minutes excess stain was washed with water then dehydrate slide with alcohol. Slides were mounted with DPX (Distrain Plasticizer Xylene) solution. Then covered with coverslip. Slides were left overnight to dry completely so that coverslips were adherent to the slides.

Determination of hematological profiles

Blood samples were taken before dissection after giving local anesthesia and blood was taken from the jugular vein. Blood for the hematological study was collected using the orbital technique (Bernardi *et al*., 1996) into a sample bottle containing ethylene diamine tetra acetic acid (EDTA). Immediately after blood collection, the sample bottle was gently shaken to mix up the blood with the EDTA to prevent clotting or lysing of the blood. Different hematological tests are determined. These hematological tests were PVC (Packed Cell Volume), hemoglobin concentration, erythrocyte count, platelet count and leukocyte count. The PVC was carried out by the micro hematocrit method (McGovern *et al*., 1955). The hemoglobin concentration was identified by using the cyanmethemoglobin method while the erythrocyte count was determined by using the hemocytometer method (Schalm *et al*., 1975). The platelet count was identified by using the Ress-Ecker method (Brown, 1976) and the total leukocyte count was carried out by

using the hemocytometer method (Schalm *et al.*, 1975) while the differential leukocyte count was determined using the Leishman technique (Mathur *et al.*, 2013).

Red blood corpuscle (RBC) count

RBC count for each group of rats was determined by Neubauer hemocytometry used to count RBC's under a microscope hemocytometer is a graded slide. Hemocytometer was washed with 70% alcohol and the blood sample was dropped by micropipette on a slide and covered with cover slide and examined under a microscope for a blood count. RBC count was determined by the following formula; Number of cells × dilution factor × depth factor / Area counted (Friedmann *et al.*, 1969).

Estimation of hemoglobin concentration (Hb)

Hemoglobin concentration was determined by sahli's hemoglobinometer method. Usually, hemoglobin was reported as a gram of Hb per 100ml (g/dl or g%). The amount of hemoglobin can be determined by the conversion of a known volume of blood into acid hematin by the addition of dilute HCl (N/10 HCl) and calorimetric comparison with a suitable standard. Draw blood in hemoglobinometer tube after 10 minutes add distilled water drop till the color of the solution is asme ass that of Comparator Box color strips. Observe the readings in hemoglobinometer in Hb gram % (Sahli, 1962).

Estimation of mean cell volume (MCV)

MCV was assessed via micro haematocrit method (Schalm *et al.*, 1975). The blood used to be unceasing of capillary pipes holding the anticoagulant, using capillary action rendering to 2/3 on their length. The tubes had been nominated to permit blood to flow in the way of giving up or after granting enough area in conformity with preventing outflow then the opposing ends were closed. The backyard concerning the capillary pipes had been spread broadly over blood and the index toe was once situated upon the poachy ends in agreement withhold the column concerning the blood into the area as the differing dead ends have been forced amongst the sealing cloth in agreement with shape a close plug. The capillary pipes then placed of the centrifuge along the sealed ends coaching form then centrifuged at 12,000 rpm for 5 minutes. MCV was decided by way of rolling the capillary tubes a reader card till the highpoint of the plasma column was united with a 100% line and the lowest about the packed erythrocytes was once of the zero lines. The block to that amount exceeded the top regarding the packed erythrocyte stupor characterized the MVC of percent.

White blood corpuscles (WBC) count

Blood is pinched from the flask of WBC pipette above to 0.5 marks and promptly the thinning liquid is broad on in conventionality with 1911 marks. The solution is mixed wholly via trembling gently. The rest on the process is equally as defined via (Davidson & amp; Henry, 1969) for RBC count. In the case of WBC, the count used to be done between larger squares on the chamber. The WBC count used to be uttered in cu mm.

Differential leukocyte count

A drop of blood was positioned on a spotless glass slide concerning 1-2 cm from one finish with the help of a dispersal slide located at an associate degree angle of 45° about the drop of blood was shown quickly on the line of management of the propagator with the slide. The slide was positioned flat on glass rods over a sink and was covered with Leishman dye. The dye was thinned by the drop by the accumulation of buffered water and stained for 5-7 minutes. The stain was shattered and washed with water and air-dried out and observed under a microscope. Counting was ongoing underneath high power oil entanglement objective from the sting of the smear moving the slur towards the center. Leucocytes were known and also the movement was frequent until a complete a hundred cells were totaled. The values of various morphological diversities were uttered because of the percentage.

