EFFECTS OF BINARY MIXTURE OF INSECTICIDES ON HEMATOLOGY AND HISTOPATHOLOGY OF LIVER IN WISTER ALBINO RATS

Zunaira Bibi

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Dedication

I pay my heartiest and sincerest gratitude to my whole family, especially to my Mother **Sughra Bibi**, Grandfather **Muhammad Siddique** and my brothers **Babar Ali** and **Mohsin Ali**. They are the symbol of success for me and their valuable guidance and financial assistance enable me to perceive and pursue higher ideas in life. My mother is a minerate for love who enlightened me with a learning spirit.

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Abstract

Insecticides are commonly used to control agricultural, home pests and boost crop yields all over the world. Increased use of insecticides is hazardous to non-target organisms and can endanger the health of animals and humans. This research was designed to study the hematological and histopathological alterations in the liver of Wistar albino rats (Rattus norvegicus) in response to Chlorpyrifos (CPF) and Fipronil (FPN). Animal samples were collected from the Animal House, UAF. Rats were acclimatized in the stainless-steel cages for one week before the commencement of the experiment and were treated with two different concentrations of a binary mixture of CPF and FPN (3.1mg/kg + 2mg/kg) and (9.7mg/kg + 6.2mg/kg), respectively. After the completion of experimental trials, rats were slaughtered, and animal samples were taken. Blood samples were taken to estimate the hematological parameters like RBCs, WBCs, hemoglobin, MCH, MCV, MCHC, and PCV. The liver tissues of rats were fixed and stained with hematoxylin-eosin for the diagnosis of histopathological alterations. The results indicated that exposure to the binary mixture of insecticides causes a significant ($p<0.05$) decrease in the RBCs count, MCH, MCHC, and hemoglobin level, while on the other hand, it causes a significant ($p<0.05$) increase in the WBCs count, MCV, and platelets in the insecticides treated groups. The exposure to insecticides also caused a reduction in the body weight of insecticide-treated groups as compared to the control. The tissue damage confirmed through histopathological examination revealed hepatocytic vacuolation, degeneration of hepatocytes, sinusoidal dilation,

and focal necrosis of the degenerated portal vein in the liver of insecticide-exposed rats. It is therefore suggested that necessary precautions should be taken whenever insecticides are used or disposed of in areas with close mammalian proximity.

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CHAPTER 1

Overview of Research

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1.1. Introduction

Insecticides are chemical or biological products that are commonly used around the world to control household and agriculture pests that pose a danger of exposure to humans and animals [1]. A major rise in the utilization of these chemicals in agriculture has resulted from an increase in global food demand. Increased insecticide use could result in irreversible ecosystem damage. Toxicity from insecticides is a global issue in countries [2]. Many pesticides are also hazardous to non-target organisms which are putting animal and human health at risk [3]. Nowadays human are exposed to diverse mixtures of hazardous substances in their homes and workplaces. Pesticides which are sometimes applied carelessly in huge quantities and generate gradual contamination are a particular route of contamination**.** Synthetic pesticides are constantly needed for worldwide food generation and their residues can be found in food from both vegetable and animal sources including in the air and water [4]. Pesticides were found in more than 90% of fish and water samples taken from many streams [5]. Pesticides are used all over the world and each one has a unique structure and nature that provides a significant environmental concern. Pesticides are used in agricultural production at a pace of around 2 million tonnes per year, with 69 % of them used in Germany and the US alone [6].

Anticholinesterase compounds include carbamate and organophosphate insecticides, which inactivate the acetylcholinesterase enzyme. OPs that inhibit AChE are phosphoric esters but CMs are carbamic acid esters. Anti-ChE pesticides are extremely toxic and lack selectivity of species posing a severe hazard to non-target organisms such as

mammals (including people), birds, animals and marine species [7]. The neurological system is the main focus of most insecticides although they can also affect other organs. Exposure biomarkers derived from animal and human fluids, tissues and excrement provide quantitative and qualitative indications of contamination to pesticides [8]. Pesticides are chemical or natural substances that are used to eliminate pests that cause diseases in plants and weeds. Pesticides are helping to meet the demand for population growth in agriculture. It is also used to control other living organisms such as worms, arthropods, insects and vertebrates which destroy our food supply and cause a range of health problems. Pesticides also have other advantages such as saving man's power, time and ensuring high efficiency. They are exceedingly dangerous by nature when used widely without protection, they pose major risks to human health and the environment. They negatively affect agricultural labourers [9]. Pesticide mixtures or combinations are a new trend in pest control that has emerged as a significant strategy for managing insecticide resistance to previously used compounds. The main objective of pesticide formulations is to create a product with maximum biological efficiency that is easy of apply and has little impact on the environment. Toxicity and environmental contamination issues have arisen due to it. Pesticides that are used in mixed formulations allow for additive and collaborative interactions amongst active components as well as long term residual effects [10]. When compared to single pesticides, mixed pesticides can have high synergetic noxiousness on targeted species. They are also harmful to valuable species [11]. The liver and kidneys are the most vulnerable to insecticide toxicity and damage [12].

Pesticides kill about 52.5 % of birds and are the largest cause of animal death worldwide. Pesticides include fungicides, rodenticides, herbicides, insecticides and fertilisers [13]. A pesticide's toxicity is the degree to which it can affect vulnerable species. Toxic effects can explain the influence on an entire organism such as an animal, bacteria or plant as well as the effect on a structure of the organism including cytotoxicity or organotoxicity such as the liver (hepatotoxicity). These phrases are specified for toxicity to specific animals after a particular exposure time [14].

Fipronil is the most widely used insecticide that belongs to the latest group of phenyl pyrazole pesticides. FPN has a wide range of applications in veterinary medicine, household pest control and agricultural pest control, allowing it to manage a wide variety of pests, including flies, ants, beetles, wasps, cockroaches, rootworms, termites, thrips, weevils, ticks, fleas and several more [15]. It is more effective than pesticides including pyrethroid, organophosphate and carbamate against a wide variety of species of coleoptera, lepidoptera and orthoptera. FPN kills insects by selectively suppressing the nervous systems. Histopathological changes in hepatic tissue can be caused by FPN [16].

Lipids, oils, proteins and lipid organic solvents are all soluble in fipronil but not in water. It can last for about a year at ambient temperature but it decomposes quickly in the presence of an alkaline environment [17]. The action of fipronil in vertebrates is relatively unknown. Fipronil is rapidly digested in rats and residues are disseminated throughout the body, particularly in fatty tissues beneath the epidermis and hair follicles. For this reason, fipronil is continuously secreted on the skin and hairs together with

sebaceous secretion where residues may still present in large proportions a week after application [18].

The inhibition of a neurotransmitter known as a GABAA-gated chloride channel is associated with the hazardous process that is triggered by FPN. This results in the animal suffering from a neurological threshold that is insufficient which eventually ends in the victim's death [19]. Fipronil can completely block the activity of the human alpha1beta, alpha1, alpha2, and alpha3 glycine enzymes [20]. The ability of chlorpyrifos oxon metabolite to bind to and block the serine hydrolase AChE is linked to its toxicity. The nervous system is the major focus of AChE because it hydrolyzes a neurotransmitter acetylcholine and hence prevents its synaptic action. When AChE is inhibited, acetylcholine accessibility at the neural synapse rises, leading to excessive cholinergic pathway activation in the peripheral and central nervous systems. Severe inactivation of AChE causes acute cholinergic effects, sickness, and death [21].

Chlorpyrifos (CPF) is an insecticide which belongs to the organophosphate group and is widely employed in both residential and commercial settings around the world. It is a class II insecticide which means it poses a moderate risk to humans but has a wide variety of uses in agriculture, including in food crops, garden plants and commercial crops [22]. CPF is an insecticide that controls a variety of pests. Chlorpyrifos is a pesticide which is non-systemic that invades the body of an insect via ingestion or contact and is taken by up the pulmonary membranes, skin and intestinal tracts to kill the insects [23].

The wide use of pesticides to control ailments and crop pests has no doubt enhanced the yield of

crops in Pakistan however they have also resulted in severe issues of pesticide residuals [24]. The chlorpyrifos molecule has limited solubility in water and easily divides from aqueous to organic phases in the environment due to its non-polar nature. Hepatic dysfunction, immunological problems, embryotoxicity, genotoxicity, teratogenicity, and neurochemical and neurobehavioral changes have all been related to CPF. CPF may also cause hematological and histopathological changes in vitro. The liver is the primary site for all metabolic activity as well as hazardous compound detoxification [25].

In anaerobic and aerobic conditions chlorpyrifos has a half-life of between sixty and one hundred days and degrades in the environment over the course of extended periods [26]. Chlorpyrifos exposure potentially causes haemorrhage, mild congestion, histopathological lesions and vacuolar deterioration of renal tubular epithelial cells [27]. Chlorpyrifos is released into the ecosystem via direct application to use site. It spreads through spray drift, vaporization, drainage and leaching into the water. Chlorpyrifos enter the body through the skin, digestive tract, and lungs. Analysis of human urine revealed that approximately 70% of chlorpyrifos is absorbed orally with less than 3% absorbed through the skin [28]. Chlorpyrifos is capable of crossing the cell membrane with ease due to its lipophilicity. Although little concentration of chlorpyrifos can accumulate in fatty tissues this build-up may not be remarkable [29].

Exposed rats and mice showed many behavioral and neurotoxic effects through many experiments are obvious effects of pesticide-based toxicity. The toxicity of chlorpyrifos

in younger animals is more prominent and found to be more sensitive and decreases their locomotor activity [30]. The administration of a lower concentration of chlorpyrifos for a longer time may cause many neurological disorders such as neurodegenerative disease and depression. These organophosphate pesticides are directly linked to diabetes, cancers, Parkinson and Alzheimer's problems and their toxic mechanism is still unknown [31].

Haematological investigations are helpful in environmental and physiological research to understand the connection between blood properties and the surroundings. They are the primary tool for selecting genetically resistant individuals to specific diseases and environmental situations. Changes in haematological indices are frequently used to assess body health and stress resulting from environmental, dietary and pathogenic variables [32]. To assess the toxic stress induced by toxicants, haematological measures such as haemoglobin, hematocrit, RBCs, WBCs and haematological indices such as MCH, MCHC and MCV are frequently analyzed. In various organisms, changes in these parameters are regarded as an anaemic state [33].

Blood cell indices such as MCV, Hb and MCHC were reduced after exposure to chlorpyrifos. These parameters are very sensitive and reflect changes in the homeostatic system, variations in these characteristics are closely related to PCV, haemoglobin concentration and erythrocyte count. Thrombocytopenia has also been observed in chlorpyrifos-induced liver disorders [34].

Chlorpyrifos exposure in albino rats and Sprague Dawley rats caused a reduction in neutrophil percentage in peripheral blood. Chlorpyrifos intoxication was reported to cause the

rupturing of neutrophils which might be due to the phagocytic function of neutrophils. This caused the neutrophil count to reduce [35]. At relatively low dosages chronic toxicity study using pesticides indicated a range of toxic potential [36]. Pesticide treatment is thought to cause changes in haematological levels which can threaten the survival of exposed species. When exposed to CPF hematotoxicity is a typical side effect [37]. Haematological measures are used as predictors of health issues and the consequence of pesticide toxicity within organisms [38].

The study of biological cells and tissues as well as the microscopic alterations or abnormalities that might arise as a result of disease is known as histopathology. The Wistar rat is an albino rat that has been outbred. The fact that this particular breed of rat was the first rat to be used as a model organism is remarkable. In order to be employed in biological and medicinal research this breed was created in 1906 at the Wistar Institute. The most common use of histology in clinical medicine is the evaluation of specimens for possible diagnosis and screening by a pathologist followed by the processing of the tissue for viewing under a microscope. Histopathological abrasions have been employed as markers for monitoring the fitness of individuals susceptible to contaminants and they can also serve as early warning indications of illness [39].

In biomedical research one of the most regularly used animals is the rat. They are the best model in a variety of fields including neurobehavioral research, cancer research and toxicology. The Wistar rat is an albino rat that has been outbred. The fact that this particular breed of rat was the first rat to be used as a model organism is remarkable. In order to be employed in biological and medicinal research this breed

was created in 1906 at the Wistar Institute. One of the most popular laboratory rats used today is the Wistar rat. Rodents with hominids share roughly thirty thousand genes that makeup almost 97% of genes mutual in both groups. It features a broad head, big ears and a tail that is always smaller than its body length [40]. Hence in the current effort, an attempt has been made to evaluate the toxic possibilities of insecticides on Wistar albino rats.

1.2. Objectives

Following were the objectives of this study

- To estimate the effect of insecticides on the haematological parameters in Wistar albino rats (*R. norvegicus*)
- To evaluate the effect of insecticides on the histopathology of liver in Wistar albino rats (*R. norvegicus*)
- To study the factors involved in the alterations of haematology and histopathology of the liver in Wistar albino rats (R. *norvegicus*)

CHAPTER 2

Review of Literature

Sepici-Dinçel, Benli [41] measured a whole series of biological, histopathological and behavioural changes in carp fingerlings to investigate the effects of a sublethal concentration of insecticides for 2 days using semi-static testing. The total antioxidant status levels in the 48-hour-exposed group dropped, chloride levels decreased in one week while salt and phosphorous levels elevated. Plasma cortisol levels were raised in both the 48-hour and one-week exposure groups. After 7 days of exposure, the brain exhibited a higher level of malondialdehyde than other tissues. Synthetic pyrethroid's potential oxidative-stress-inducing effect is described for the first time as an alternative mode of action and also described as sodium channel blockage.

Kundu and Roychoudhury [42] evaluated the effect of insecticides on the haematological parameters of cricket frogs. Malathion at sub-deadly focuses (0.006 ppm) was concentrated on the haematological factors of the cricketfrog (Fejervaryalimnocharis) for 24 to 240 hours of exposure and critical haematological alterations were identified. The haematological factors study demonstrates a fundamental ($P<0.01$) decline in total erythrocyte count inebriation of malathion from 24 hours to 96 hours of exposure when contrasted with the control group. A critical decline ($P<0.01$) was observed in packed cell volume and haemoglobin from 480 hours to 240 hours. There was a critical increment ($P<0.01$) in leucocyte count during the exposure time. Enhanced numbers of eosinophils and lymphocyte were identified in the current examination, which exhibited eosinophils and lymphocytes, inferring that this was the outcome of direct immune defence stimulation attributable to the presence of a toxic material or was related to injury of

tissue. The cyto neurotic and morphological assessment of leucocytes and erythrocytes in Malathion examined frogs at an amount of 0.0061 revealed Oppm an assortment of cytotoxic impacts at different exposure periods. It was seen that the shape and size of erythrocyte varieties rely upon the kind of blood infection.

Tripathi and Srivastav [43] assessed the effect on the liver of rats due to long-lasting consumption of various concentrations of chlorpyrifos. Before the test, Wistar male rats were distributed into 3 groups. During experimentation, group A acted as the control group while group B and C got the oral dose of insecticide daily. After 24 hours of the last treatment, rats were slaughtered. Small liver fragments were taken out and fixed in Bouin's solution. After the processing of tissue histological examinations were done. According to the results, Chlorpyrifos treatment caused histopathological changes in rats' livers. However, the strength of these effects varied depending on the dosages and period of treatment.

Savithri, Sekhar [44] observed that the chlorpyrifos noxiousness caused alterations in the blood profile of Wistar rats. Rats were given a sub-lethal dose of insecticide orally after the interval of 2 days. After the test, the reduction in the packed cell volume, red blood cell and Hb was observed and it could be regarded as delayed hemopoiesis impairment. The contraction of MCHC, RBC and MCH revealed a substantial decrease in all concentrations which is due to impairment in the shape and size of RBCs and a reduction in the production of haemoglobin. These symptoms infer anaemia. The chlorpyrifos toxicity also causes the reduction in the %age of neutrophils in the blood which suggests that neutrophils are

involved in phagocytosis throughout the xenobiotic toxicity and neutrophils could have busted.

