

**CYPERMETHRIN-INDUCED CHANGES IN THE BEHAVIOURAL,  
MORPHOMETRIC, HAEMATOLOGICAL AND HISTOLOGICAL  
PROFILES IN MICE *MUS MUSCULUS* L.**



**A Thesis Submitted To**

Biotechnology and Genetic Engineering Discipline, Life Science School,  
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requirements for the degree of Master of Science (MS) in Biotechnology and  
Genetic Engineering

**Submitted By**

Muhammad Mizanul Hoque  
Examination Roll No: MS-100707  
Session: 2010-2011



**BIOTECHNOLOGY AND GENETIC ENGINEERING DISCIPLINE  
LIFE SCIENCE SCHOOL, KHULNA UNIVERSITY,  
KHULNA-9208, BANGLADESH  
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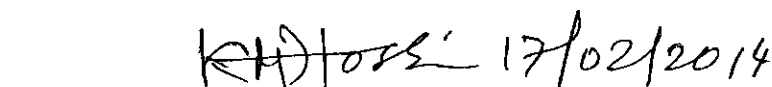
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# DECLARATION

I do hereby declare that the entire work submitted as a thesis entitled “**CYPERMETHRIN-INDUCED CHANGES IN THE BEHAVIOURAL, MORPHOMETRIC, HAEMATOLOGICAL AND HISTOLOGICAL PROFILES IN MICE (*Mus musculus* L.)**” for the Degree of Masters of Science in the Biotechnology and Genetic Engineering Discipline, Khulna University, is the result of my own investigation. To the best of my knowledge and belief, it contains no material previously published by any other person except when due reference is made in the text.

*Muhammed*

16.02.2014

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*The Research Work is*

*DEDICATED*

*TO*

*MY BELOVED PARENTS*

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#### **The Author**

## ABSTRACT

Cypermethrin, a synthetic pyrethroid insecticide having low mammalian toxicity, is a fast-acting neurotoxin and is known to cause free radical-mediated tissue damage. It is used worldwide for pest control in agriculture and households. The present study investigated the cypermethrin-induced clinical and behavioural symptoms, accompanied by morphometric, histological and haematological changes in the albino mice (*Mus musculus* L.) Twenty five adult male albino mice were divided equally into five groups where each 20g mice received: distilled water only (Group I, served as control); 0.10 ml cypermethrin (Group II); 0.15 ml cypermethrin (Group III); 0.20 ml cypermethrin (Group IV); and 0.25 ml cypermethrin (Group V). Treatments continued intraperitoneally each week for four consecutive weeks. During the experimental period, there were significant decreases between the initial and final body weights in the cypermethrin-treated mice but no abnormalities were observed in the control group. At the end of the 4<sup>th</sup> week, all the mice were sacrificed; their brain, lungs, heart, kidneys, liver, viscera and gonads were dissected out, weighed and preserved in 70% ethanol. The samples of liver and kidneys were processed for histological studies. Cypermethrin treatment exhibited severe histopathological changes especially in the liver and kidneys accompanied by weight loss of various organs. Cypermethrin-induced necrosis of various body parts, degeneration and necrosis of hepatic cells, dilatation of sinusoids and dissociated remark cordons in livers, sloughing off of epithelial cells, shrinkage of glomeruli, and necrosis of renal tubules in kidneys were noticeable. These changes were due presumably to the oxidative stress that modified biochemical parameters and histological aspects of liver and kidneys. Just before sacrifice, blood was collected from the experimental mice for the estimation of major haematological profiles. The results revealed statistically significant decrease in the red blood cells, platelets, haemoglobin, neutrophils and basophils but increase in the white blood cells such as lymphocytes, monocytes and eosinophils. It was observed from the present study that repeated exposure to cypermethrin affected the behavioural, morphometric, histopathological and haematological profiles of the albino mice. From these findings, it is postulated that cypermethrin and other synthetic pyrethroids might cause hazardous effects in mammals including human beings and therefore, judicious use of this insecticide should be assured while applying them at agricultural field and household purposes.