Determination of Biochemical Profiles of Blood Serum

The rodents from each group were relinquished when

light ether anesthesia by severance of the jugular veins toward the finish of a month dosing residency. 3 millimeters of the blood test were gathered from each rodent into an axis test tube that was down and out of anticoagulant medicine. The blood tests were permitted to cluster and were hatched at room temperature for thirty min. A short time later, the blood tests were centrifuged at 1000 x g for five min to get a straightforward straw hued blood serum that was utilized to quantify biochemical parameters like centralizations of all-out protein, egg whites, urea and creatinine. Elective biochemical parameters measured inside the blood serum were the exercises of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and soluble phosphatase (ALP). The biochemical parameters were measured with bayer explicit and Clinical Chemistry Autoanalyzer (Bayer & Germany). Though blood serum bilirubin focus was estimated utilizing the methodology. AST and height exercises were assessed as depicted by abuse the indistinguishable auto-analyzer as announced. High mountain fixation was measurable dependent on the enzymatic hydrolysis system (Waziri *et al.*, 2010).

Results

Body weight Gain/Loss

Before the start of the experiment on rats, the initial weight of each group was measured by physical balance to compare it to the end weight of labeled rats. And the weight was measured after 1st week, 2nd week, 3rd week and 4th of the experiment. Weight is measured to check the physiological changes taking place between different labeled groups of rats.

After 1st week of CPF dose to the experimental group weight of the control group increased because of proper feed and proper environment. But the weight of other experimental groups starts decreasing according to the oral CPF dose taken. Groups of high concentration of dose decrease more weight because of infectious effect CPF on the metabolic system of the body. After second week weight of control was increased.

Table 1. Weekly body weight (g) of rats exposed to chlorpyrifos for 28 days.

Groups	Control	CPF 14mg/Kg	CPF 15.5mg/Kg	CPF 17.5mg/Kg	CPF 20mg/Kg	CPF 23.33mg/Kg	CPF 28mg/Kg	CPF 35mg/Kg
Initial Weight	118 ±1.00	118.6 ±2.50	117.33 ±2.51	122 .66±3.05	120 ±3.60	119 ±3.60	121.66 ±1.53	121.66 ±3.05
First Week	125.33 ±1.50	126.33 ±1.53	119.66 ±3.05	126 ±1.00	123 ±3.06	119.3 ±2.80	124 ±2.00	118 ±1.00
Second Week	130.66 ±1.53	129.33 ± 2.52	129.33 ± 2.52	128.33 ± .57	119..33 ± 2.08	117.66 ± 1.53	114.66 ± 1.53	109.66 ± 3.05
Third Week	137.33 ±1.53	119.66 ±1.53	116.66 ±2.52	112.33 ±.58	115.33 ±1.53	112.66 ±1.52	111 ±1.00	101.33 ±2.09
Fourth Week	142.33 ±1.53	112 ±1.00	114.33 ±2.09	111.33 ±1.50	110 ±1.00	109.33 ±2.53	104.33 ±1.52	101.33 ±.59

Data are represented as mean ±S. E

After the second, third and fourth weeks, the significant difference in the body mass of control and treated groups was observed. The weight of the control group increased progressively but in treated groups, the weight was not increased because of the pathological effect of chlorpyrifos on the body.

In the group treated with the higher dose of CPF (chlorpyrifos) the weight reduced according to the dose concentration because of highly hazardous effect CPF. Weight was reduced according to the dose given to the rats. The weight of all groups including control groups and experimental groups during the whole time of the experiment is given in (Table 1).

Biochemical analysis

Table 2 showed the biochemical results of the blood of rats (Sprague Dawely) after 28 days of treatment. After 28 days of treatment of albino mice, the significant differences in the biochemical parameters of the blood of treated rats and the control mice were recorded. The total sugar level, bilirubin, AST, ALT, ALP increases significantly ($P<0.05$) among all treated rats in comparison with all control groups.