Shalaby, Farrag [45] tested the effect of pesticides on the haematology and histopathology of albino rats. The activities of ALT and amylase were significantly increased by thiamethoxam. Also, the creatinine levels increased. Triglycerides showed a considerable drop. There were no significant changes in albumin concentration. The hepatic lobule structure was disrupted and there were vacuoles in the cytoplasm of hepatocytes according to microscopic analysis of the liver. The kidney section revealed lobulated glomeruli, a substantial region of haemorrhage and clogged blood vessels with thickened walls. The findings revealed that this insecticide harmed the physiological and histological characteristics of albino rats.

Khalifa, Khalil [46] assessed the antioxidant role of wheat germ oil (WGO) and grape seed oil (GSO) in chlorpyrifos-induced oxidative stress and biochemical and histological changes in liver in male albino rats. Chlorpyrifos (CPF) was added to the different experimental tested diets at two levels of low and high doses.WGO and GSO were added to the experimental diets at a level of 200 mg/kg diet. The results showed that enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase were significantly increased in rats administrated only by chlorpyrifos, while total proteins, albumin, and globulin showed a significant decrease at high and low doses of CPF groups. In addition, CPF caused a significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione- s-transferase (GST).Wheat germ oil and grape

seed oil supplementation caused significant improvement in different biochemical parameters of all rat groups.

Demir, Uzun [37] examined the effects of chlorpyrifos in the rat erythrocyte antioxidant system and evaluated the ameliorating effects of catechin and quercetin on the oxidative damage induced by chlorpyrifos. Sexually mature male Wistar rats were given chlorpyrifos (5.4 mg/kg, 1/25 of the oral LD50), catechin (20 mg/kg), quercetin (20 mg/kg), catechin plus chlorpyrifos, and quercetin plus chlorpyrifos daily via gavage for four weeks. No statistical differences were found in the catechin-only and quercetin-only groups compared with the control group. By the end of the fourth week, chlorpyrifos alone increased the levels of malondialdehyde (MDA) and decreased superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities compared with the control group in rat erythrocytes. In the catechin-plus-chlorpyrifos and quercetin-plus-chlorpyrifos groups, there were statistically significantly decreased MDA levels and increased SOD, CAT, and GPx activities compared with the chlorpyrifos-only group. Thus, it appears that catechin and quercetin ameliorate chlorpyrifos-induced oxidative stress in rat erythrocytes in vivo.

Modaresi and Seif [47] studied the toxicological impacts of pesticides on the haematological parameters of the rat. This poison can be ingested by humans and animals by food, inhalation or skin contact. They investigated the long-term effects of insecticide on blood factors in rats. They divided 50 rats into five groups. The control group received commercial feed and the other 4 groups were treated with different concentrations of the pesticide via gavages for 21 days. They discovered that insecticide has long-term impacts on blood

factors that cause substantial harm and even anaemia as well as related maladies in rats. They determined that using organochlorine slump-dais in homes and farms is hazardous to human and animal health, particularly rats.

Gül, Benli [48] reported that carp was utilised as a model animal to examine the sub-lethal toxicity and genotoxic potential of a routinely used insecticide in veterinary medicine and public health. Fish were divided into several groups before the experiment. For three days, fish were exposed to various concentrations of carbamate pesticide. Histological studies of the spleen, gut, muscle and skin tissues revealed no notable changes. The frequency of micronuclei did not rise in a statistically meaningful way. Haematological tests revealed lower hematocrit and mean corpuscular volume values also higher WBCs and RBCs count. There were rises in glucose, cholesterol, triglyceride and aspartate aminotransferase levels, phosphorus, salt and total plasma protein.

Ural [49] investigated the effects of lycopene on chlorpyrifos caused alterations in oxidant and antioxidant status as well as haematological markers in Cyprinus carpio. Fish were placed into seven experimental groups before the experiment. Control received no treatment, treatment 2 received maize oil orally, treatment 3 received lycopene orally, treatment 4 received CPF, treatment 5 received CPF plus an oral dose of lycopene, treatment 6 received CPF and treatment 7 received CPF plus an oral dose of lycopene for two weeks. After the experiment, blood and tissue samples were taken. The samples were also tested for variations in blood parameters such as RBCs, WBCs, haem content, hematocrit and erythrocyte indices such as mean corpuscular, MCH and

MCHC. According to the results, chlorpyrifos has a deleterious influence on hematologic indices.

Uzun and Kalender [50] documented that chlorpyrifos induced hepatotoxic and hematologic changes in rats: The role of quercetin and catechin. Catechin, quercetin, chlorpyrifos, catechin + chlorpyrifos and quercetin + chlorpyrifos were given to male rats through gavage for 4 weeks. Serum total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, triglyceride, total cholesterol levels, haematological changes, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase activities and malondialdehyde content in liver tissues and also histopathological changes of liver were investigated in the rats compared to the control group. No significant differences in all investigated parameters were observed between the control, catechin and quercetin groups. There were statistically significant changes in liver function tests, some haematological parameters, antioxidant enzyme activities and malondialdehyde levels in the chlorpyrifos-treated group compared to the control group. In the catechin + chlorpyrifos treated group and quercetin + chlorpyrifos treated group, we observed the protective effects of catechin and quercetin on examining parameters but not completely. While some histopathological changes were detected in liver tissues in chlorpyrifos treated group, fewer histopathological changes were observed in catechin + chlorpyrifos and quercetin + chlorpyrifos treated groups at the end of the 4th week. As a result, catechin and quercetin significantly reduce chlorpyrifos-induced hepatotoxicity in rats.

Yıldırım, Baydan [51] investigated the effects of chlorpyrifos on the thoracic aorta and the level of NO in plasma and aorta. The effect of chlorpyrifos on the thoracic aorta in organ baths was determined in 10 rats. Another 45 rats were assigned to 3 groups with 15 rats each, control group 1 received distilled water, control group 2 was given corn oil and the last group was given 13.5 mg/kg chlorpyrifos dissolved in corn oil every other day for 8 weeks orally. Chlorpyrifos showed no effect on isolated thoracic aorta. Plasma AChE activity was decreased while LDH, ALT, GGT and AST activities were increased in the chlorpyrifos group compared to control groups. Plasma NO level was increased in the chlorpyrifos group compared to control groups. iNOS expression was present in all groups in the cytoplasm of the endothelial and the smooth muscle cells of the aorta. According to semiquantitative histomorphological analysis, iNOS immunopositive reactions were seen in decreasing order in chlorpyrifos, control 2 and control 1 groups. In conclusion, chlorpyrifos induced NO production in the aorta following an increase in NOS expression.

Adhikari, Agarwal [52] analyzed the haematological effects of fipronil poisoning in white leghorn cockerels. A total of 20 one-month-old white-leg horn male chickens were separated into four equal groups each with five birds. Various concentrations of FPN were supplied orally through feed for 3 months after a one-week acclimation period. Each bird had blood taken every 20 days for the purpose of testing various parameters. On the day of blood collection, haemoglobin, PCV, total erythrocyte count and differential leukocyte count were all assessed as per standard protocol. According to the results, there were significant decreases in haematological

parameters which led to anaemia and also caused a considerable fall in total leucocyte count, which resulted in a suppression of the cell-mediated immune response.

Hedayati and Tarkhani [53] evaluated the impact of several pesticides on haematological parameters and gill morphology in iridescent sharks. Over a week, fish were treated with various pesticide concentrations. According to the results, insecticides induced leukocytosis, lymphopenia, and neutrophilia, increased Red blood cells, PCV, haemoglobin and mean corpuscular volume. In pesticide-exposed fish, morphological gill damage was detected. They also produced immune system changes and gill injury, which could explain the rise in red blood cells, Hematocrit, Hemoglobin level and MCV in sharks. bin Abdul Razak [54] used rats to illustrate the development of atherosclerosis in the aorta after contact with CPF. In this experiment, he used high-profile instruments like TEM and SEM. He aimed to see modifications in the ultrastructure of the intima layer that covers modern atherosclerotic plaques. The Aorta of mice administered in vehicle showed no evident ultrastructural variations in Foam cells & crystals of calcium identified in CPF susceptible rats but not in vehicle rats or rats that had not been handled. In the CPF-treated rat aorta, histological labelling with toluidine blue revealed sites of intimal bulges and elastic fibre displacement. He concluded that continuous access to CPF induces arterial wall deterioration, which might be one of the systems involved in CPF-provoked atherosclerosis

Yonar, Ural [55] investigated the potential for pesticide toxicity to be mitigated in the blood and other tissues of carp was studied. For ten days, they exposed fish to sub-lethal amounts of malathion. After the experimentation, they took

samples of the blood and tissue. The fish's immunological response and blood profile were analysed by using the blood samples that were taken. This analysis includes the determination of the number of RBCs, the haematocrit level, erythrocyte indices, haemoglobin concentration and mean corpuscular volume. Malathion harms the immune system of fish also the activity of antioxidant enzymes and haematological markers.

El-Tawil [56] conducted an experiment on adult male and female albino rats that were fed with a poisoned diet containing chlorpyrifos (at 200ppm), chlorpyrifos-methyl (at 2000ppm) for 12 weeks followed by 3 weeks of the recovery period. At 2, 4, 6, 8, 10 and 12 weeks post-treatment and after 3 weeks of recovery periods, a number of male and female rats were sacrificed, blood samples were collected and their organs were dissected out and weighed. Total protein, total lipid and glucose contents were determined in the plasma, liver and brain of treated and control rats. Results showed that no toxic symptoms were observed during the experimentation period, but the body weight gains of treated rats were less than that of control. In treated rats, the relative weights of the heart, lungs, liver, spleen, kidneys and brain of both sexes as well as ovaries showed significant increases, whereas male testes and sperm counts showed significant decreases compared to those of control rats. For both treated sexes, there were significant increases in total lipid content while the total protein and glucose contents were significantly decreased compared to those of control rats. During the recovery period, all these adverse effects were gradually ameliorated.

Mossa, Swelam [16] revealed that chronic exposure to fipronil can cause histological alterations, biochemical changes and

oxidative stress in the liver and kidney of albino rats. During the test, different concentrations of insecticide were given to rats along with drinking water for about one and a half months. After the test, the rats were sampled and changes in the treated groups were observed. When compared to the respective control, it is observed that FPN initiated histopathological changes in the kidney and liver of rats. According to the results, it was deduced that FPN can cause injury in the kidneys and liver of rats. The toxic action of FPN may be responsible for these alterations in the liver and renal tissues.

El-Murr, Imam [57] assessed the effects of fipronil on histopathology, immunology, haematology and biochemical effects on Nile Tilapia. 240 Oreochromis niloticus were divided into four groups and exposure with varied concentrations of FPN in duplicate. According to the findings, fish exposed to fipronil had pale gills and neurological symptoms as well as congestion and haemorrhages in various inner organs. In comparison to the control group, there was a substantial drop in Immunoglobulin and Lysozyme levels with a simultaneous increase in serum nitric oxide levels. All of the FPN-exposed groups had significantly higher serum levels of AST, ALT and cortisol compared to the control group. Fish exposed to FPN had various histological changes in their liver and gills.

Khan, Uggini [58] documented that in-ovo treatment of CPF and cypermethrin in a mixture changes the haematological indices in 2 generations of domestic hen. In generations of hens, the impacts of in-ovo intoxication with an insecticide combination (chlorpyrifos 50 per cent EC + cypermethrin 5 per cent EC) were studied. On day zero of incubating, the

experimental groups of eggs received dosages of a mixture of insecticide while the group received maize oil. Blood was taken from the brachial vein of a 25-week-old parental Progenies and a 4-week-old chick and conventional haematological parameters such as TRBC, TWBC, DC, Hb, and PCV were recorded. The combined usage of chlorpyrifos and cypermethrin resulted in a reduction in RBC, PCV, lymphocytes and platelets also caused a decrease in WBC count.

Begum, Upadhyaya [59] studied that oral chlorpyrifos (CPF) dosing affects indigenous chicken. Hb, TEC, TLC, and biochemical elements such as ALP, AST, total protein, and uric acid were all measured in blood samples. In CPF-treated birds, serum ALP, AST, ALT, and uric acid levels increased and CHE levels dropped. The protein concentration remained constant. The results show Chronic CPF poisoning causes haematological, biochemical and pathological alterations in treated birds.

Shahzad, Khan [60] reported that orally administered chlorpyrifos (CPF) had immunologic effects in day-old broiler chicks tested that orally administered chlorpyrifos (CPF) had immunologic effects in day-old broiler chicks. The lymph proliferative responses to avian tuberculin were used to assess cell-mediated immunity. A carbon clearance assay was used to assess leukocyte phagocytic capacity. CPF reduced humoral immunity, cell-mediated immunity, and phagocytic activity in broiler chicks in a dose-dependent manner. In treated birds, dose- and time-related pathological alterations were found in the bursa of Fabricius, spleen and thymus. The Bursa of Fabricius demonstrated enhanced inter-follicular

connective tissue growth and degenerative alterations such as pyknosis and nuclei fragmentation.

Ali, Mohamed [61] assessed the sublethal effect of FPN exposure on liver and renal tissues using Japanese quail recovery abilities. Fipronil's toxic effects on tissue histology and clinical biochemistry in Japanese quail were investigated in a two-week gavage trial and a two-month degradation period. The FPN and recovery groups' liver and kidney relative weights did not differ significantly from those of the control group when compared to those of the control group. According to the findings, the fipronil group had histological abnormalities in the liver and kidney. Clinical alterations in serum enzyme indicators such as alanine transaminase, alkaline phosphatase, aspartate transaminase and lactate dehydrogenase occurred concurrently with these variations. Fipronil exposure resulted in severe liver damage markers as well as histological abnormalities in Japanese quail liver and kidney tissues.

Qureshi, Bibi [62] evaluated that acute dosages of fipronil and ibuprofen either together or separately cause biochemical, haematological, histopathological and genotoxic harm in common carp. Carps were divided into different groups and then treated with wide concentrations of fipronil and buprofezin singly or in a mixture for 3 days. According to the results, fish treated with FPN had considerably lower serum protein and globulin levels, whereas albumin levels were unaffected by any of the treatments. Hematological analysis revealed a significant drop in RBCs, thrombocyte counts, haemoglobin level and Hct % in all treatment groups while WBCs count increased. Histopathological alterations in the gills were detected with all treatments.

Sharma, Tiwari [63] assessed the harmful effects of insecticides on white blood cells in different groups of human blood. For evaluating insecticide content in human blood plasma, they used spectroscopic methods and Fourier transform infrared coupled with solid phase extraction. SPE was used to extract spiking amounts of insecticides in the range of 0.15-1.71 mg/ml. From blood plasma samples. They defined active functional groups. The recovery rate of malathion was 75%. They showed that insecticides decreased the number of white blood cell counts. They concluded that insecticides cause haematological problems in leukocytes in human blood by a reduction in number and chromosomal abnormalities in peripheral leukocytes.

Hendawi, Alam [64] evaluated that haematological, histological and biochemical aberrations in male Wistar albino rats as a result of insecticide poisoning as well as the potential protective function of flaxseed oil. Subacute thiacloprid poisoning resulted in a considerable increase in RBCs, Hb, PCV and WBC counts as well as the production of bone marrow micronuclei. Furthermore, blood biochemical markers associated with liver injury such as alanine aminotransferase and alkaline phosphatase increased dramatically. Blood total protein and albumin levels were significantly lower. Insecticides caused histological liver damage which was reduced by therapy with flaxseed oil. Flaxseed oil was found to be capable of protecting against thiacloprid-induced hepatoxicity.

Hocine, Merzouk [65] evaluated that insecticides impact certain biochemical parameters in Wistar rats. Recent research revealed the effects of repeated simultaneous exposure to large amounts of deposits and endosulfan on specific

metabolic markers in male Wistar rats, Rats in group I were given untreated water, while rats in group II were given water lead-based on lead acetic acid 1000 ppm (Pb1000). The third group was fed a diet treated with 10 ppm industrial grade endosulfan (E100), Ph (1000) + E is assigned to group IV (100). Collect blood and target organs for biochemical parameter estimation to determine the toxicity of these two compounds when combined at higher doses. The research outcomes revealed the utilizing a larger dose of endosulfan alone or in combination and the results changed the general metabolic characteristics of the current study.