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## **ABBREVIATIONS USED IN THE TEXT**

°C = Degree Celsius

ACI = Advanced Chemical Industries

ANOVA = Analysis of Variance

AST = Aspartate Aminotransferase

BW = Body Weight

cm = Centimetre

CRD = Completely Randomized Design

cumm = Cubic Millimeter

CYP = Cypermethrin

DC = Differential Counts

df = Degrees of Freedom

DHC = Degenerated hepatic cells

dl = Decilitre

DLC = Differential Leucocyte Count

DPX = Digital Picture Exchange

DT = Degradation Time

EDTA = Ethylene Diamine Tetra Acetic Acid

EPA = Environment Protection Agency

ESR = Erythrocyte Sedimentation Rate

FCE = Feed Conversion Efficiency

FCR = Feed Conversion Ratio

g = Gram

Hb = Haemoglobin

Hr = Hour

Kg = Kilogram

LD<sub>50</sub> = Lethal Dose 50

LSD = Least Significant Difference

MCHC = Mean Corpuscular Hemoglobin Concentration

MCV = Mean Corpuscular Volume

mg = Milligram

ml = Millilitre

mm = Millimetre

NaCl = Sodium Chloride

NC = Necrotic Cells

NPTN = National Pesticides Telecommunications Network

OSI = Organ-Somatic Index

RBC = Red Blood Corpuscles

RH = Relative Humidity

SD = Standard Deviations

SG = Shrinkage of Glomeruli

Sig.=Significance

SPSS=Statistical Package for Social Sciences

TC=Total Count

TEC=Total Erythrocyte Count

TLC=Total Leucocyte Count

WBC=White Blood Corpuscles

WHO= World Health Organization

# CHAPTER ONE

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## INTRODUCTION

## CHAPTER ONE

### INTRODUCTION

Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting insecticide. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. CYP is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN). It is found in many household ant and cockroach killers, including Raid and ant chalk.

Cypermethrin (Plate 1) is classified by the World Health Organization (WHO, 1995) as “moderately hazardous”. It is considered to be environmentally safe and widely used in agriculture and veterinary medicine (Shukla *et al.*, 2002). It is a fast-acting neurotoxin and is known to cause free radical-mediated tissue damage (Patel *et al.*, 2006). Like other pyrethroids, cypermethrin kills the insects by interacting with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds instead of the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long-lasting trains of repetitive impulses in sense organs (Rodriguez *et al.*, 2008). Similar to other synthetic pyrethroids cypermethrin is highly non-polar with a Log value of around 5.3 (Pesticide Properties Database, 2011). As a result it has very low water solubility and a tendency to attach to the top soil layer. It is moderately persistent in soil with a field degradation half-life ( $DT_{50}$ ) of 69 days (Pesticide Properties Database, 2011). CYP has for many years been the pyrethroid used in the largest amounts in Denmark (USEPA, 2011), but in 2011 the market share dropped considerably making taufluvinate, which causes a lower environmental stress than cypermethrin, the most used

pyrethroid (Kjolholt *et al.*, 2012). The material shall consist of alpha-cypermethrin together with related manufacturing impurities and shall be a white- to cream-coloured crystalline powder with characteristic odor, free from visible extraneous matter and added modifying agents (WHO, 2013).



**Plate 1:** Cypermethrin in a 100mL bottle, commercially available as Caught 10 EC

## **1.1 Albino Mice (*Mus musculus* L.) as a model laboratory animal**

### **1.1.1 General Information**

The mouse was first domesticated several hundred years ago by mouse fanciers who bred mutants with various colored hair coats. The albino laboratory mouse was derived from these mice in the early 1900s. Today, there is a wide variety of inbred and transgenic mice (mice carrying genes from another organism) that are prominent models for studying human diseases, such as diabetes, cancer, and muscular dystrophy. Healthy mice should be alert and curious. The hair coat should be smooth and shiny (Katherine *et al.*, 2011).

House mice (scientific name *Mus musculus*) (Plate 2) originated in the central and southern regions of Asia. Because they are very adaptable, they have spread all over the world. House mice were domesticated around 1800 by both Europeans and Asians who began to selectively breed them for their fur and coloring.



**Plate 2:** Albino Mice (*Mus musculus*)

### 1.1.2 Taxonomic Position

The albino mouse is a member of the Family Muridae under the Order Rodentia and Class Mammalia. The Genus *Mus* includes several other thirty (30) known species.

- *Mus musculus* - House Mice
- *Mus sylvaticus* - Field Mice
- *Mus minutus* - Harvest Mice
- *Mus musculus domesticus* - are the "Pet Mice" or "Fancy Mice" (Wikipedia, 2012).