According to experimental trials after 28 days of different dose concentration in the experimental groups, blood biochemical analysis of glucose level shows that control group shows normal glucose level

because of no pesticides are given orally and proper feed and environment was given. But in the case of experimental groups shows a high level of glucose in blood samples which are taken after 28 days. In control group glucose level observed was 21 mg/dL, while groups of rats treated with 14mg/Kg of body weight, 15.5 mg/kg of body weight, 17.5mg/kg of body weight, 20mg/kg of body weight, 23mg/kg of body weight, 28mg/kg of body weight and 35mg/kg of body weight of CPF had glucose level of 22mg/dL, 28 mg/dL, 31 mg/dL,34 mg/dL, 74 mg/dL, 77 mg/dL and 83 mg/dL respectively.

These results clearly show that a high level of chlorpyrifos (CPF) increases the level of glucose in the blood as compared to the low concentration of CPF.

Table 2. Mean ± SD of biochemical parameters among control and treated groups after 28 days.

Treatment	Unit	To	T1	T2	T3	T4	T5	T6	T7
Sugar	(mg/dL)	21.00 ± 0.63 e	22.00 ± 0.45 de	28.00 ± 0.89 cd	31.00 ± 1.00 c	34.00 ± 0.71 c	74.00 ± 2.30 b	77.00 ± 2.35 ab	83.00 ± 1.90 a
Total bilrubin	bilrubin (mg/dL)	0.50 ± 0.009 e	0.58 ± 0.007 d	0.63 ± 0.008 d	0.70 ± 0.015 c	0.78 ± 0.015 b	0.80 ± 0.009 ab	0.82 ± 0.011 ab	0.85 ± 0.014 a
ALP	(IU/L)	252.0 ± 7.66 d	273.0 ± 5.81 cd	294.0 ± 4.82 c	303.0 ± 4.94 c	338.0 ± 4.95 b	371.0 ± 7.07 a	389.0 ± 5.39 a	401.0 ± 10.88 a
ALT	(IU/L)	24.00 ± 0.45 g	33.00 ± 0.45 f	39.00 ± 3.02 ef	47.00 ± 0.71 e	61.00 ± 0.89 d	73.00 ± 0.89 c	87.00 ± 3.39 b	104.00 ± 2.41 a
AST	(IU/L)	148.0 ± 4.29 f	162.0 ± 2.83 ef	167.0 ± 2.68 de	180.0 ± 2.98 cd	192.0 ± 2.28 c	217.0 ± 2.28 b	228.0 ± 3.91 b	249.0 ± 5.55 a

Means sharing similar letters in a row are statistically non-significant (P>0.05).

After 28 days of trial, a significant increase in the level of bilirubin was observed in experimental groups given with different concentrations of CPF as compared with control groups because control groups were not given with any pesticide given with normal feed and water. In the control group, the observed bilirubin level was 0.5 mg/dL.While in group of rats treated with 14mg/Kgb.w of CPF, 15 5mg/Kg b.w of CPF, 17.5mg/Kg b.w of CPF, 20mg/Kg b.w of CPF, 23.33 mg/Kg b.w of CPF, 28 mg/Kg b.w of CPF and 35 mg/Kg b.w of CPF were 0. 58 mg/dl, 0.63 mg/dL, 0.70mg/dL, 0.78mg/dL, 0.80mg/dL, 0.82mg/dL and 0.85mg/dL of bilirubin level in the blood samples of rats respectively. The result showed a significant difference in bilirubin levels in the treated group as compared to the control group.

According to (Table 2) (after 28 days of trial application, a significant increase in ALP was observed in treated groups as compared to the control groups. In the control group, a simple diet was given without pesticide application. In the control group, the observed ALP level was 252 IU/L. While in a group of rats treated with 14mg/Kg of CPF,15.5mg/Kg of CPF,17.5mg/Kg of body weight of CPF, 20mg/Kg of body weight of CPF, 23.33 mg/Kg of body weight of CPF, 28 mg/Kg of body weight of CPF and 35 mg/Kg of body weight of CPF had ALP level of 273 IU/L, 294 IU/L, 303 IU/L, 338 IU/L, 371 IU/L, 389 IU/L, 401 IU/L respectively. The outcomes showed a significant difference in ALP level according to the concentration of chlorpyrifos (CPF) in the experimental group as compared to the control group.