Deeba, Raza [66] evaluated the effects of locally used insecticides chlorpyrifos (CPF) and lambda-cyhalothrin (LCT) on oxidative stress biomarkers in human erythrocytes. The activity of catalase (CAT), superoxide dismutase (SOD) and protein contents, as well as the levels of malondialdehyde (MDA) and osmotic fragility (OF), were measured in human erythrocytes exposed to CPF at concentrations of 0, 100, 500, 1000 and 2000 ppm and LCT at concentrations of 0, 100, 300, 600 and 800 ppm for 1 h and 3 h. MDA levels of erythrocytes were significantly higher in erythrocytes incubated with CPF and LCT at increasing concentrations of both insecticides and increased incubation time. However, erythrocyte CAT and SOD activities were decreased at all concentrations of CPF and LCT tested. Protein oxidation products were decreased at lower doses of CPF (100 and 500 ppm); at higher doses (1000 and 2000 ppm), total protein content was increased compared with control. In contrast, LCT was associated with a decreased in protein contents at all concentrations. These results demonstrated that CPF and LCT can induce oxidative stress in human erythrocytes (in vitro).

Nath, Anshu [67] examined that insecticides caused abnormality in Microtubules in Swiss Albino Mice. The objective of the recent research was assumed; towards detecting the noxious consequences of endosulfan in the genitalia of albino mice and to discover its potential role in sterility. Swiss albino mice were given 3 mg/kg body weight of endosulfan for 2, 4, 6, 8, 10, and 12 weeks. Blood and tissue samples were obtained after endosulfan injection and Biochemical, electron microscopic, hormonal and histological evaluations were done. In each group of endosulfan-treated mice, significant decreases in testosterone and sperm total were seen, whereas MDA levels were markedly increased. Endosulfan-treated Swiss albino mice died less sperin at 6 weeks, 8 weeks, 10 weeks and 12 weeks. In 12 weeks of endosulfan-treated Swiss albino mice, destruction of the 9+2' preparation of spermatozoa microtubule was observed under an electron microscope (TEM). As a result of the current study, it has been determined that endosulfan decreases testosterone, sperm total and MDA levels in Swiss albino mice It also altered the histopathology of testicular erection at the cellular and subcellular levels. After administration of endosulfan, low sperm amount, low sperm movement ability and annihilation of the 9+2 prearrangement of microtubule of spermatozoa confirmed that its poisonousness caused asthenozoospermia, resulting in sterility in Swiss albino mice. Therefore, it was concluded that endosulfan affects microtubules in Swiss albino mice and impacts testosterone levels.

Yadav [68] studied the effect on broiler chicks fed 45 ppm chlorpyrifos-containing diets. Periportal fibrosis, mononuclear cell infiltration, hepatocyte necrosis and bile duct hyperplasia

were among the histopathological abnormalities seen in the liver. Tubular epithelial deterioration and necrosis were seen in the kidney. Myocytes in the hearts of all toxin-treated birds revealed vacuolar degeneration. The gizzard had glandular interstitial fibrosis as well as heterophil and mononuclear cell infiltration. The harmful effects of these toxins in numerous organs of broiler chickens were discovered in the investigation at low dose levels. Karami-Mohajeri, Ahmadipour [69] estimated the genotoxic effects and histopathological changes due to exposure to chlorpyrifos in rats. Insecticides in various concentrations were given to rats Insecticides were orally given to treated groups including 6 test species for about a month. A few haematological changes and histopathological alterations were also assessed. Increase in the WBCs and Inflammation was indicated by infiltration of lymphoid in liver and rise in the parameter of white blood cells. A substantial rise in PCE and platelet count was also noticed in insecticide-treated groups. According to the result, chlorpyrifos caused histopathological changes in the parenchyma of the liver.

Peiris and Dhanushka [70] assessed the effects of sub-chronic chlorpyrifos exposure on male rats' reproductive toxicology. Male rats were given 0, 2.7, 5.4, and 12.8 mg/kg orally for 90 days. In exposed males, chlorpyrifos caused a significant decrease in testicular sperm counts and a large increase in sperm malformation rate. At various dose levels, histopathological analysis of testes revealed mild to severe degenerative alterations in seminiferous tubules. Follicle-stimulating hormone (FSH) levels were considerably higher in the 5.4 and 12.8 mg/kg groups. This indicates that chlorpyrifos hurts the reproductive system of male rats.

Ardeshir, Zolgharnein [71] estimated the comparison of intravenous and waterborne exposure of fipronil in the Caspian whit fish on acute toxicity and histology. There were 133 fish divided into 19 tanks (seven fish per container), with one control and six treatment groups. Fish were given varying doses of FPN and were observed for three days. According to the findings, the degree of cellular change in important organs from moribund fish exposed through waterborne exposure demonstrated substantial damage in the gill. The fish that was exposed intraperitoneally seemed to have severe kidney injury. Although nicely staining showed modest changes in Purkinje cell distribution, hematoxylin and eosin staining of the brain indicated no histological changes.

Fayinminnu, Tijani [72] assessed that the sub-lethal dosage of chlorpyrifos causes toxicity in the kidney and liver of *R.norvegicus*. The lethal dose of insecticide is orally administered daily for a month. After the experiment, the noxiousness of chlorpyrifos was evaluated in rats through haematology and histopathological examination. Results indicated that lymphocytes, Hb and Packed cell volume displayed a remarkable decrease as compared to the control. The findings also assessed the rise in the histopathological changes depending upon dose. The liver indicated reasonable vacuolar variation of hepatocytes kidneys indicated the slight focal shedding off of epithelium. The study revealed that a wide range of concentrations of chlorpyrifos cause noticeable changes even at the lowest most tested concentration in the tested animals thus disturbing the performance in meaning of in terms of healthiness and comfort. Therefore, it could also

cause similar environmental hazards to individuals even at low concentrations.

El-Sawasany, El Okle [73] reported that TMX has no negative effects on the reproductive system in adult male rabbits. The hormonal study demonstrated that TMX caused a considerable increase in blood testosterone levels. While FSH and LH hormone concentrations did not differ between the treated and control groups. The findings also revealed that while TMX therapy did not produce significant sperm DNA fragmentation, it did create significant changes in sperm parameters. The main histological abnormalities found in the testis of inebriated animals were spermatogenic cell degeneration and necrosis, as well as inter-tubular oedema and vacuolations.

Menezes, Qadir [74] described the mechanism of pesticide toxicity and histopathological findings. Endosulfan has extensively used in spite of its life-hazardous effects. This study revealed the toxicity mechanism, histopathological findings, the pharmacokinetics of endosulfan; clinical demonstrations and management were defined. The lethal outcomes of endosulfan disturbed several organs and structures giving in an inclusive display of signs and indications. Even though named a limited Classed insecticide, it remains to be used, exclusively in the rising world, owing to its advantageous outcomes on agriculture. Intoxication of endosulfan had been testified from dissimilar areas of the ecosphere. Endosulfan has a lethal impact on different organs. When there was currently no antidote for endosulfan poisoning, management was mostly supportive. To reduce the present sickness and damage from this insecticide, the use of endosulfan must be strictly restricted and eventually stopped

completely. Moreover, monitoring of biological trials with non-aggressive means such as breast milk samples provided an effective method of detecting the elimination of this ecologically persistent organic poison from a large population. Therefore it was concluded that endosulfan had a lethal impact on different organs in organisms.

Sánchez, Alvarez Sedó [75] evaluated the impact of endosulfan on mammalian sperm in vitro study. Previous studies revealed that endosulfan might disrupt animal and human development, the objective of this study was to observe that endosulfan exerts an impact on sperm parameters and the development of chromatin de-condensation in sperm of mice. The Al (active ingredient) or two commercial preparations (CFS) of endosulfan (Zebra Ciagro and Master) were used to incubate spermatozoa from the caudal epididymis of mature male mice. In comparison to controls, a significant decline in the percentage of motility and viability of Spermatozoa was noticed. In the presence of heparin and glutathione, in vitro decondensation was done. The Al, endosulfan Master and endosulfan (Zebra Ciagro) were increased chromatin de-condensation in spermatozoa. Furthermore, when sperm were hatched with any of the CFs, fragmentation of DNA was considerably higher than when they were incubated with the Al or controls, according to the TUNEL assay. The structure of the membranes of acrosome, plasma and membranes of sperm treated with endosulfan Al or the CF's was: evident in ultrastructure examination of sperm cells. Therefore, it was concluded that Endosulfan had been shown to impact sperm integrity, DNA fragmentation and chromatin DE condensation in vitro.

El-Wakf, El-Habibi [76] investigated the prolonged intake of either pomegranate peel methanolic extract (PPME) or pomegranate juice (PJ) could protect against CPF-induced cardiotoxicity and restore cardiac functions up to normal status. Thirty-six male albino rats were equally allocated into 6 groups, the first four ones were used as control, PJ, PPME and CPF groups while the two last groups were given CPF+PJ and CPF+PPME at the same mentioned doses daily for 60 successive days. The CPF-exposed group showed electrocardiograph (ECG) alterations manifested as ST-segment elevation, prolonged corrected QTc interval and shortened RR interval, along with elevation in heart rate and blood pressure. Further elevation in cardiac marker enzymes and myocardium-specific troponin isoform were noticed. Results also showed decreased cardiac antioxidants. Cardiac histopathological changes characterized by disorganization and degeneration in myocardial fibres with cytoplasmic vacuolization and separation of cardiac myofibrils were observed. Congested thickened arteries with extravasation of the blood in between the cardiac myofibrils were also noticed following CPF exposure. In contrast, co-administration of CPF and either PJ or PPME tended to protect against CPF-induced cardiotoxicity as manifested by improved ECG alterations, oxidative status apoptotic biomarkers and histological characters. Thus, administration of PPME or PJ seems to offer a greater protective effect against CPF-induced myocardial.

Kartheek and David [77] evaluated changes in the histopathology and biochemical aspects due to the sub-chronic exposure of fipronil in Wistar albino rats. Different concentrations of insecticide were given to rats orally. The

damage of tissues in the liver of insecticide-treated rats was verified by histological investigation which revealed abnormalities such as necrosis and deteriorated portal vein. However, alterations in histoarchitecture demonstrated the chances of structural damage to the liver at any time throughout the exposure duration. The results showed that FPN is hazardous to rats when exposed to it for an extended period of time. As a result, it was recommended that adequate safety measures should be taken if insecticides were employed or discarded in locations where animal populations were dense.

Aroonvilairat, Tangjarukij [78] evaluated that chlorpyrifos and their mixture with other insecticides cause immunological and hematological alteration in rats. 3 groups of rats were exposed dermally to the kidney, adrenals and liver. Substantial alterations were detected in the numerous biological criteria including hemolytic toxicity, rise in platelet count and leukocytes, %age of neutrophils and reduction in % age of lymphocytes, RBC and eosinophil. Results concluded that pesticides and their mixture are hazardous to the health of the individuals.

Hussain, Ghaffar [79] analyzed the fipronil's effects on hemato-chemistry, growth, protoplasm and reproduction in adult cockerels. Fipronil was combined with maize oil and given orally to separate groups in various concentrations. Birds exposed to higher amounts showed signs of poisoning such as anaemic comb, gasping, tremors and watery droppings. According to the results, when birds were exposed to increased quantities, their feed ingestion, the weight of the body and both absolute and relative organ weights all experienced significant reductions. Birds had congestion in

their livers, kidneys and pyknosis of hepatocytes. These organs also showed signs of necrosis in the tubular epithelial cells as well as widening urine gaps. Haematological indicators like erythrocyte counts, haemoglobin concentrations and Hct were drastically reduced in birds while total leukocyte counts increased.

Ghaffar, Hussain [80] assessed that modest dosages of fipronil cause hemato-biochemical, histological and genetic harm in common carp. Fish were placed into 6 groups in glass tanks after two weeks of acclimation and treated with varied amounts of fipronil for two weeks. Hemato-biochemical variables were evaluated using blood samples taken every four days. Clinical-hematological and biochemical markers in fish treated with large doses show serious aberrations. According to the results, there was a substantial decrease in erythrocyte count, haemoglobin and hct nevertheless, there was a significant increase in MCV, TLC , neutrophils, monocytes and lymphocytes.High-dose treated groups also had more nuclear and intracellular aberrations. In C.carpio, fipronil causes clinical-hematological and serum biochemical alterations.

Uchendu, Ambali [81] evaluated the effect of the combination of low levels of chlorpyrifos (CPF) and deltamethrin (DLT) on body weight and haematological parameters in Wistar rats and the effect of alphalipoic acid (ALA). Male Wistar rats were divided into six groups o feight each. Group I was given soya oil at 2 ml kg−1, group II (ALA) received ALA at 60 mgkg−1. Group III (DLT) received DLT at 6.25 mg kg−1 while group IV (CPF) was given CPF at 4.75 mg kg−1 (1/20th LD50). Rats in group V (CPF+DLT) received the combination of CPF and DLT while rats in group VI (ALA+CPF+DLT)

were pretreated with ALA at 60 mg kg−1 and then co-exposed to CPF and DLT for 45 min later. The regimens were administered once daily by gavage for 4 months. Co-exposure to CPF and DLT caused significant ($P< 0.05$) changes in body weight and some haematological parameters (red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV) and neutrophil/lymphocyte ratio (NLR)). Pretreatment with ALA did not significantly ameliorate most of the alterations induced by the pesticide combination. It was concluded that co-exposure to CPF and DLT caused more adverse effects on body weight and some haematological parameters than single pesticide exposure; pretreatment with ALA at 60 mg kg−1 did not significantly ameliorate the changes induced by the pesticide co-exposure.

Fredianelli, Pierin [82] documented the toxicity of FPN for *Rhamdia quelen* using hematologic, biochemical, genetic and histological indicators. Fipronil was exposed to 42 juveniles in separate groups. Thirty-six silver catfish were placed into control groups for further tests. Blood, kidneys, livers and gills were taken after the experiment to be evaluated. The results revealed a decrease in hematocrit and a rise in liver enzymes. Alterations seen in liver samples were cytoplasmic vacuolization and histological degradation. Gills displayed clear signs of vascular congestion, complete fusion of secondary lamellae and epithelial cell expansion. Anemia, as well as changes in the levels of liver enzymes, erythrocyte modification, and damage to the liver, gills, and kidneys, are all caused by FPN in silver catfish.

Akpa, Ayo [83] evaluated the effect of sub-chronic oral CPF administration on haematological and serum biochemical indices, and the possible

ameliorating effect of vitamin C on the indices in mice. Thirty mice divided into 3 groups of 10 mice each were used for this study. Mice in group I (control) were dosed with vegetable oil while those in group II were given CPF (21.3 mg/kg~ $1/5^{th}$ LD50) only. Mice in group III were pretreated with vitamin C (100 mg/kg) before dosing with CPF 30 min later (Vitamin C + CPF-treated group). This regime was given to each group of mice three times a week for ten weeks. At the end of the study period, blood samples were collected from the mice and analyzed for packed cell volume (PCV), total red blood cell (RBC), white blood cell (WBC) and total protein. The results showed that mice in the vitamin C + CPF-treated group exhibited milder signs of toxicity and a significant increase in weight gain ($p<0.01$) compared to the CPF-treated group. No significant increase in weight in the CPF-treated group was observed compared to the control. There was a significant increase in PCV, RBC, Hb, TP and creatinine, but a significant decrease was obtained in WBC, ALT and AST in the CPF-treated group compared to the control. All the parameters with the exception of WBC, ALT and AST (which increased significantly), were significantly decreased in the vitamin C + CPF-treated group compared to the CPF-treated group.