#### **Classification**

- |               |                          |
|---------------|--------------------------|
| ▪ Kingdom     | : Animalia               |
| ▪ Phylum      | : Chordata               |
| ▪ Class       | : Mammalia               |
| ▪ Order       | : Rodentia               |
| ▪ Superfamily | : Muroidea               |
| ▪ Family      | : Muridae                |
| ▪ Subfamily   | : Murinae                |
| ▪ Genus       | : <i>Mus</i>             |
| ▪ Species     | : <i>Mus musculus</i> L. |

### 1.1.3 Suitability of Albino Mice for Testing

Albino mice are easy to breed, virtually all are the same & because they are quite tough & resilient creatures. Mice have limited ability to regulate their body temperature, and they are very sensitive to heat and cold. They regulate their body temperature through dilation or constriction of the veins in their tails. They should be kept in a temperature range of 64 to 72°F (18 to 22°C), with a humidity level of 40 to 70%. Temperatures above 86°F (30°C) can cause heat stroke, especially if there is overcrowding in the cage. Although good lighting is important, mice should be kept away from direct sunlight (Wikipedia, 2012).

### 1.2 Post-Treatment Clinical and Behavioural Symptoms

Clinical signs of toxicity such as slight nervousness, mild depression, reduced feed intake, rough hair coat, and abnormal gait were observed in cypermethrin-exposed mice. At term, there was a significant decrease in body weight of animals treated with cypermethrin alone as compared to untreated and vehicle control groups (both the control groups hereafter referred as control). The lower dose produced very mild toxicosis characterized by intermittent diarrhea, decreased feed intake, and thick eye discharge, whereas higher dose displayed mild to moderate toxicosis with diarrhea, decreased feed intake, loss of body weight, dyspnoea, ataxia, eye discharge, and salivation (Grewal *et al.*, 2010).

### 1.3 Morphological parameters

In chronic toxicity studies, dietary administration of alpha-cypermethrin to mice, rats and dogs resulted in clinical signs of treatment that were limited to adverse effects on the skin and hair. Decreases in body weight gains were observed in mice treated with doses \_100ppm (approximately 14.3 mg/kg bw/day). The body weight gain in treated rats was lower at both 2 and 4 weeks as compared to the control rats. The weight of liver and spleen decreased, while that of kidneys increased as compared to the control rats (Sangha *et al.*, 2011).

degenerative changes in the liver and kidneys were observed. Repeated oral doses of cypermethrin for 30 days enhanced the relative weight of liver and heart, but significantly decreased that of brain, kidneys, and testes (Ince *et al.*, 2012).

### 1.7 Aims of the Study

Introduced commercially about 40 years ago, synthetic pyrethroids account for approximately one-third of insecticides used worldwide in agriculture, domestic and veterinary applications, thus having a high potential risk for human exposure. Cypermethrin (empirical formula  $C_{22}H_{19}Cl_2NO_3$ ; molecular wt. 416.3) a type II pyrethroid, is used to control pests of a variety of crops. It demonstrates species-specific toxicity by exerting its neurological effects principally on the central nervous system. Cumulative research findings suggest that cypermethrin is capable of inducing a host of adverse effects on a number of experimental animals including rats and mice. These include: free radical-mediated tissue damage, mutagenesis and tumour promotion, genotoxicity of various organs like lungs, liver, kidneys and brain, nephrotoxicity, alteration of haematological profiles, induced immunosuppression, degenerative tissue morphology, cell metabolism toxicosis leading to physiological impairment, decrease in body weight and histopathological changes of different vital organs. Human populations at highest risk of high-dose cypermethrin exposure are particularly pesticide workers and small farm owners applying the insecticide for their plant protection. Because of its widespread use nowadays, therefore, evaluation of the toxic effects of cypermethrin is of major concern to public health.

Keeping the above information in mind, the present MS research was designed to investigate the following aspects of cypermethrin-induced changes in the albino mice under laboratory conditions:

- To record all recognizable behavioural and clinical signs in post-treated mice;

- To assess various morphometric parameters related to individual growth and development over a period of four weeks;
- To estimate organ/somatic indices at the end of cypermethrin exposures;
- To assess the effects of cypermethrin on different haematological profiles; and
- To investigate the histopathological abnormalities associated with cypermethrin toxicity in the test animals.

## **CHAPTER TWO**

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### **REVIEW OF LITERATURE**

## CHAPTER TWO

### REVIEW OF LITERATURE

#### 2.1 Review of Theories and Concepts

##### 2.1.1 Historical Background

In 1977, cypermethrin was allowed for use as a very active synthetic pyrethrin insecticide, effective in the control of many pest species in agriculture, animal breeding, and the household (Leng, 1996). In spite of their low mammalian toxicity, retention of pyrethroids in mammalian tissues may be potentially dangerous.