After 28 days of experimental trial application significant enhancementin ALT level was identified in experimental groups as compared to the control groups. In the control group, a proper fee was given without CPF application. In the control group, the observed ALT level was 24 IU/L. While in a group of rats treated with 14mg/Kg of CPF, 15.5mg/Kg of CPF, 17.5mg/Kg of body weight of CPF, 20mg/Kg of body weight of CPF, 23.33 mg/Kg of body weight of CPF, 28 mg/Kg of body weight of CPF and 35 mg/Kg of body weight of CPF had ALT level of 33 IU/L, 39 IU/L, 47 IU/L,61 IU/L,73 IU/L, 87 IU/L,104 IU/L respectively. The result showed a major deviation in ALT concentration according to the concentration of chlorpyrifos in the experimental group as compared to the control group.

After 28 days of rats, experimental trial application major increase in AST was observed in different experimental groups as compared to the control groups. In control groups, a proper diet was given to the rats without CPF pesticide dose. In the control

group, the identified AST level was 148 IU/L. While in a group of rats treated with a dose of 14mg/Kg of CPF, 15.5mg/Kg of CPF, 17.5mg/Kg of body weight of CPF, 20mg/Kg of body weight of CPF, 23.33 mg/Kg of body weight of CPF, 28 mg/Kg of body weight of CPF and 35 mg/Kg of body weight of CPF had AST level of 162 IU/L, 167 IU/L. 180 IU/L, 192 IU/L,217 IU/L, 228 IU/L, 249 IU/L respectively. The result showed a clear difference in AST level in experimental groups as compared to the control group because in experimental groups different concentrations of chlorpyrifos (CPF) given to the rats which are harmful to the health of living organisms. Table 2 shows the clear difference in the level of sugar, total bilirubin, ALP, ALT, AST as compared with biochemical analysis of control groups of Sprague Dawely rats.

Table 3. Mean ± SD of hematological parameters among control and treated groups after 28 days.

Treatment	Unit	T0	T1	T2	T3	T4	T5	T6	T7
WBC	(x103/L)	6.50 ± 0.14 f	7.60 ± 0.12 ef	8.70 ± 0.14 de	9.80 ± 0.14 d	12.60 ± 0.21 c	12.70 ± 0.43 c	14.40 ± 0.44 b	16.90 ± 0.58 a
RBC	(x106/L)	8.84 ± 0.11 a	8.49 ± 0.16 ab	8.29 ± 0.14 bc	7.94 ± 0.08 cd	7.54 ± 0.10 de	7.35 ± 0.12 e	7.32 ± 0.12 e	6.76 ± 0.08 f
HGB	(g/dL)	16.60 ± 0.31 a	16.50 ± 0.18 ab	16.30 ± 0.24 ab	15.80 ± 0.23 abc	15.50 ± 0.24 bc	15.00 ± 0.20 cd	14.40 ± 0.15 d	13.30 ± 0.23 e
HCT	(%)	53.60 ± 1.74 a	51.40 ± 1.10 a	50.80 ± 1.00 a	48.80 ± 0.67 ab	47.30 ± 1.28 abc	43.40 ± 1.37 bcd	41.20 ± 2.68 cd	37.70 ± 1.95 d
MCV	(fl)	65.60 ± 1.63 a	64.30 ± 1.55 a	63.50 ± 1.19 a	63.10 ± 1.12 a	62.70 ± 0.76 a	55.30 ± 1.02 b	53.10 ± 0.91 b	50.80 ± 1.97 b
MCH	(pg)	21.50 ± 0.60 a	20.40 ± 0.47 a	18.30 ± 0.22 b	17.90 ± 0.15 bc	17.60 ± 0.27 bc	17.10 ± 0.31 bcd	16.60 ± 0.23 cd	15.70 ± 0.21 d
MCHC	(g/dl)	35.20 ± 0.50 a	31.70 ± 0.69 b	31.30 ± 0.32 b	31.00 ± 0.31 b	32.80 ± 0.65 ab	32.60 ± 0.24 ab	32.00 ± 1.20 b	31.30 ± 0.38 b
PLT	(x103/L)	761 ± 16.26 d	825 ± 14.38 d	871 ± 20.86 cd	882 ± 12.75 cd	1,015 ± 33.88 bc	1,079 ± 47.34 ab	1,097 ± 49.44 ab	1,197 ± 42.20 a
LYM	(%)	91.60 ± 2.99 a	54.20 ± 0.94 c	95.90 ± 3.59 a	94.10 ± 1.62 a	89.90 ± 3.05 a	91.80 ± 1.18 a	91.70 ± 1.21 a	70.30 ± 1.58 b