Ravikumar, Madhuri [84] documented the haematological alteration due to individual toxicities of cadmium, chlorpyrifos and their combination in albino wistar rats. The experiment was carried out for 28 days. Group 1 - Control. Group 2 - Cadmium chloride (Cd) 22.5mg/ kg b.wt /per oral / day. Group 3 - Chlorpyrifos (CPF) 25 mg/ kg b.wt /per oral / day. Group 4 - Cadmium chloride (Cd) 22.5 mg + Chlorpyrifos (CPF) 25 mg/ kg b.wt /per oral / day. The

weekly body weight gains were significantly ($P < 0.05$) decreased in groups 4, 3 and 2 when compared with the control group. Haematological observations revealed a significant ($P < 0.05$) decrease in overall means of TEC, Hb, and PCV except TLC in groups 4, 2 and 3 when compared with the control group. TLC values were increased by 4, 2 and 3. These results revealed that combined toxicity of cadmium and chlorpyrifos is significant than exposure of individual components which resulted in alterations in haematological parameters.

Severcan, Ekremoglu [85] evaluated the insecticide's effects on liver tissues in rats. The rats were divided into 4-groups of six animals each. Group 1 was given corn oil, while groups 2, 3 and 4 were given Malathion dissolved in corn oil at doses of 100, 200, and 400 mg/kg, respectively. After 24 hours of Malathion exposure animals were slaughtered and tissues of liver were collected. Histopathologically and biochemically liver tissue was examined. The level of tumour necrosis a factor and status of total oxidant were increased significantly in group IV as compared to group I. The activities of catalase enzyme in groups III and IV were statistically increased as compared to group I. level of aryl esterase in groups III and IV was significantly lower than that of group I. In group IV degeneration was seen while some hepatic vacuoles were remarked.

Jalili, Farzaei [86] stated that insecticides exert potential impacts on the liver of rats. The impact of Malathion has been observed as an antioxidants. Red grapes produced a natural phenol known as Resveratrol which had been used as an antioxidant. This experiment was designed to observe the effects of resveratrol as an antioxidant in contradiction of

toxicity on Malathion in the liver of some rats. In this experiment forty-eight rats were divided into eight groups saline-treated, controlled (Malathion 50 mg), resveratrol-treated group (2, 8 and 20mg) and Malathion + resveratrol-treated groups (2, 8 and 20 mg/kg). The prepared treatments were given on a daily basis for fourteen days through the abdominal peritoneum. To determine nitrite oxide serum level Gries technique was used. Alanine aminotransferase, Aspartate aminotransferase and concentrations of alkaline phosphate were noticed to affect liver functioning. Administration of Malathion enhanced the level of nitric oxide and MDA (malondialdehyde) of the liver, CHV diameter and also caused a decrease in Frap (tissue ferric-reducing ability of plasma). Malathion and Resveratrol treated group also showed a decrease in the diameter of CHV and hepatocyte, the enzymatic activity of the liver, MDA and enhanced FRAP. So there was clear evidence of improvement in the injury of the liver which was induced by Malathion.

Abdelgadir, Al-Qudsi [87]2020) observed that acute treatment of insecticide caused histological and biochemical alterations in the heart, kidney and liver of albino rats. The rats were distributed into 4 groups. One group is regarded as control while the rest of the groups were gavaged orally with a wide range of fipronil for about a month. After 12 days from the initiation of the trial, the haematological values of MCHC and packed cell volume were high in the exposed groups when compared to the control. After a month from the initiation of the trial there were no substantial outcomes in the haematological parameters. During the trial, no animal was deceased. FPN also induced histopathological changes in the organs of rats. According to the results, it was considered that

histopathological alterations show the hazardous effect of FPN.

Silva, Fraga [88] documented the genotoxic effect of the insecticide chlorpyrifos on the erythrocytes of *Odontophrynus carvalhoi* tadpoles. Over three days, tadpoles were exposed to different concentrations of this insecticide. After the experiments, the tadpoles were sedated and their blood was obtained through heart incision followed by cyto smears and May Grunwald Giemsa staining. When contrasted to the control group, the tadpoles exposed to chlorpyrifos had a higher frequency of genetic mutations. After two days of exposure, there were statistically significant variations in the frequency of micronuclei. According to the findings, O. carvalhoi tadpoles were vulnerable to genetic changes caused by the use of a standard chlorpyrifos mixture which had a carcinogenic and mutagenic effect in erythrocytes.

Abouelghar, El-Bermawy [89] evaluated the changes in the biochemical activities and hematology due to the short-term exposure to fipronil in mice. Mice were exposed to diverse lethal concentrations for about a month. High and medium exposure to FIP indicated lower values of Hb, RBCs, platelets and white blood cells as compared to the control group. The sublethal concentration lower than the marketable formulation of fipronil might have no observable serious effect under our current experimental conditions. On the contrary, the high concentration might cause substantial effects on biomarkers and it may be toxic to health the of humans under long exposure to insecticide.

Farkhondeh, Amirabadizadeh [90] investigated the blood glucose levels in rats exposed to chlorpyrifos. Meta-analysis of 7 animal studies indicated the dose-dependence manner of

chlorpyrifos exposure on the blood glucose levels. The subgroup analysis indicated that exposure to low doses of chlorpyrifos significantly increased the blood glucose levels in exposed animals versus the non-exposed (0.11; 95% CI: − 1.14, 1.36, z = 2.25, p =0.03, I2 =90.1%, p < 0.001) and high doses markedly decreased blood glucose levels in exposed rats versus the nonexposed (7.34; 95%CI: − 9.35, − 5.32, z = 6.41, p < 0.001, I2 = 96.9%, p < 0.001). The random effects and pooled analysis indicated that the blood glucose levels were 4.22-fold lower in exposed animals versus the non-exposed ones (95% CI: − 5.59,− 2.85; Z = 3.97; p < 0.001); therefore, heterogeneity was significant (I2 = 96.5%, p < 0.001). The present finding indicated the association between chlorpyrifos exposure and a decrease in blood glucose levels.

Roshanravan, Mehrpour [91] evaluated the impact of sub-chronic exposure to chlorpyrifos (CPF), a potent OP, on the hematological parameters of male rats of different ages. 48 male Wistar rats including young (2-month old), middle-aged (10-month old) and aged (20-month old), were randomly divided into three control and three treatment groups as follows (n¼8 per each group): young control group (C2), young CPF-treated group (CPF2), middle-aged control group (C10), middle-aged CPF-treated group (CPF10), aged control group (C20) and aged CPF-treated group (CPF20). CPF (5mg/kg, orally) was administrated for 45 days. The control animals were given olive oil as a vehicle. The blood samples were gathered from the heart to assess values of the mean platelet volume (MPV), mean corpuscular haemoglobin (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), platelets (PLT), hematocrit (Hct), haemoglobin (Hb) and the number of red blood cells (RBCs).

The findings showed that CPF dramatically reduced the number of RBCs, Hct, Hb, MCH and PLT values in the young rats versus the age-matched control rats. However, no marked differences were found between haematological parameters in ageing rats versus age-matched controls. The present findings indicated that sub-chronic exposure to CPF caused anemia in young animals. It is proposed that young animals are more sensitive to the hepatotoxic impact of CPF than ageing rats. However, more studies are needed to confirm the current findings.

Roshanravan, Mehrpour [91] assessed the chlorpyrifos toxicity in the hematology of rats depending on their age. During the test, rats belonging to different age groups were distributed randomly into 3 groups and each group contained eight rats. Different concentration of insecticide was given to the rats belonging to the different age groups for about one and a half months. Samples of blood were collected from the heart to evaluate the value of Hb, MCV, MCH, HCT, MCHC, MPV, RBCs and the number of platelets. The study revealed that insecticide dramatically decreases the values in young groups of rats. On the other hand, no significant change was observed in old ones. It indicated that anemia is caused in young ones due to chronic experience to CPF. It was concluded that young ones are a bit more sensitive to the toxicity of insecticides.

Akram, Iqbal [92] evaluated the effects of pesticides on haematological, serum biochemical and histological indicators in bighead carp. Total eighty bighead carps were randomly divides in different 4 groups. Fish were subjected to different doses of pesticides for 2 month. Fish in group A acted as a control treatment. Results showed that erythrocyte counts,

haemoglobin level, lymphocytes and monocytes markedly reduced while total leukocyte and neutrophil counts significantly raised in treated fish. Various blood chemistry indicators such as serum albumin and total proteins drastically reduced. Histological examination of the brains of fish that had been exposed to the toxin revealed several abnormalities including the death of neurons in the cerebellum, a buildup of lipofuscin, necrotic neurons, microgliosis and inflammatory cells as well as haemorrhage.

Tahir, Ghaffar [93] determined that insecticides caused toxicity in different organs of Nile tilapia. The goal of this study was to look at the toxicity of endosulfan, an organochlorine-pesticide, upon Nile-tilapia (Oreochromis niloticus, a freshwater fish) as well as lycopene's impacts towards toxicity/harmfulness. The following 4 treatmenting groups of fish were considered: (1) a control group. (2) an endosulfan-exposed/experienced, (3) a basal-eating accompanied with lycopene and (4) a basal-eating provided with lycopene and acquainted with endosulfan group. Over the course of four weeks, the experiment was conducted Endosulfan impacted liver function, particularly liver enzymes and plasma proteins, in a negative way. Endosulfan influenced fish blood-factors, lowering RBC and WBC numbers while also affecting immunological markers. Endosulfan raised the amount of lipid peroxide malondialdehyde and lowered the levels of antioxidants catalase, superoxide dismutase, and glutathione peroxidase, causing oxidative stress (MDA). Endosulfan also raised cytochrome P450 (CYP450) levels while reducing GST (glutathione S-transferase) mRNA transcript levels and disrupting the histo-logical structure of afflicted fish's spleen, gills and liver.

CHAPTER 3

Materials and Methodology

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Insecticides are chemical or biological products that are commonly used around the world to control household and agriculture pests that pose a danger of exposure to humans and animals. The present study was designed to study the effects of a binary mixture of insecticides (Chlorpyrifos+Fipronil) on haematology and histopathology in the Liver of Wistar Albino rats. The research was carried out at the Animal House, University of Agriculture Faisalabad.

3.1. Test Animal and Acclimatization

Male albino rats weighing 125 ± 6.2 g were purchased from Animal House, University of Agriculture Faisalabad. None of the animals had been diagnosed with a disease. The experiment was performed for 28 days in an animal house facility, UAF. The rats were sorted into three groups of similar size, each consisting of five rats and housed in three separate cages at the ideal temperature for rats in the room (22°C- 25°C)Before the experimentation, rats were acclimatized for one week in a stainless steel cages. They were given water and conventional food with twelve-hour light and dark cycles. The UAF ethical committee oversaw all animal ethics throughout the entire study period.

Figure 3.1: *Commercial Feed along with Acclimatization of Wistar Albino Rats*

3.2. Chemicals and Stock Solution

Fipronil and Chlorpyrifos were purchased from Sigma-Aldrich. Sigma-Aldrich was the vendor for the acquisition of all other chemicals. Corn oil was used in the preparation of the stock solution. Stock solution for low dose was prepared by dissolving chlorpyrifos and fipronil (3.1mg/kg + 2mg/kg) in 10ml of corn oil while on the other hand stock solution for high dose was prepared by dissolving chlorpyrifos and fipronil (9.7mg/kg + 6.2mg/kg) in 10ml of corn oil.

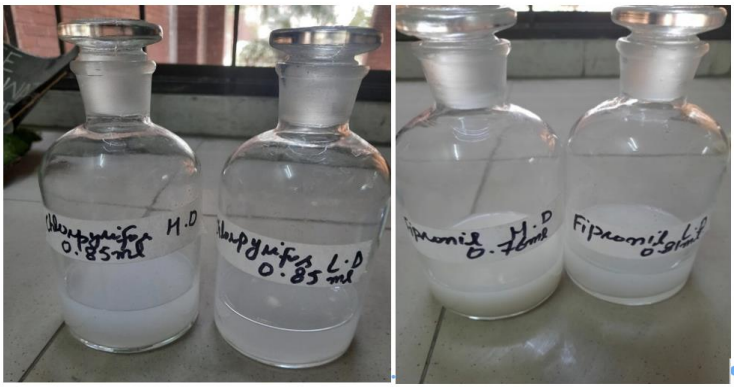

Figure 3.2: *Preparation of Stock Solution*

3.3. Exposure of insecticides

Rats were divided into three treatments T_o, T_1 and T_2 (5 rats/group). T_o was considered as control. It was provided only with water and commercial feed in an aerated and clean environment. T_1 and T_2 were regarded as treated groups and each rat was gavaged orally with a binary mixture of CPF + FPN dissolved in corn oil for 28 days. Treatment 1 was exposed to the low dose of binary mixture of chlorpyrifos and fipronil (3.1mg/kg + 2mg/kg at the rate of $1/50^{th}$ of known

LD_{50} of respective insecticides) along with commercial feed. Treatment 2 was treated with commercial feed along with a high dose of binary mixture of chlorpyrifos and fipronil (9.7mg/kg + 6.2mg/kg at the rate of $1/10^{th}$ and $1/25^{th}$ of known LD_{50} of insecticides respectively. Throughout the course of the investigation, weekly measurements of body weight were taken. Throughout the experiment, each rat was monitored for any symptoms of poisoning or signals of impending death.

3.4. Measurement of organ and body weight:

During the study time of 4 weeks, the body weight of rats was recorded at the start of the experiment and at the time of sacrifice using the analytical balance. At that time, the weight gain of the animals was calculated by the following formula:

Weight gain (g) = (body weight on specific week (g) − initial body weight (g))

At the termination of particular periods of treatments, the exposed albino rats were sedated with ether before their kidneys were extracted through a midline incision in the abdomen. The kidneys were weighed on a Sartorius balance after being dried in filter paper. The mean weight of the kidneys of treated albino rats from all treatments was measured at the time of sacrifice. The relative weight of the kidney was measured from the equation as follow:

$$\text{Relative weight of Kidney (g)} = \frac{Mean\ Absolute\ \ weight\ of\ Kidney\ (g)}{Bodyweight\ on\ sacrifice\ day\ (g)} \times 100$$

3.5. Blood sample collection

A the trial, rats were starved overnight and on the next day anaesthetized by means of diethyl ether. The rats were sacrificed and then 10ml of blood samples were collected from the abdominal artery with the help of a sterilized

heparinized syringe and consequently distributed into 5ml test tubes each. First, the blood samples were transferred into germ-free capped flasks holding anticoagulant potassium ethylene diamine tetra acetic acid (EDTA) for haematological examination.

Figure 3.3: *Blood Sampling*

3.6. Hematological examination

The RBC count was determined using a Neubauer crystalline counting chamber. On the RBC's pipette, blood was sucked up to the 0.5 point, and then Hayem's fluid (RBCs diluting fluid) was pulled up to the 101 marks. To ensure adequate mixing pipette was rotated between the thumb and fingers. Over the governed area, a cover glass was placed. While holding the pipette at a 45° angle, a drop of blood was put towards the edge of the coverslip. It was allowed to rest for 2 to 3 minutes. Under a light microscope, the charged chamber of the hemocytometer was magnified and the number of RBCs was counted in 80 tiny squares. Counting was done on the cells that had come into touch with both the upper and lower lines. The lines on the bottom and the right were used to remove any cells that came into touch with them. The number of RBCS per cubic millimetre was calculated as follows:

The total number of RBCs per square millimetre was calculated by using the following equation:

$$\text{Total No. of RBCS} = \frac{N \times 4000 \times 200}{80}$$

N = Number of total cells in 80 small squares

3.7. Determination of White Blood Cell Count (WBC)

The WBC count was determined using a hemocytometer [94]. As described by Donald and Bonford, (1963) the blood was drawn up to the 0.5 mark of the White blood cell pipette and the Turk's fluid (WBC diluting fluid) was drawn up to the 11 mark above the bulb. The solution was allowed to mix and stand for 2 to 3 minutes. A drop of blood was put beneath the pipette's coverslip while holding the pipette at a 40° angle. It was left to stand for 2 to 3 minutes before being removed. To check for equal cell distribution the ruled region of the hemocytometer was focused using a low-power magnification and dim light. The number of white cells was counted in each of the four 1 sq mm corner regions. Those white square cells that touched the top and right centre lines were counted. White cells that touched the bottom and left lines were not included in the analysis. The number of WBC per cubic millimetre was calculated as follows.