Insecticide toxicity is a global problem with maximum number of poisonings occurring in the developing nations. Pyrethroids are preferred over organochlorines, organophosphates and carbamates due to their high efficacy against a large variety of insects, low mammalian toxicity and easy biodegradability. However, these claims of low mammalian toxicity no longer holds true as reports of toxicity are gradually accumulating against pyrethroids. According to the Agency for Toxic substances and Disease Registry (ASTDR, 2003) US department of health and human services, Atlanta, GA, pyrethroids are widely used in forestry, agriculture, veterinary and human medicine and household pest control (Soderlund *et al.*, 2003). Pyrethroid can elicit a range of immunotoxic and neurotoxic effects (Galloway *et al.*, 2003). Pyrethroid metabolites have been measured in the urine of 1177 persons without any apparent exposure in Germany (Heudorf *et al.*, 2004). Their exposure has also been well documented in potentially sensitive subpopulations including pregnant women, infants and children (Yanez *et al.*, 2004). Their use is continuously increasing since 1998 due to the phase out of older drugs like organophosphorus compounds (Amveg *et al.*, 2005). Pyrethroid residues have been reported to cause epilepsy, liver and kidney dysfunction (Pandit *et al.*, 2005), somatic growth depression, neuritis and testicular cancer and produce dose-dependent decreases in motor activity of rats (Woolansky *et al.*, 2006).

Pyrethroids are synthetic chemicals similar to pyrethrins in the pyrethrum extract which is obtained from *Chrysanthemum* plant. Historically, pyrethroids have been classified into two classes that differ in their chemical structure and symptoms of exposure:

Type I pyrethroids include allethrin, tetramethrin, d-phenothrin, permethrin, and bioresmethrin.

Type II pyrethroids include cypermethrin, cyphenothrin, deltamethrin, cyfluthrin, fenvalerate, (WHO, 2013).

Cypermethrin is a type II pyrethroid with potent insecticidal property. It is extensively used as an ectoparasiticide in animals, agriculture and public health programmes. It causes hazardous effects in different levels to non-target species, producing significant histopathological changes (WHO, 2013).

Cypermethrin has become one of the most important insecticides in wide scale use. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry. Cypermethrin, an analogue of pyrethrins is classified by the World Health Organization as moderately hazardous (class II). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds compared to the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long lasting trains of repetitive impulses in sense organ (WHO, 2013).

## **2.1.2 Basic Information on Cypermethrin**

### **2.1.2.1 Toxicological Information**

A number of toxicological studies carried out by manufacturers and researchers in support of registration applications in many countries, shows cypermethrin (Table 1) to be one of the safest synthetic pyrethroids (WHO, 2013).

**Table 1:** The main toxicity values of cypermethrin are given in the table

|                                   |                |
|-----------------------------------|----------------|
| LD <sub>50</sub> oral (rat)       | >4150 mg/kg    |
| LD <sub>50</sub> oral (mice)      | >138 mg/kg     |
| LD <sub>50</sub> dermal (rat)     | >4920 mg/kg    |
| LD <sub>50</sub> dermal (rabbit)  | >2460 mg/kg    |
| LC <sub>50</sub> Inhalation (rat) | >2.5 mg/l (4h) |

### 2.1.2.2 Uses of Cypermethrin

Alpha-cypermethrin is a non-systemic, broad spectrum, insecticidal pyrethroid, with rapid knockdown activity. It is effective by contact and ingestion against target pests at relatively low application rates. It acts by preventing transmission of nerve impulses, by blocking the passage of sodium ions through channels in nerve membranes, thus preventing signals passing down axons. Typically this intoxication results in a rapid “knockdown” and mortality (WHO, 2013).

It is used in to control a wide range of chewing and sucking insects (particularly Lepidoptera, Coleoptera and Hemiptera) in fruit (including citrus), vegetables, vines, cereals, maize, beet, oilseed rape, potatoes, cotton, rice, soya beans, forestry and other crops. In public health it is used to control cockroaches, mosquitoes, flies and other insect pests. It is also used in animal health as an ectoparasiticide (WHO, 2013).

### 2.1.2.3 Health Issues of Cypermethrin

Cypermethrin is classified by the WHO as 'moderately hazardous' chemical belonging to Class II category (WHO, 1995). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds, compared to the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long-lasting trains of repetitive impulses in sense organs.

Symptoms of poisoning include abnormal facial sensations, dizziness, headache, nausea, anorexia and fatigue, vomiting and increased stomach secretion. Cypermethrin is also a skin and eye irritant. The toxic responses in all species were found to be qualitatively similar. The clinical signs observed after oral and inhalation exposure were indicative for an action on the central nervous system and consisted of salivation, ataxia, splayed gait, hyper-excitability to auditory stimuli, tremors, convulsions and choreoathetosis (Agriphar, 2010).