Hematological analysis

Hematological analysis of blood was determined after completion of the duration of the experiment. Hematological analysis experimental groups also show a major deviation of results as compared with the control group of experimental animal models. Which was definitely due to the action of chlorpyrifos with different concentrations for different groups of rats labeled according to the name of their groups.

In the experimental groups of rats, there is a variation of results. On the other hand, WBC count in the control group was seen to be normal during the whole period. In experimental animals, tests identified that with the increase in the dose concentration of CPF WBC count also increased. Low concentration of dose in experimental rats show fewer increments in the WBC count. Results of WBC count of all groups of rats were given in (Table 3).

Red blood cell counts also disturbed by the application of pesticide dose to the experimental groups. The Control group of rats show a very little deviation in RBC's count because no harmful drug or chemical was added in the dose during the whole period of research. As compared with the control group other experimental groups show a great variation according to the dose given to the rats. RBC's count in experimental groups decreased with the increase in the concentration of CPF dose which is given in (Table 3).

As chlorpyrifos harm the living body, if the high concentration of Chlorpyrifos was exposed to a body, metabolic and physiological changes occurring in the body and it disturb the cellular function completely or partially. This chemical had also affected the concentration of hemoglobin levels. For the control group of rats, it was not changed because these rats were not exposed to CPF dose. Experimental animals were affected with CPF dose according to the concentration of dose. Hemoglobin level decreased by the hazardous effect of CPF which is shown in (Table 3).

Hematocrit levels also disturbed with the exposure of CPF on experimental rats. But test results of the control group show the normal value of hematocrit level (HCT), because of no exposure of pesticides. Groups with a very low amount of concentration of CPF show a very low deviation of hematocrit percentage from normal values of HCT. But with a

high concentration of chlorpyrifos in the dose of rats shows a very high deviation of hematocrit as shown in (Table 3).

Chlorpyrifos had also bad effects on the mean cell volume of blood samples of experimental rats giving a dose of chlorpyrifos. The mean cell volume of blood of a group of rats that are given the high dose of CPF decreased from the normal value of MCV. But the MCV of the controlled group remains to normal mean cell volume of blood. The mean cell volume of controlled and experimental groups is given in (Table 3).

The mean cell hemoglobin level also decreased by the increase in the Chlorpyrifos concentration in the experimental rats. Mean cell hemoglobin for a dose of 35mg/kg of CPF the mean cell hemoglobin was 15.70 pg which is abnormally decreased concentration as compared with a controlled group which is 21.50 pg. MCH value is given in (Table 3).

Mean cell hemoglobin concentration for a controlled group of rats was 35.20g/dL, for 14 mg/kg of CPF concentration was 31.70g/dL and for 35 mg/kg the mean cell hemoglobin concentration was 31.30 g/dl. It shows that MCHC value decreased by the increase in the pesticide concentration in the dose. MCHC value of all groups is given in (Table 3).

When infectious chemicals affect the body, platelets count also increased. As chlorpyrifos was an infectious pesticide it affects the metabolic system of the body of experimental rats. In the control group of rats as no pesticide was given in the dose the platelet count remains in a normal range. But in experimental groups, as chlorpyrifos was given to each group with different concentrations, hazardous pesticides have harmful effects in the body of rats which is shown in (Table 3).