The depth of the counting chamber: is 0.1 mm

- Area counted: 4 sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore, 4 x 1.0 sq mm = a total of 4 sq mm).
- Volume counted = area counted x depth = 4 sq mm x 0.1 mm = 0.4 cu mm
- Volume correction factor = 1/0.4-2.5
- Number of Cells per cu mm = n x dilution factor x volume correction factor= n x 20 x 2.5

- n = number of cells in 0.4 cu mm of diluted blood

3.7.1. Determination of Platelet Counts

For the manual determination of platelet count, a hemocytometer was used [95]. In the first step, a white blood cell tube was packed with the blood sample up to 0.5 markings and then filled with the platelet diluting solution (Rees - Ecker Fluid) up to 11 markings. After that, the tube containing the specimen and solution is circled between palms for their proper combination. Holding the tube at 45^{o}, a droplet of blood sample was poured below the coverslip from the tube. After pouring the stimulated chamber was retained in a humid surrounding for about ten to fifteen minutes and the cells were permitted to set without losing moisture. The stimulated hemocytometer was then set under the light microscope to count the platelet numbers.

The number of platelets per unit volume can be calculated as follows:

$$\text{Total platelets count} = n \times \text{Dilution factor} / (\text{Area} \times \text{Depth})$$

- n = Number of platelets counted
- Area = Area of 5 square = 1/ 5 sq. mm
- Depth of chamber = 0.1 mm
- Dilution Factor = 20

3.7.2. Packed cell Volume (PCV)

%Age of PCV will be checked by using the ICSH method. Blood samples will be moved from an EDTA tube to a capillary tube with a microhematocrit. After that, each sample will be centrifuged for 5 minutes at 1000 RPM. Measurementswere made on the length of the red blood cell

column and the whole column of red blood cells and plasma inside the tube.

3.7.3. Mean Corpuscular Volume (MCV)

MCV measures the average size of the sample's RBCs. It was calculated by the given formula:

$$MCV = \frac{\text{Hematocrit} \times 10}{\text{Red blood cells per ul}}$$

3.7.4. Determinant of Hemoglobin Concentration (Hb)

The cyanmethemoglobin Technique was employed to conclude the haemoglobin concentration in the blood sample. In a glass tube 5ml of Drabkin's solution was placed and then 20 µl of sample blood was combined along with EDTA and a dilution in a ratio of 1:251 was synthesized. Then the mixture is permitted to settle down for a maximum of five minutes after mixing. The time of settling down was required to convert the haemoglobin into haemoglobin cyanide. Then the blood sample was into the cuvette and the absorbance of the sample solution was by means of a spectrophotometer fixed to 540nm. The absorbance of the standard solution was also calculated for comparison. Absorbance was also measured in comparison with Drabkin's solution.

Hemoglobin concentration was measured via the equation below:

$$Hb(g/dl) = \frac{\text{The absorbance of the test sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} \times \frac{\text{Dilution factor}}{1000}$$

3.7.5. Mean Corpuscular Hemoglobin (MCH)

The average mass of haemoglobin in a sample is known as MCH. It was calculated through the given formula:

$$MCH = \frac{\text{Hemoglobin} \times 10}{\text{Red blood cells per ul}}$$

3.7.6. Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC is a way to measure how much haemoglobin is in a certain amount of packed RBCs. It was calculated through the given formula:

$$MCHC = \frac{\text{Hemoglobin} \times 10}{\text{Hematocrit per ul}}$$

3.7.7. Hematocrit (PCV)

Packed cell volume (PCV) was calculated with the help of a microhematocrit procedure [96]. The blood sample was first combined with an anti-coagulant and then sucked up into two micro-hematocrit tubes via capillary action to avoid any bubble formation. The test tubes were filled up to 3/4 height. Then one end of the test tube was covered tightly with a little soil substance perpendicularly so that the covering must be completely even bottom. The totally covered tubes having the sample were then placed in the centrifuge in a way that the covered face was directed toward the external side of the centrifuge. Rotation of samples in a centrifuge parted the red blood cells from plasma fluid and a patch of buffy coats of white blood cells and platelets was formed. After taking out the sampling tubes from the centrifuge machine, a hematocrit value was attained on the hematocrit reader.

3.8. Histopathological Analysis of liver

At the end of the trial, the rats were sacrificed and all livers were extracted. Histopathological analysis was carried out by using the protocol of Michael [97] with minor variations. All the samples were placed into bottles and immediately fixed in formalin for the whole night. Small sections of livers were

taken, embedded in paraffin and stained with hematoxylin-eosin stain to determine the abnormalities of tissues by microscopic study.

Figure 3.4: *Dissection of Albino rats for Histopathological evaluation of liver*

Histopathological evaluation was done according to the following steps:

3.8.1. Fixation of Liver tissues

The samples were fixed to 10% formalin solution and allowed to fully immerse at room temperature for at least 24 hours. Formalin was used to stiffen the tissues for proper staining.

3.8.2. Post-fixation treatment of tissues

The selected portion of liver tissue slices was $0.1cm^2$ and these portions were washed with water for 6-7 hours. The tissues were dehydrated with paraffin, ethanol and xylenes of different concentrations.

Figure 3.5: *Fixation of liver sample*

Table 3.1. *Post-fixation treatment protocol for histopathology*

Dehydrating agent	**Time (hour)**
Alcohol 25-30%	One
Alcohol 45-50%	One
Alcohol 65-70%	Two
Alcohol 75-80%	One
Alcohol 90-95%	One
Absolute alcohol-1	Two
Absolute alcohol-2	Two
Xylene-Alcohol	One-half
Xylene-1	One
Xylene-2	One
Paraffin-1	Two
Paraffin-2	Two

3.8.3. Embedding

After extraction, small sections (5µm) of livers were placed in an embedding agent named paraffin wax for the protection of homogenous mass.

Procedure

- The tissues were placed in the core of the steel frame
- Over the steel frame, a plastic frame was placed
- Liquid paraffin was placed in the mould of plastic around the tissues and left to set
- Allowed it to cool
- When paraffin solidified then separated the block from the mould
- The excess tissues were removed on all sides
- Identification was set with the help of a spatula to aside block
- These blocks were placed in the refrigerator until used for protection from dust particles

Figure 3.6: *Organ Embedding*

3.8.4. Sectioning

A transparent slice of organ tissue placed on a glass plate and covered with a cover slip and this slide refers to a section. A mechanical instrument microtome is used for the cutting of extremely thin slices named sections. The tissue was sectioned scopmicroscopically after embedding.

Procedure

- The microtome was cleaned with xylene and lubricated with oil.
- A three-micrometre thick section was managed to achieve.
- The section was held in place in the tissue carrier on the disc block.
- Tissue block was created by placing a screw block against the blad's cutting edge.
- The microtome was used to cut the strip-forming section. Floating in warm water in the
- water bath helped to flatten the sections.
- The Sections were directly placed on the slides.
- The pieces of paraffin were excised by inserting them into the 45°C temperature of the oven.
- The blade was washed with xylene after each block-splitting

3.8.5. Mounting

With the help of a sticky Meyer's egg white, the samples of cut tissues were placed on glass slides.

Procedure

- The slides were cleaned with xylene, labelled and recognized
- A thin smear of albumin was stained on the slide surface

- A cutter is used to separate the necessary portion of a strip
- The sections were floated in warm water at 35°C in a plastic container
- The slides that were prepared started sinking below the sectioning
- Allowed them to float on the slide cover
- The assembly of slide sections were removed from the water and then dried for 24 hours at 37 ^{0}C
- The slides were incubated for 25-30 minutes after drying
- Water evaporated from the sections then placed carefully on the surface of the glass
- Completely dried sections were mounted on the slides

3.8.6. Staining

The tissues were stained with hematoxylin and eosin as given below:

Table.3.2: *Hematoxylin-eosin staining*

Staining Agent	**Time**
Xylene-1	7 minutes
Xylene-2	7 minutes
Absolute alcohol-1	3-time dip
Absolute alcohol-2	3-time dip
Alcohol 65-70%	3-time dip
Washing	5-6 time
Hematoxylin	12-13 minute
Washing	3-4 time
Acid alcohol	2-3 dip
Washing with ammonia	5 time
Alcohol 65-70%	5 time
Eosin	50-60sec
Absolute alcohol-1	3-4 dip
Absolute alcohol-2	3-4 dip

Clean and dry	
Xylene -1	5-6 time
Xylene-2	5-6 time
Mounting	
Heating	8-10 minutes

Procedure

The slides were stained with Hematoxylin-Eosin and ncovered with a cover slip. A light microscope was used for the examination of sections.

The following steps were taken:

- A sharp cutter was used to cut the small pieces of about 5 µm of liver
- After that, the slices of kidney were washed in distilled water for 6-7 hours
- After washing with distilled water slices of the kidney were washed with 70%, 50% and 30% alcoholic solutions
- Kidney slices were dipped for 5-6 hours in an alcoholic solution.Dipping the sample in solution for 4-5 hours than in 80% and 95% alcoholic solution for 1-2 hours respectively
- Then dipped in 95 % and 80% solution for 2-3 hours in alcoholic solution
- After that dip in absolute alcohol one and two for 1-2 hours respectively
- Then small pieces of liver were dipped in the mixture of alcohol and xylene solution for 30 minutes
- After that, these slices were dipped in the solution of Xylene 1, and Xylene 2 for sixty minutes
- The pieces were then immersed in paraffin 1 for 1-2 hours and then in paraffin 2 for 2-3 hours

- Liver slices that were wax moulded, cut with HM 325 of 3µm
- After that finally fixed in the slides
- Then stained with hematoxylin-eosin stain
- The light microscope was used for the final examination

Figure 3.7: *Preparation of slides for histopathological examination*

3.9. Statistical Analysis

For statistical analysis, all the derived data were stated as mean ± standard deviation. The values of the treatment groups were compared with the control group. Data were analyzed by one-way ANOVA with repeated measures under CRD and comparisons of mean were carried out by Tukey test by using software Statistix 8.1.

CHAPTER 4

Results and Discussion

Insecticides are chemical or biological products that are commonly used around the world to control household and agriculture pests that provide a danger of exposure to humans and animals Wang, Xing [1]. A major rise in the utilization of these chemicals in agriculture has resulted from an increase in global food demand. Increased insecticide use could result in irreversible ecosystem damage. Toxicity from insecticides is a global issue that affects developing countries Soliman, Attia [2]. Many pesticides are also hazardous to non-target organisms, putting animal and human health at risk Kim, Kabir [3].

The present research work was carried out to study the effects of a binary mixture of insecticides (Chlorpyrifos Fipronil) on haematology and histopathology of the liver in Wistar Albino rats. Generally, the health of the rats was usual throughout the trial period with no cause of death in any treated group during the chronic exposure of 28 days.

4.1. Effects on the Body Weight and size of the organ

All animal's body weights were comparable at the beginning of the experiment. In each of the three groups, the animals' weights climbed correspondingly. Both mortality and morbidity were undetectable in the treated animals. Generally from the second week after exposure, rats exposed to greater concentrations of binary mixtures of insecticides showed indicators of toxicity including a change in activity and aberrant gait. We observed an increase in the relative liver weight of treated rats with the high dose of the binary mixture of chlorpyrifos and fipronil in comparison with control and other treated groups.

4.2. Hematology

In albino rats, haematological measures such as RBCs, haemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), mean corpuscle volume, mean corpuscle haemoglobin (MCH) and mean corpuscle haemoglobin concentration (MCHC) were used as biomarkers of pesticide toxicity. The oral treatment of a binary pesticide mixture resulted in hematotoxicity in rats.

Table 4.1: *Analysis indicating the body weight in the treated groups and control groups of Albino rats*

	Body Weight(g)		
Treatments	**Initial body weight**	**Final body weight**	**Weight gain**
Control	127.6±2.88	174±2.74	46.4 ± 2.30^{a}
T1	127.6±3.05	164.8±1.48	37.2 ± 3.19^{b}
T2	129.4±2.07	157.2±1.30	27.8 ± 3.27^{c}

Results of the present study regarding weight gain of the albino rats treated with various doses of chlorpyrifos and fipronil were depicted in Table 4.1. The table shows that the weight gain in the low and high-dose treatments of insecticides T1 and T2 were significantly lowered as compared to the untreated control group To after treatment with the binary mixture of Chlorpyrifos and Fipronil as depicted. Different letters indicate statistical differences among control and insecticide treatment groups within a measured parameter. The significantly lowered weight gain was observed in low dose T1 and high dose T2 with the

values of 37.2±3.19 g and 27.8±3.27 g respectively. Maximum weight gain among all the treatments was found to be 46.4±2.30 g in untreated control treatment To. The values of the weight gain ranged between 46.4±2.30 to 27.8±3.27. The trend of the weight gain among albino rats of different treatments treated with low and high doses of insecticides chlorpyrifos and fipronil was To>T1>T2. Exposure to a binary mixture of chlorpyrifos and fipronil has affected the growth of rats but the maximum effect was observed in the treatment exposed to the high dose of insecticides.

Table 4.2: *Analysis of Variance (ANOVA) indicating the body weight in the treated groups and control groups of Albino rats*

Source of Variance	SS	MS	F-value P-value
Treatment 2	864.9	432.467	49.52* p<0.05
Error 12	104.8	8.733	
Total 14	969.7		

*= significant (p<0.05)

The statistical analysis demonstrating the body weight in the treated groups and control groups of Albino rats is represented in table 4.2. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides (chlorpyrifos and fipronil) has affected the growth of rats.

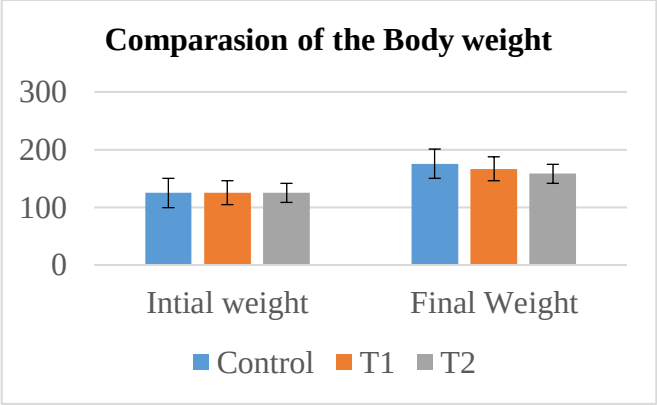

Figure 4.1: *Comparison of Initial and Final weight of the Albino rats after 28 days exposure of binary mixture of Insecticides*

Graphical representation of the comparison of initial and final body weight of all the treatments treated with various doses of insecticides for 28 days were presented in Figure 4.1. Error bars depict the standard error of the mean. Weight gain was increased significantly in all treatments at the end of the trial when compared to the non-treated groups. The figure showed that the final body weights of high and low doses of chlorpyrifos and Fipronil treated rats in treatment T1 and T2 were significantly lowered than the control treatment To. Minimum weight gain in the albino rats was observed in the high dose of chlorpyrifos and fipronil treatment T2. Maximum weight gain was observed in the untreated control treatment which was provided with only commercial feed. Weight gain in different treatments of albino rats was increased in an order of To>T1>T2. Exposure to a binary mixture of chlorpyrifos and fipronil has affected the body weight of rats but the maximum effect was observed in the treatment exposed to the high dose of insecticides.

Table 4.3: *Analysis indicating the organ (Liver) weight in the treated groups and control groups of Albino rats*

Organ Weight (g)	
Treatments	**Relative organ weight(g)**
Control	5.28 ± 0.14^{c}
T1	6.2 ± 0.11^{b}
T2	7.3 ± 0.15^{a}

The outcome of the current research exhibited increased liver weight in all the treatments at the completion of trials as depicted in table 4.2. Table illustrates that the rats exposed to the high dose of the binary mixture of the chlorpyrifos and fipronil in T2 have significantly ($p < 0.05$) increased liver weight at the end of experimental trials as compared to the control To. Different letters indicate statistical differences among control and insecticide-treated groups within a measured parameter. Values of liver weight gain recorded in control, low dose treatment of (chlorpyrifos + fipronil) and high dose treatment of (chlorpyrifos + fipronil) are 5.28 ± 0.14g, 6.2 ± 0.11^{g} and 7.3 ± 0.15 g respectively. The minimum value of liver weight gain was observed in the untreated control treatment To. The kidney weight in all the treatments of insecticide (chlorpyrifos and fipronil) treated albino rats followed the trend of $T_2>T_1>T_0$.

Table 4.4: *Analysis of Variance (ANOVA) indicating the organ weight in the treated groups and control groups of Albino rats*

Source of Variance	SS	MS	F-value
	P-value		

Treatment 2	9.9213 $p < 0.05$	19.4447	883.85*
Error 12	0.3160	0.0220	
Total 14	10.2373		

*= significant ($p < 0.05$)

The statistical analysis demonstrating the organ weight in the treated groups and control groups of Albino rats is represented in Table 4.4. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < 0.05$).

The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides (chlorpyrifos + fipronil) has affected the weight of organs in albino rats.

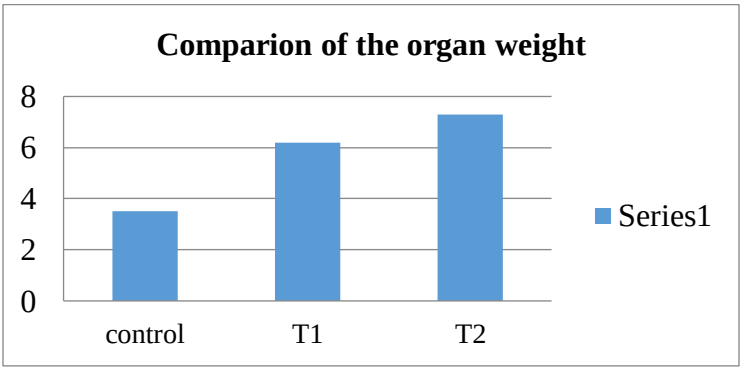

Figure 4.2: *Comparison of Initial and Final weight of the organ (Liver) of the Albino rats after 28 days exposure of binary mixture of Insecticides.*

Figure 4.2 represents the comparison of weight gain of the liver in insecticide chlorpyrifos and fipronil treated different

treatments of albino rats. Error bars depict the standard error of the mean. The figure shows that the final weight of the liver increased significantly in all the treatments including control at the completion of the trial. Maximum liver weight was recorded for T2 treated with a high-dose binary mixture of chlorpyrifos and fipronil. Minimum liver weight was recorded in the untreated control treatment which was only provided with the commercial feed. The figure concludes that both the low dosage and high dosage of poisonous insecticides chlorpyrifos and fipronil resulted in significant increase in liver weight of in albino rats. The organ weight was increased in different treatments of albino rats in an order of $T_2 > T_1 > T_0$. Exposure to a binary mixture of chlorpyrifos and fipronil has affected the body weight of rats but the maximum effect was observed in the treatment exposed to the high dose of insecticides.

Table 4.5: *Analysis indicating the RBCs (10^6/µl) count in the treated groups and control groups of Albino rats*

RBCs (10^6/µl) Normal Range (6-8)			
No. of Animals	**Control**	**T1**	**T2**
1.	6.13	4.41	3.95
2.	6.28	4.64	3.76
3.	6.85	4.95	4.03
4.	7.02	5.02	3.62
5.	6.95	4.17	3.56
Mean±SD	6.65±0.41	4.63±0.35	3.78±0.20

Table 4.4 shows that the treatments treated with insecticides chlorpyrifos and fipronil declined the RBCs count in the blood of albino rats. The red blood cell count has decreased significantly in low and high-dose treatments of chlorpyrifos and Fipronil with values of 4.63±0.35 and 3.78±0.20 respectively. Treatments T1 (low dose) and T2 (high dose) exhibited significant differences when compared to the control group. Control group To provides a higher concentration of RBCs count than insecticide groups with a value of 6.65±0.41. The level RBC (10^6/µl) in the treated groups of Albino rats decreased significantly when compared to the control group (table:4.6).

Table 4.6: *Analysis of Variance (ANOVA) indicating the level RBCs (10^6 /µl) in the treated groups of Albino rats*

Source of Variance	SS	MS	F-value
	P-value		
Treatment 2	21.5874 p<0.05	10.7937	95.7*
Error 12	1.3541	0.1128	
Total 14	22.9415		

*= Significant (P<0.05)

The statistical analysis of data relating to RBCs count in the treated groups of albino rats has been elaborated in Table 4.6. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides (chlorpyrifos and fipronil) has affected the level RBCs in albino rats.

Table 4.7: *Comparisons of means values of the level RBCs (10^6 /µl) in the treated groups of Albino rats*

Treatment	Mean Values
Control	6.65±0.41[a]
T1	4.63±0.35[b]
T2	3.78±0.20[c]

Values are expressed as Mean±SD.

Different letters indicate statistical differences among control and insecticide treatment groups within a measured parameter. All means having different letters are statistically significant.

Table 4.7 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

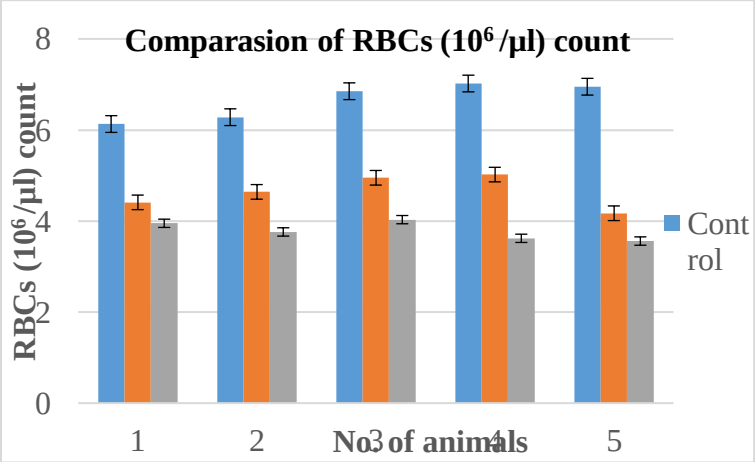

Figure 4.3: *Graphical representation of Red Blood cells (RBCs) of Albino rats after lethal exposure to insecticides*

Figure 4.3: depicts the level of Red Blood cells (RBCs) in the blood of albino rats after treating them with high and low doses of insecticides Chlorpyrifos and Fipronil. A comparison of the treatments revealed that minimum RBCs were found in

the high-dose treatment of Chlorpyrifos and Fipronil in the treatment T2. Maximum value of RBCs was found in untreated treatment control To. Significant differences were present among the untreated control treatment To and the insecticide-treated treatments T1 and T2.

Table 4.8: *Analysis of WBCs (10^3 /µl) count in treated groups of albino rats*

WBCs (10^3 /µl) Normal Range (8-11)			
No. of Animals	**Control**	**T1**	**T2**
1.	8.94	12.11	13.2
2.	8.64	12.99	14.3
3.	9.2	11.84	14.0
4.	8.62	12.63	15.1
5.	8.85	12.5	14.85
Mean±SD	8.85±0.2	12.41±0.41	14.29±0.67

The statistical analysis of data relating to WBCs count in the blood of albino rats has been presented in Table 4.8. A significantly higher concentration of white blood cell count was recorded in chlorpyrifos and fipronil treated treatments T1 and T2 than in the control treatment To which was not treated with the insecticides. WBCs count was recorded as 12.41±0.41 in low-dose treatment T1 and 14.29±0.67 in high dose treatment T2 of chlorpyrifos and fipronil. In untreated treatment To, an 8.85±0.2 value of WBCs count was observed. The increasing value of the WBCs count in all the treatments followed the trend of $T_2 > T_1 > To$.

Table 4.9: *Analysis of Variance (ANOVA) indicating the level WBCs (10^3 /µl) in the treated groups of Albino rats*

Source of Variance	DF	SS	MS	F-value	P- value
Treatment	2	76.3585	38.1792	140*	p<0.05
Error	12	3.2773	0.2731		
Total	14	79.635			

* = Significant (P<0.05)

The statistical analysis of data relating toWBCs count in the treated groups of albino rats has been elaborated in Table 4.7. The p-value was less than 0.05 which showed that the results were significant.

Table 4.10: *Comparisons of means values of WBCs (10^3 /µl) in the treated groups of Albino rats*

Treatment	Mean Values
Control	8.85 ± 0.2^a
T1	12.41 ± 0.41^b
T2	14.29 ± 0.67^c

Values are expressed as Mean±SD. All means having different letters are statistically significant.

Table 4.8 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

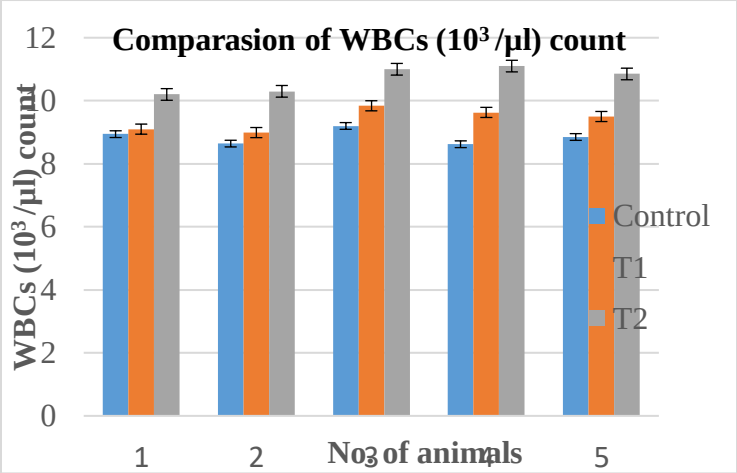

Figure 4.4: *Graphical representation of White Blood Cells (WBCs) of Albino rats after lethal exposure to insecticides*

Figure 4.4 describes the level of white blood cell (WBCs) count in low and high doses of Chlorpyrifos and Fipronil treatments of albino rats. The overall concentration of WBCs increased significantly in insecticide-treated treatments T1(treated with low dose binary mixture of CPF and FPN) and T2 (treated with ea high dose of CPF and FPN). White blood cell (WBCs) count decreased significantly in control treatment To.

Table 4.11: *Analysis of Platelets (10^3 /µl) count in treated groups of albino rats*

PLT (10^3 /µl) Normal Range (600-900)			
No. of Animals	**Control**	**T1**	**T2**
1.	894	902	915
2.	885	906	918
3.	888	899	920
4.	874	910	911
5.	880	907	917

DOI:10.64060/978-627-7898-02-1

Mean±SD	868±6.8	904.8±3.8	916±3.05

Findings of the present work revealed that platelet count was increased significantly in icinsecticide-treated treatments T1 and T2. Results showed that the recorded value for platelet count in the control treatment which was not exposed to the insecticides Chlopyrifos and Fipronil was 868±6.8 (103 /µl). In insecticide groups T1 and T2, the mean value of platelets recorded for T1 which was provided with the low dose of the binary mixture of the insecticide (CPF and FPN) was 904.8±3.8 while the mean value of platelets recorded for T2 which was provided with the high dose of the binary mixture of the insecticide (CPF and FPN) was 916±3.05 (Table 4.11).

Table 4.12: *Analysis of Variance (ANOVA) indicating the level of Platelets (10^3 /µl) in the treated groups of Albino rats*

Source of Variance	**DF**	**SS**	**MS**	**F-value**	**P-value**
Treatment	2	2630.53	1315.27	44.5 *	p<0.05
Error	12	354.40	29.53		
Total	14	2984.93			

* = Significant (P<0.05)

The statistical analysis of data relating to platelets count in the blood of albino rats has been elaborated in Table 4.12. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides has affected the level of platelets in rats.

Table 4.13: *Comparisons of Means of Platelets (10^3 /µl) in the treated groups of Albino rats*

Treatment	Mean Values
Control	868±6.8[c]
T1	904.8±3.8[b]
T2	916±3.05[a]

Values are expressed as Mean±SD. All means having different letters are statistically significant.

Table 4.11 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

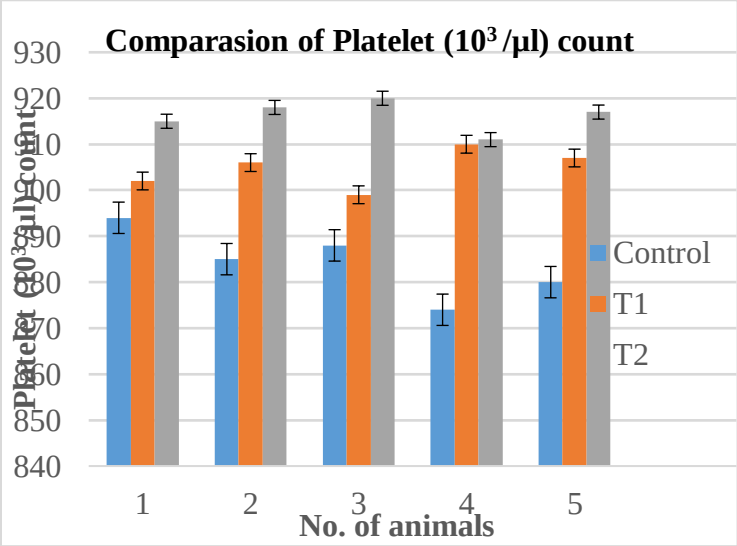

Figure 4.5: *Graphical representation platelets of Albino rats after lethal exposure to insecticides*

Figure 4.5 exhibits that the control treatment To showed a significantly lower concentration of platelets count than

insecticide treated groups T1 and T2. The platelets elevated significantly in both treatments T1 and T2 when compared to the control group to at the end of the trials. T2 showed higher concentrations of platelets. The error bar depicts the standard mean.

Table 4.14: *Analysis indicating the Hemoglobin level (g /dl) in the treated groups and control groups of Albino rats*

Hb (g /dl) Normal Range (11-15)			
No. of Animals	**Control**	**T1**	**T2**
1.	14.61	11.31	9.23
2.	14.45	10.23	8.76
3.	13.78	10.45	8.33
4.	13.23	10.89	8.98
5.	12.23	10.15	8.43
Mean±SD	13.66±0.97	10.61±0.48	8.75±0.37

An interpretation of current research regarding the level of haemoglobin is exhibited in Table 4.14. The untreated control treatment showed a higher concentration of haemoglobin level than low and high dose insecticides treated treatments T1 and T2. In the untreated group, the mean values of the Hb level were recorded as 13.66±0.97. In insecticide-treated treatments, the mean Hb level count recorded for the low dose of the binary mixture of the insecticide was 10.61±0.48 while the mean Hb level recorded for the high dose of the binary mixture of the insecticide was 8.75±0.37 (Table 4.12). The Hb level showed a trend of To>T1>T2.

Table 4.15: *Analysis of Variance (ANOVA) indicating the level of Hb (g/dl) in the treated groups of Albino rats*

Source of Variance	DF	SS	MS	F-value	P-value
Treatment	2	61.5565	30.7	69.9*	p<0.05
Error	12	5.2828	0.4402		
Total	14	66.8394			

* = Significant (P<0.05)

The statistical analysis of data relating to Hb level in the blood of albino rats has been elaborated in Table 4.15. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides (chlorpyrifos and fipronil) has affected the Hb level of albino rats.

Table 4.16: *Comparisons of means of Hb (g/dl) Level in the treated groups of Albino rats*

Treatment	Mean Values
Control	13.66 ± 0.97^{a}
T1	10.61 ± 0.48^{b}
T2	8.75 ± 0.37^{c}

Values are expressed as Mean±SD. All means having different letters are statistically significant.