Lymphocytes in the samples of the control group and experimental groups show a major deviation in the percentage of Lymphocytes level in the blood. Lymphocytes level in the control group was determined as 91.60 % and in the first group of dose 14mg/kg of CPF was checked as 54.20% and a group of dose 35mg/kg of CPF was identified as 70.30% which shows a clear deviation in the results due to high concentration of clorpyrifos which is shown in (Table 3).

(Table 3) shows a deviation in results of White Blood Cells, Red blood cells, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets and lymphocytes level the groups of experimental rats exposed with chlorpyrifos. The RBC's and HGB, HCT, MCV, MCH, MCHC diminished ($p<0.05$) among control and experimental groups. While the results of WBC's, PLT, LYM increment among control and experimental rats.

Histologcal analysis

(a) Shows sections of the testis of the control group showing the normal structure of Sertoli cells of spermatogenic cells that develops into spermatozoa. The Control group testes histopathology was not affected as shown in the because this control group was not exposed by Chlorpyrifos.

(b) Shows of histology of testis of an experimental group of T1 which was exposed with 14mg/kg of CPF showing an abnormal structure of Sertoli cells spermatogenic cells that develops into spermatozoa.

(c) Shows the histology of the group of T4 which was exposed to 20mg/kg of CPF shows Epithelium and interstitial tissues are damaged due to severe effects of chlorpyriphos.

(d) Shows the histology of the experimental group of T7 which was exposed with 35mg/kg of CPF shows the testis section of rats treated with insecticides showed significant depletion in the brown granolas with the primary stages of germ cells.

Histopathological study of testis of control and normal groups shows that there was a harmful effect of CPF dose on the testicular structure of rat, and is

the major cause of infertility in rats. CPF had an infectious and damaging effect on the testicular study as clearly shows in the (fig. 1).

Discussion

Significant decrease in body weight was seen (50-60%) at higher dose 35mg/kg of body weight of chlorpyrifos in rats and Chlorpyrifos is appeared to deliver wellbeing dangers like blood coagulating in vessels, degenerative change in the testis of living beings may demonstrate the side effects including degeneration in seminiferous tubules and germinal epithelium.

There was an increment in total bilirubin, Glucose, ALP, ALT, and lactate dehydrogenase due to damage in the liver. The generous decrease in RBC's, HCT, MCV, MCH, MCHC, and HGB and increase in WBC's and ALP was noticed as these results were also observed that gave fumes of chlorpyrifos and fed pelleted mice feed for 28 days, observed decrease in weight of mice and also observe an increase in WBC's and decrease in RBC's count and also semen examination exposed reduced sperm activity and irregular sperm cells.

In the case of determining hematology, various dosages of chlorpyrifos were given to rodents for 28 days on consistent schedule orally in eating regimen. Hematological and serum compound profiles of rodents show increment in WBC's, PLT, LYM, Total Bilirubin, Glucose, ALP, ALT, and AST and diminishing in the dimension of HCT, MCV, MCH, MCHC, RBC's and HGB was noted.