Table 4.16 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the

treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

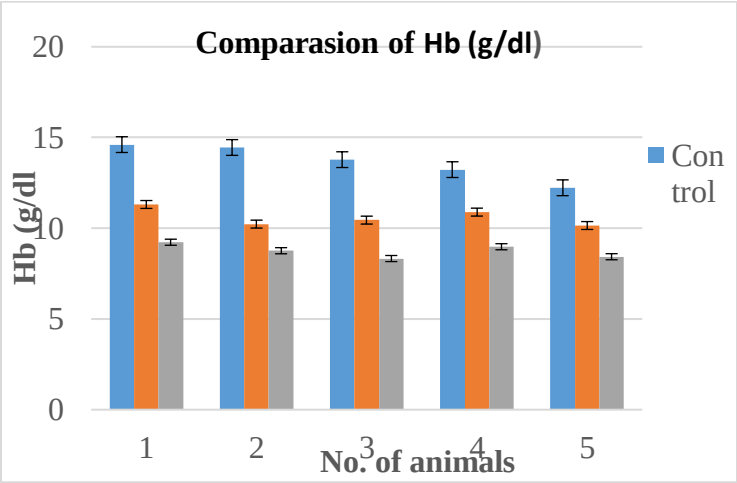

Figure 4.6: *Graphical representation of Hemoglobin level of Albino rats after lethal exposure of insecticides*

The graphical presentation of the data related to haemoglobin levels in low and high-dose insecticide treatments of albino rats has been shown in Figure 4.6. Significant reduction in Hb level in both high dose and low dose of chlorpyrifos and fipronil is depicted clearly in Figure 4.6. Hb value of the untreated control treatment remained under the normal range. CPF and FIP significantly declined the hb level in insecticide-treated rats.

Table 4.17: *Analysis indicating the Mean corpuscular volume in the treated groups and control groups of Albino rats*

MCV Normal Range (50-75)			
No. of Animals	**Control**	**T1**	**T2**

1.	75.21	78.34	80.23
2.	74.23	77.78	81.46
3.	75.43	78.34	80.45
4.	73.67	76.33	81.78
5.	74.49	76.44	81.75
Mean±SD	74.61±0.72	77.44±0.99	81.13±0.74

Results show that 28 days exposure of to different concentrations of a binary mixture of chlorpyrifos and fipronil significantly affected the mean corpuscle volume (MCV) in the blood of Albino rats when compared to the control. The untreated control treatment exhibited a lower concentration of MCV than low-dose and high doses of insecticides chlorpyrifos and fipronil treatments. The control treatment has an MCV value of 74.61±0.72 g/dL while low dose treatment T1 and high dose treatment T2 have 77.44±0.99 and 81.13±0.74 values respectively. Insecticides significantly increased MCV values in albino rats.

Table 4.18: Analysis of Variance (ANOVA) indicating Mean corpuscular volume in the treated groups of Albino rats

Source of Variance	**DF**	**SS**	**MS**	**F-value**	**P-value**
Treatment	2	107.136	53.5681	78.1*	p<0.05
Error	12	8.230	0.6859		
Total	14	115.367			

* = Significant (P<0.05)

The statistical analysis of data relating to MCV in the blood of albino rats has been elaborated in Table 4.18. Significant

changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides has affected the level of Mean corpuscular volume in rats.

Table 4.19: *Comparisons of Means of MCV level in the treated groups of Albino rats*

Treatment	Mean Values
Control	74.61 ± 0.72^{c}
T1	77.44 ± 0.99^{b}
T2	81.13 ± 0.74^{a}

Values are expressed as Mean±SD. All means having different letters are statistically significant

Table 4.17 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

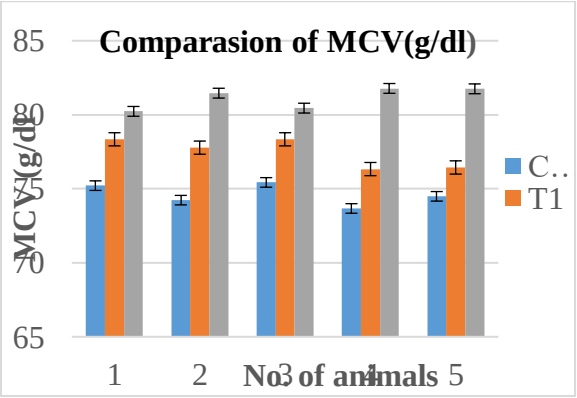

Figure 4.7: *Graphical representation of MCV levels of Albino rats after lethal exposure to insecticides*

The graphical illustration of MCV levels in different treated groups of Aalbino rats is exhibited in Figure 4.7. A significant increase in the low-dose T1 and high doses treatment T2 of chlorpyrifos and fipronil was depicted relative to the control treatment To (Figure 4.7). The highest value of MCV was observed to be 81.13±0.74 in high-dose treatment T2 treated with insecticides chlorpyrifos and fipronil.

Table 4.20: *Analysis of Mean corpuscular Hemoglobin in treated groups of albino rats*

MCH Normal Range (17-23pg/cell)			
No. of Animals	**Control**	**T1**	**T2**
1.	18	16	15
2.	19	15	16
3.	18	17	16
4.	17	16	17
5.	18	16	17
Mean±SD	18±0.63	16±0.6	16.2±0.7

Results relative to the MCH values of albino rats are presented in Table 4.18. The untreated control group To exhibited a higher concentration of mean cell hemoglobin than the insecticide treated groups T1 and T2 when compared to the non-treated group. In the control group, the mean value of the MCH was calculated as 18±0.63g/dl as shown in Table 4.20. In insecticide-treated groups, the mean MCH count

recorded for the low dose of the binary mixture of the insecticide chlorpyrifos and fipronil was 16.0±.0.6, while the mean cell haemoglobin concentration recorded for the high dose of the binary mixture of the insecticide chlorpyrifos and fipronil was measured as 16.2±0.7 (Table 4.20).

Table 4.21: *Analysis of Variance (ANOVA) indicating Mean Corpuscular Hemoglobin in the treated groups of Albino rats*

Source of Variance	DF	SS	MS	F-value	P-value
Treatment	2	12.1333	6.06667	10.7*	p<0.05
Error	12	6.8000	0.56667		
Total	14	18.9333			

* = Significant (P<0.05)

The statistical analysis of data relating to MCH in the blood of albino rats has been elaborated in Table 4.19. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides has affected the Mean Corpuscular Hemoglobin in rats.

Table 4.22: *Comparisons of Means of MCH values in the treated groups of Albino rats*

Treatment	Mean Values
Control	18 ± 0.63^{a}
T1	16 ± 0.6^{c}
T2	16.2 ± 0.7^{b}

Values are expressed as Mean±SD. All means having different letters are statistically significant.

Table 4.22 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

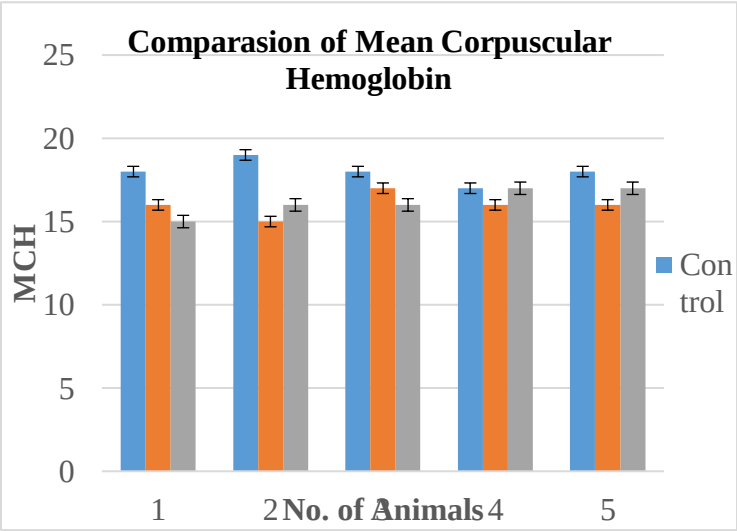

Figure 4.8: *Graphical representation of Mean Corpuscular Hemoglobin of Albino rats after lethal exposure to insecticides*

Figure 4.8 depicts the level of mean cell haemoglobin in different insecticide-treated treatments of albino rats. The significant difference was recorded between control and insecticide-treated treatments. Figure 4.8 clearly illustrates that the level of mean cell volume decreased in both low-dose treatment T1 and high-dose treatment T2 of insecticide chlorpyrifos and fipronil after the trial.

Table 4.23: *Analysis of Mean Corpuscular Hemoglobin Concentration in treated groups of albino rats*

MCHC Normal Range(32-38g/dL)			
No. of Animals	**Control**	**T1**	**T2**
1.	33.61	31.31	29.23
2.	33.45	31.23	28.76
3.	34.78	32.45	29.33
4.	32.23	31.89	27.98
5.	33.23	31.15	29.43
Mean±SD	33.46±0.91	31.61±0.55	28.95±0.59

The 28 days exposure of to different concentrations of a binary mixture of chlorpyrifos and fipronil significantly affected the mean corpuscle haemoglobin concentration in Albino rats as shown in Table 4.23. The untreated control treatment exhibited a higher concentration of mean cell hemoglobin concentration than the insecticide-treated treatments with the mean value of the MCHC calculated as 33.46±0.91 g/dl. In insecticide treated groups, the mean MCHC count recorded for the low dose of the binary mixture of chlorpyrifos and fipronil was 31.61±0.55 while the mean MCHC count recorded for the high dose was 28.95±0.59 (Table 4.23).

Table 4.24: *Analysis of Variance (ANOVA) indicating Mean Corpuscular Hemoglobin concentration in the treated groups of Albino rats*

Source of Variance	DF	SS	MS	F-value	P-value
Treatment	2	51.4819	25.7409	51.6*	p<0.05
Error	12	5.9908	0.4992		
Total	14	57.4727			

* = Significant (P<0.05)

The statistical analysis of data relating to MCHC in the blood of albino rats has been elaborated in Table 4.24. The p-value was less than 0.05 which showed that the results were significant.

Table 4.25: *Comparisons of Means MCHC concentration in the treated groups of Albino rats*

Treatment	Mean Values
Control	33.46±0.91[a]
T1	31.61±0.55[b]
T2	28.95±0.59[c]

Values are expressed as Mean±SD. All means having different letters are statistically significant

Table 4.25 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

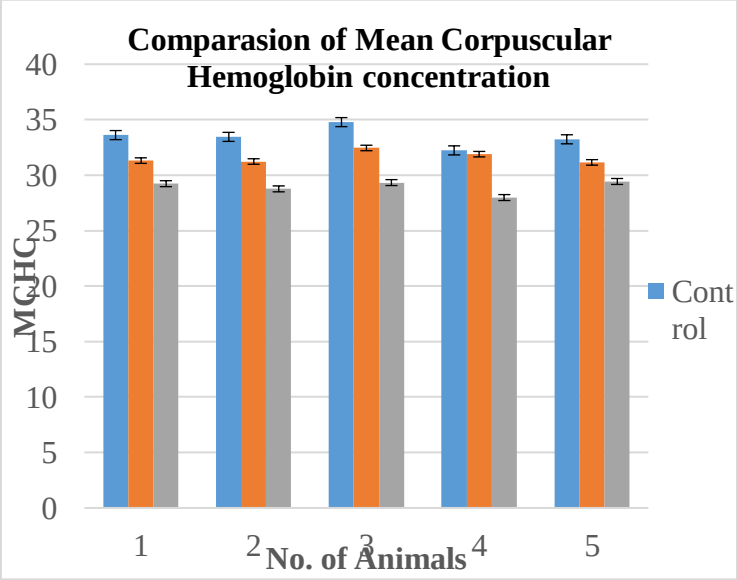

Figure 4.9: *Graphical representation of Mean Corpuscular Hemoglobin Concentration of Albino rats after lethal exposure to insecticides*

A graphical representation regarding the value of MCHC concentration in the blood of albino rats was given in Figure

4.9. The figure depicts the level of MCHC in the control treatment as well as low and high-dose treatments of CPF and FPN in albino rats. Maximum value of MCHC was observed for the untreated control treatment To and the minimum value was found in the high dose treatment T2 of insecticide chlorpyrifos and fipronil. Significant differences among treatments can be seen in Figure 4.9.

Table 4.26: *Analysis of Hematocrit level in treated groups of albino rats*

HCT Normal Range (30-35%)			
No. of Animals	**Control**	**T1**	**T2**
1.	33.91	32.16	30.12
2.	33.52	30.61	29.16
3.	34.17	30.12	30
4.	34.82	31.62	29.13
5.	33.67	30.12	30.15
Mean±SD	34.01±0.51	30.08±0.85	29.71±0.46

The 28 days exposure of to different concentrations of a binary mixture of chlorpyrifos and fipronil significantly affected the hematocrit level in Albino rats as given in Table 4.24. The control group exhibited a higher concentration of hematocrit than insecticide-treated treatments of chlorpyrifos and fipronil. In the control group, the mean calculated value of the hematocrit was calculated as 34.01±0.51 g/dl. In insecticide treated groups, the mean value of hematocrit level for the low dose of the binary mixture of the insecticide was 30.08±0.85 while the mean hematocrit level recorded for the high dose of the binary mixture of the insecticide was 29.71±0.46 (Table 4.26).

Table 4.27: *Analysis of Variance (ANOVA) indicating Hematocrit level in the treated groups of Albino rats*

Source of Variance	DF	SS	MS	F-value	P-value
Treatment	2	49.2932	24.6466	53.4*	p<0.05
Error	12	5.5353	0.4613		
Total	14	54.8284			

* = Significant (P<0.05)

The statistical analysis of data relating to Hematocrit (HCT) in the blood of albino rats has been elaborated in Table 4.25. Significant changes were determined using a one-way ANOVA to identify specific differences between treatments ($p < .05$). The -p-value was less than 0.05 which showed that the results were significant. It means that exposure to a binary mixture of insecticides (chlorpyrifos and fipronil) has affected the level of hematocrit in albino rats.

Table 4.27: *Comparisons of Means of hematocrit level in the treated groups of Albino rats*

Treatment	Mean Values
Control	34.01 ± 0.51^{a}
T1	30.08 ± 0.85^{b}
T2	29.71 ± 0.46^{c}

Values are expressed as Mean±SD. All means having different letters are statistically significant.

Table 4.28 gives the comparison of all three treatments of albino rats. A comparison of treatment shows that all the treatments To, T1 and T2 are significantly different from each other as they do not share a common alphabet .

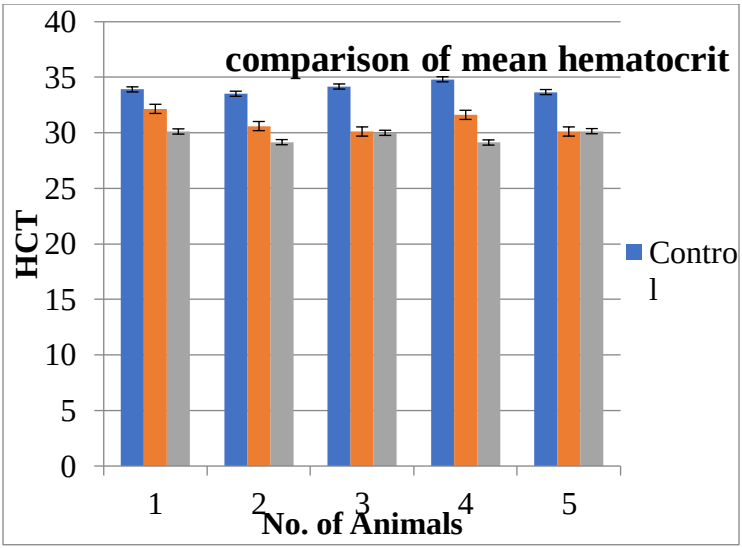

Figure 4.10: *Graphical representation of Hematocrit level of Albino rats after lethal exposure to insecticides*

Figure 4.10 shows the level of hematocrit (HCT) in the control and insecticide-treated treatments of chlorpyrifos and Fipronil in the albino rats. The level of hematocrit declined significantly in both low-dose treatment T1 and high-dose treatment T2 when compared to the control group To. A maximum value of hematocrit was obtained in treatment To which received no insecticide.

4.3. Histopathological Analysis

Histopathological alterations in the livers of rats treated with the binary mixture of chlorpyrifos and fipronil were observed under light microscopic exams when compared to the control group. The histological alterations changes were observed in the rat liver of all three groups with H&E stain, x200 and x 400. The histopathological investigation revealed findings in liver histoarchitecture of rats under T1 and T2 Groups

(exposed to the binary mixture of chlorpyrifos and fipronil groups unlike that of the Control group.