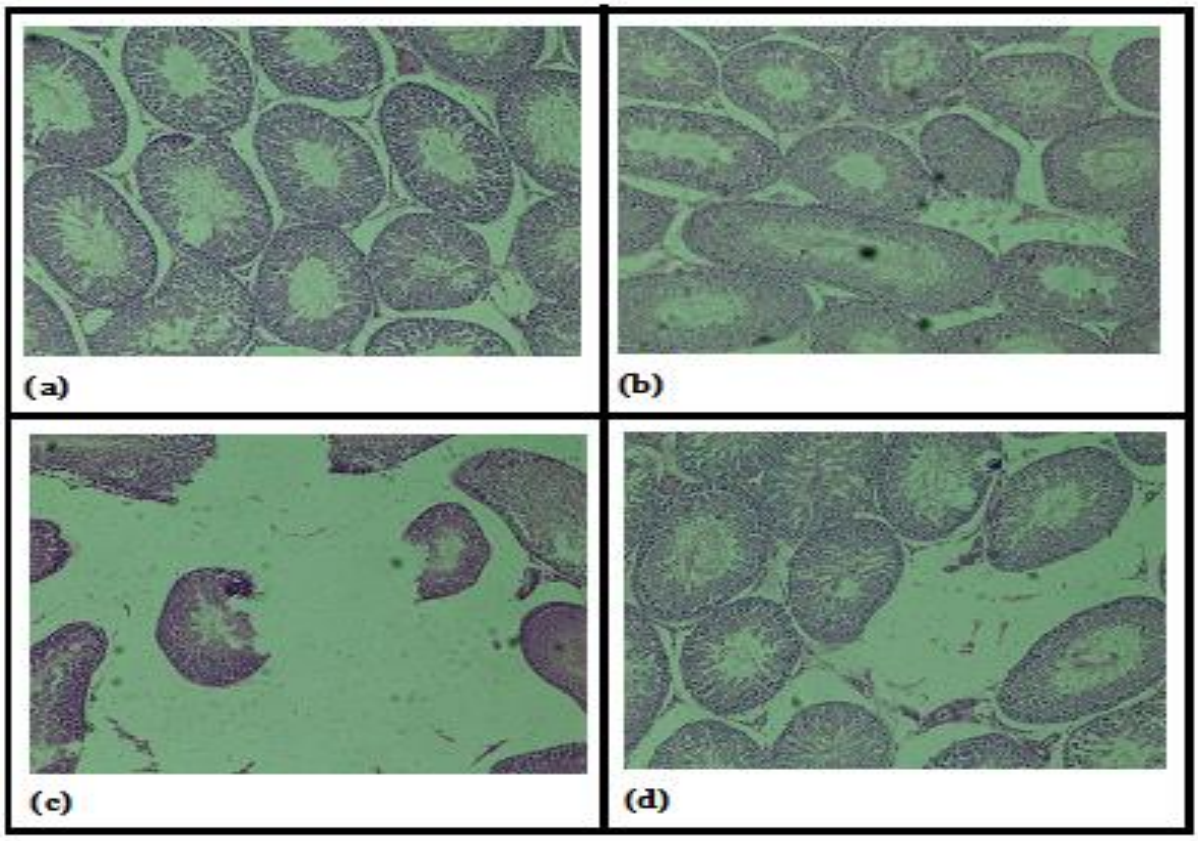

Fig. 1. Shows the microscopic picture of slides of testis cells of control group, T1, T4 and T7 group exposed with different concentrations of CPF.

Histopathological assessment photomicrographs of the testis section in the control group show a normal structure of seminiferous tubules and normal growth of testis. The section of the testis of mice group exposed to 14mg/Kg b.w of chlorpyrifos shows an abnormal structure of Sertoli cells spermatogenic cells that develop into spermatozoa Testis of rats group exposed to 20mg/Kg b.w of chlorpyrifos shows

Epithelium and interstitial tissues are damaged due to severe effects of CPF. Testis of mice group exposed to 35mg/Kg b.w of chlorpyrifos shows significant depletion in the brown granolas with the primary stages of germ cells.

It is experimentally proved that chlorpyrifos have degenerative effects in the hematological, biochemical and histological paramenters of male albino dawely rats. Consistent exposure of CPF may also cause serious health issues in the human body and can effect on testicular physiology and damage testis spermetogonial cells that can lead to the infertility. Therefore it is important to control the over or misuse of these pesticides all over the world to beware from other dangerous effects of these poisonous chemicals.

Conclusion

It is obvious that chlorpyrifos at 14mg/kg, 15.5mg/kg, 17.5mg/kg, 20mg/kg, 23.33mg/kg, 28mg/kg and 35mg/kg had very bad hematological and histological effects on rats in experimental group.

There was a significant increase in ALP, ALT, AST shows the serious damage in the body organs that results in the breakage of these enzymes into the bloodstream. Also, there is a decrease in RBC's, HGB, MCH, and MCHC was observed which shows the anemic condition in treated rats. Significant damage can also be seen in the figures of testis due to degeneration, hemorrhage, in seminiferous tubules and germline wall. Also, Sertoli cells and a decrease in the number of sperm count was obvious.

These changes were more obvious in rats: treated with a higher dose of chlorpyrifos. So, it is concluded that chlorpyrifos has degenerative and harmful effects in living organisms including humans.

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