The liver segment of the control group rats is shown in a histological study of the liver. The control rats' livers had a normal pattern of hepatocytes with evident sinusoids in most areas. The parenchyma was found to be normal with normal sinusoidal spacing. Hepatocyte nuclei had normal vesicular structure and the cytoplasm was homogenous and normal. Control rats' livers are made up of cords that run from the central vein to the portal triads. Hepatocytes are organised into cords that are divided by sinusoids. Kupffer cells can also be seen in the sinusoidal gaps. Each hepatocyte is a polygonal cell with a huge spheroid nucleus in the centre that has a chromatin structure and a unique nucleolus. Hepatocytes appeared normal and had a lot of granular cytoplasm. Even though the cytoplasm of hepatocytes is mildly grainy, no alterations were seen in the control group (Figure 4.11).

Changes in the liver's histo-architecture were shown by things like enlarged blood sinusoids, broken clogs in the bile duct and portal vein and damage to the hepatocytes. Hepatocyte nuclei were in abnormal structure. Lower doses of CPF and FPN caused moderate hepatic parenchyma changes. With lymphoid infiltration and cytoplasm vacuolization, hepatocyte parenchyma appears constricted. The sinusoids were more dilated and had a high density of Kupffer cells along the walls. Glycogen deposits were found in a few cells as well as sinusoids, causing these gaps to be destroyed. Several shrunk nuclei with uneven borders, indicating degeneration have been seen. Erythrocyte disruption and acute cell swelling were detected. Individual cell necrosis is shown by pyknotic nuclei. Nuclear degeneration can be found in a few sites. The

chromatin in hepatocytes was clumped, the nuclear borders were uneven and the cytoplasm was heavily vacuolated. The cytoplasm had become more granular. There was a lot of focal necrosis. Between hepatocytes, lymphocytic infiltration was seen. The hepatic portal was discovered to be intact as were the hepatic sinusoids showing normal liver histoarchitecture. Insecticide-treated rats had histological changes in the liver resulting in severe consequences (Figure 4.13). Photomicrograph of liver sections stained with H&E depicts the histological abnormalities for low dose (Figure 4.12).

Figure 4.11. *Photomicrograph of liver sections stained by H&E for histopathological changes showing: control*

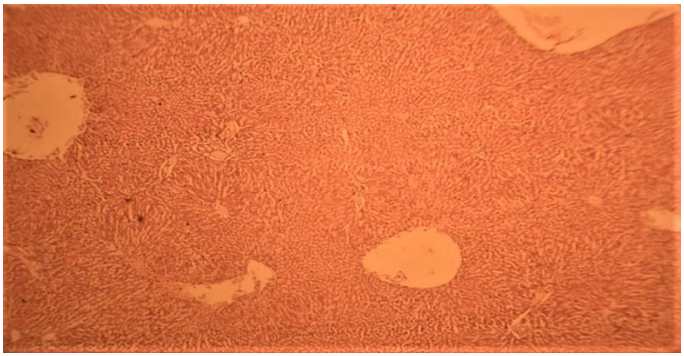

Figure 4.12. *Photomicrograph of liver sections stained by H&E for histopathological changes showing: Treatment 1 (low Dose)*

The intensity of damage noticed in T_2 was comparatively higher than that of T_1 and comprised similar findings with higher prominence. These findings included increased sinusoidal dilation (DS), nuclear degeneration of hepatocytes (ND), degeneration and shrinkage of hepatocytes (DH), degenerated portal vein (DPV) and mild cytoplasmic degeneration (CD). Among all the exposure groups, the highest damage due to ingestion of a binary mixture of the insecticides was witnessed in T_2. In some places, sinusoids were noticed filled with glycogen. Similar glycogen deposits were also noticed in hepatocytes. Minute cell infiltration was observed throughout the parenchyma. The higher dose of a binary mixture of CPF and FYP induced the formation of oedema and the dislocation of the wall of the central vein as shown in Figure 4.13.

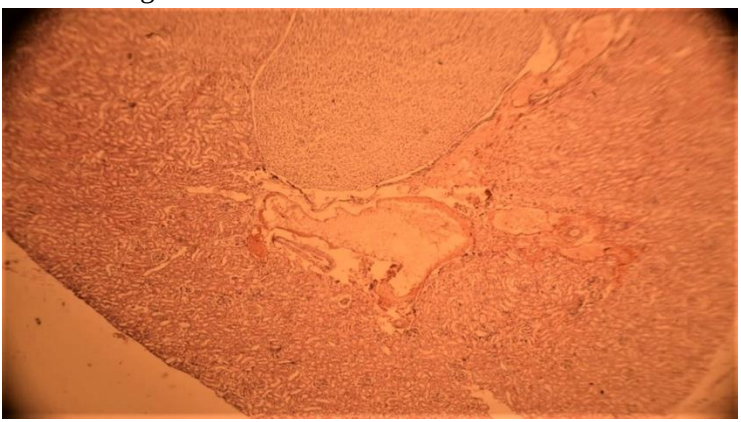

Figure 4.13. *Photomicrograph of liver sections stained by H&E for histopathological changes showing: Treatment 2 (High Dose)*

The liver of rats treated with the low dose of the binary mixture of the insecticides showed moderate injury. In contrast, the liver of rats treated with the high dose of the

binary mixture of the insecticides -treated rats showed severe injury. The histopathological alterations for high doses are represented in Photomicrograph of liver sections stained by hematoxylin and eosin (Figure4.13)

4.4. Discussion

Insecticides are the chemical or biological products that are commonly used around the world to control household and agriculture pests which provide a danger of exposure to humans and animals [1]. A major rise in the utilization of these chemicals in agriculture has resulted from an increase in global food demand. Increased insecticide usage results in irreversible ecosystem damage. Toxicity from insecticides is a global issue [2]. Many pesticides are also hazardous to non-target organisms which are putting animals and human health at risk [3]. Nowadays human are exposed to diverse mixtures of hazardous substances in their homes and workplaces. Pesticides which are sometimes applied carelessly in huge quantities and generate gradual contamination are a particular route of contamination**.** The present study was designed to study the effects of a binary mixture of insecticides (chlorpyrifos and fipronil) on haematology and histopathology of the liver in Wistar Albino Rats.

The findings of the experimental trials provided evidence that exposure to insecticides caused toxicity in albino rats. The outcome of the present study showed that when the binary mixture of (chlorpyrifos + fipronil) was given orally to the rats both the low-dose group and the -high-dosage group experienced significantly less weight gain than the control group To. It was observed that the relative weight of the liver was significantly increased in the treatment groups T1 and T2 as compared to those of the control To. These changes in

body weight gain and relative weights of internal organs indicated some general toxicity in the animals after exposure to insecticides. Fluctuations in the body weight or organ weight of animals might be due to internal damage. Another possible reason for reduced weight gain in metal-treated treatments might be the decreased muscle mass and cachexia due to oxidative stress induced by insecticides. Similar findings were reported by UÜndeger, Institoris [98] who concluded that significant body weight decreased but relative weights of the liver increased in rats when treated orally with OP insecticides methyl parathion and dimethoate. A substantial decrease in RBCs count was seen in rats after the exposure of chlorpyrifos and fipronil. The drop in RBC count after chlorpyrifos administration might be related to hemolysis as it induces haemorrhage and a decrease in erythropoiesis due to increased oxidative strain and suppression of cell activity. RBCs depression in rats at a surplus dose of chlorpyrifos and fipronil has also been observed by Sayim, Yavasoglu [99]. Similar results were also found by Aroonvilairat, Tangjarukij [78] who studied the impact of various doses of pesticide mixture in rats. He observed that the total RBC count decreased significantly ($p<0.05$) from the dose level of 1/9 LD50 to 1/4 LD50 in male rats.

The fundamental purpose of white blood cells is to protect the body from invading organisms such as bacteria and viruses which are produced as a byproduct of leukocytosis and antibody synthesis. In the current study WBCs and platelets increased when rats were exposed to varied concentrations of the insecticides but concentrations of haemoglobin, MCHC, MCH and RBCs dramatically reduced. The rise in WBC count

might be due to toxicant-induced tissue injury or immune system disruption. It is possible that this was leading to an increase in the discharge of WBC from the storage pool in the bone marrow into the bloodstream. Pathological leukocytosis can occur as a result of chemical exposure or abrupt haemorrhages and hemolysis. Severe haemorrhages in the liver and lungs are another probable cause of leukocytosis. Similar results were reported by Ambali, Ayo [100] who observeda decrease in neutrophil, lymphocyte and monocyte counts in male rats after being chronically exposed to chlorpyrifos. Luty, Latuszynska [101] reported a statistically significant increase in the number of WBCs count when exposed to different concentrations of cypermethrin at a dose rate of 10 mg/kg body weight and 25 mg/kg body weight orally for 28 days in Swiss mice.

At the end of trials, Hb concentration was decreased significantly in treatment T1 (10.61±0.48) and T2 (8.75±0.37) as compared to control To with a value of 13.66±0.97. The reduction of Hb content might be due to the impaired biosynthesis of heme in the bone marrow. Significant decrease in Hb resulted of the accumulation of insecticides inside RBCs which prevented Hb formation. CPF and FPN restrain the synthesis of Hb through the inhibition of different vital enzymes involved in the heme synthesis pathway. Reductions in the erythrocyte count, hematocrit and haemoglobin concentration were observed by Manna, Bhattacharyya [102] when WWistar rats were intoxicated with cypermethrin orally at a rate of 14.5 mg/kg body weight. The outcome of the current study revealed that the MCV values of the treated groups were significantly higher than the control group. The main cause of the increase in the MCV values is anaemia

which is caused by the insecticides. These are similar to the work of Abouelghar, El-Bermawy [89] who also found elevated MCV values in pesticide-treated rats. Singh and Srivastava (2010) observed an increase in MCV while a decrease in MCHC and Hb contents in C. striatus treated with metasystox's deadly 48 h LC50. Adhikari, Sarkar [103] recorded significantly increased levels of MCV in *L. rohita* post-exposure to carbofuran. Results showed that treatments with low and high doses of chlorpyrifos and fipronil resulted in lower MCH readings. This is due to the disruption of erythropoiesis and increase in the rate of erythrocyte destruction. Abouelghar, El-Bermawy [89] also reported the same findings and observed significantly decreased mean corpuscular hemoglobin (MCH) in the Fipronil groups exposed to 1/20 and 1/50 LD50 as compared to those in the control. HCT is primarily associated with the transport of oxygen and absorbed nutrients. The present investigation was recorded for increased Hct values in low and high-dose treatments of Chlorpyrifos and Fipronil as compared to control. Decline in this indice might be due to iron deficiency in RBCs and Hb which reduces oxygen carrying capability of blood and causes anemia. Our findings are similar to the Aroonvilairat, Tangjarukij [78] he reported that when rats were exposed to the different concentrations of the chlorpyrifos and cypermethrin there was significantly ($p<0.05$) increased in the Hct concentration.

Histopathological abrasions have been employed as markers for monitoring the fitness of individuals susceptible to contaminants and they can also serve as early warning indications of illness [39]. According to the findings of the histological examinations, CPF and FPN were responsible for

the structural alterations seen in the liver tissue of Wistar rats. These alterations in tissues were dose-dependent. Current results showed that the control treatment was with normal hepatocytes and central vein. There was no lymphoid infiltration and necrosis observed in control. Hepatic parenchyma was altered to a moderate degree as a result of the administration of the lower dose of CPF and FPN. An observation of lymphoid infiltration and vacuolization of the cytoplasm was made. In comparison to the low dose of insecticides, higher doses of CPF and FPN caused severe damage such as oedema, necrosis and displacement of the wall of the central vein. These histological alterations could be the result of cell degeneration or the harmful effects of pesticides notably the production of reactive oxygen species which causes damage to various components of the cell membrane. Our results are in the same line as Tripathi and Srivastav [43] who concluded that all liver sections of the treated rats with chlorpyrifos have shown an abundance of dilated sinusoids, dilated central veins, necrosis and hypertrophy of portal triad. He also observed that variable intensities of these changes depend upon the dosage of the treatment.

Campos-Pereira, Oliveira [104] also reported that CPF caused structural changes in the liver tissue of Wistar rats. CPF treatment generated intense cytoplasmic vacuolation and the appearance of moderate necrotic cell foci. He also observed that Necrosis was produced as a result of cell degeneration. Mansour and Mossa (2010) discovered that oral treatment of CPF produced the emergence of oedema in the portal area in male and female rats. Another study discovered that fenitrothion injection caused oedema and hemorrhagic regions

to form in liver cells (Elhalwagy et al., 2008). Co-administration of cypermethrin and chlorpyrifos produced histopathological lesions like degeneration and necrotic changes in hepatic cells, necrosis and infiltration of phagocytic cells, disruption of basement membrane along with oedema, haemorrhages and hepatic congestion [105]. It is clear from the results of the current study that the liver is a sensitive organ to the toxic action of the insecticides Fipronil and Chlorpyrifos.

CHAPTER 5

Summary

The purpose of the present study was to evaluate the effects of a binary mixture of insecticides chlorpyrifos and fipronil on haematology and histopathology of the liver in Wistar Albino Rats. The research was performed at Animal House, University of Agriculture Faisalabad. Firstly, rats were acclimatized according to the laboratory conditions the commencement of the trials. After acclimatization, rats were divided into three groups. T_0 was controlled, T1 was treated with a low dose of chlorpyrifos and fipronil (3.1mg/kg + 2mg/kg) while T2 was treated with a high dose of chlorpyrifos and fipronil (9.7mg/kg + 6.2mg/kg). After 28-day trials, rats were dissected then organ (liver) and blood were collected for analyzing the target. Hematology and histopathology of the liver were analyzed by using their respective methods. Slides of the liver were prepared for the histopathological study of the liver under light microscope. The blood samples were collected in the EDTA Tubes to prevent coagulation for the examination of haematological parameters such as haemoglobin (Hb), hematocrit, platelets, MCH, MCHC, MCV, RBCs and WBCs. The variations in hematological parameters are used as indicators for the toxicity of insecticides. The results indicated that when the rats were exposed to the binary mixture of insecticides for about 28 days, it caused a significant decrease in the RBCs count, MCH and MCHC while on the other hand, the exposure of rats with insecticides caused a significant increase in WBCs count, MCV and platelets in the treated groups when compared to the control. The exposure to insecticides also causes a reduction in the body weight of the insecticide treated group as compared to the control. The tissue damage confirmed through histopathological examination revealed

findings like hepatocytic vacuolation, degeneration of hepatocytes and their nuclei, hyperchromatic, hypertrophied nuclei at an earlier stage of treatment, sinusoidal dilation, focal necrosis degenerated portal vein and necrosis in liver of insecticide exposed rats.

The following are the conclusions:

- The exposure to insecticides has affected the body weight of rats. The rats exposed to insecticides exhibited a decrease in body weight of rats to the control group.
- The exposure of insecticides has affected the organ (liver) weight of rats. The rats exposed to insecticides exhibited an increase in organ weight of rats to the control group.

- Exposure to the lethal doses of the binary mixture of chlorpyrifos and fipronil causes fluctuations in the haematological parameter.
- The exposure of rats to insecticides causes a significant decrease in the RBCs count. The total mean values for RBCs count are 4.63±0.35 in T_1 and 3.78±0.20 in T_2.
- The exposure of rats to the insecticides caused a significant decrease in the MCH and haemoglobin levels in the insecticide-treated groups as compared to control
- A notable total increase in the WBCs count observed in the rats treated with the low and high dose of binary mixture of insecticides was 12.41±0.41 and 14.29±0.67 respectively.

- The exposure of rats to the insecticides causes a significant increase in the MCV and platelets in the insecticide-treated groups.
- A notable total decrease in the HCT observed in the rats treated with the low and high doses of a binary mixture of insecticides was 30.08±0.85 and 29.71±0.46 respectively.
- The exposure of insecticides caused noticeable alterations in the histopathology of the liver of insecticide-treated rats.
- Prominent alterations such as hepatocytic vacuolation, degeneration of hepatocytes, sinusoidal dilation and focal necrosis degenerated portal vein were observed.

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