The toxicology of pyrethroids has been well researched due to their widespread use. Algae (and plants) are not especially sensitive, and neither are mollusks. Cypermethrin is moderately toxic to mammals and less toxic to birds, but arthropods are extremely sensitive, some in the ng/L range (Maund *et al.*, 2012).

Previous studies revealed that exposure to pesticides has been associated with reproductive dysfunction, such as spontaneous abortions, infant prematurity, congenital malformations, reduced fertility, sperm mortality decrease and hyperploidy/polyploidy in the spermatozoa of exposed men (Chauhan and Gupta 2005; Qua *et al.*, 2008; Nada *et al.*, 2010).

The clinical signs observed are characteristic for the acute poisoning with a type II pyrethroid: choreoathetosis accompanied by salivation (CS syndrome). In the rat, cypermethrin cis:trans/40:60 also produces epileptic activity during repeated administration. The neurotoxic effect of cypermethrin cis:trans/40:60 on peripheral nerves (axons, endoneurium) was highly correlated with exposure time. Cypermethrin cis:trans/40:60 exerts its toxicity by opening the voltage-gated sodium channel slowly for extended times, leading to a prolonged sodium current in the target neurons. Furthermore, the decrease in the Na<sup>+</sup>, K<sup>+</sup>-ATPase pump activity is involved in the paroxysmal epileptic activity induced by cypermethrin cis:trans/40:60 (Chakraborty *et al.*, 2010).

Cypermethrin cis:trans/40:60 shows oral acute toxicity to bird a dose above 1376mg /kg/d or 5620 mg/kg feed. There were no treatment-related effects upon reproductive performance at

Although substantial information is available regarding their environmental and ecological impact, not much is known in regard to its toxicity in the mammalian system (Patel *et al.*, 2007). The general population is potentially chronically exposed to pyrethroids through food consumption (Fortina *et al.*, 2008). A study by Liu *et al.* (2009) investigated the combined effect of cypermethrin and copper on soil catalase activity and found that copper and cypermethrin affected soil catalase negatively in this order: copper exposure > mix exposure > cypermethrin exposure. Most studies have been on the combination of cypermethrin and other pesticides, but there have also been a couple of studies concerning cypermethrin and heavy metals. All these compounds are widely distributed in the market and used continuously in our houses. Thus, risks of these pesticides surround us and our sons daily (Kaya *et al.*, 2010).

Mehler *et al.*, (2011) investigated the combination of cypermethrin and lead on the benthic invertebrate *Chironomus dilutus*, and found that the joint toxicity was antagonistic. When adsorbed by suspended particles it will eventually settle and reach the sediment, where the DT<sub>50</sub> (sediment) increase to 17 days (Pesticide Properties Database, 2011). Paracampo *et al.* (2012) investigated pesticide runoff and found an increased mortality of the amphipod *H. curvispina* up to 98 days after the application of cypermethrin in the plots (Paracampo *et al.*, 2012). This investigation also showed that it is especially after a runoff event that cypermethrin affects the amphipods in the nearby watershed. They also found that cypermethrin and other pyrethroids have a negative effect on the aquatic environment. The reason arthropods are more sensitive than fish, birds and mammals is their increased sodium channel sensitivity, smaller body size and difference in metabolism, with the risk increasing with decreasing metabolic rate (Maund *et al.*, 2012). For these reasons pyrethroids pose negligible risk to avian wildlife, some risk towards fish and a high risk towards arthropods. Pyrethroids are considered one of the more environmental friendly pesticides (Paracampo *et*

*al.*, 2012). It is very potent against insects, but not very toxic towards other organisms, which is a desirable property for insecticides (Backhaus *et al.* 2012).

## 2.2 Trade and Other Names

Trade names include Ammo, Arrivo, Barricade, Basathrin, CCN52, Cymbush, Cymperator, Cynoff, Cypercopal, Cyperguard 25EC, Cyperhard Tech, Cyperkill, Cypermar, Demon, Electron, Fligene CI, Folcord, Kafil Super, NRDC 149, Polytrin, PP 383, Ripcord, Siperin, Stockade, and Super (WHO, 2013).

### 2.1.2.2 Some Products That Contain Cypermethrin

- Termiticides
- Household insecticides
- Outdoor insecticides
- Ammo<sup>tm</sup>
- Cybush<sup>r</sup>
- Cynoff<sup>tm</sup>
- Cyperkill
- Demon<sup>R</sup>

Reference: (WHO, 2013).

## 2.4 Regulatory Status

Many products containing cypermethrin are classified as Restricted Use Pesticides (RUP) by the EPA because of cypermethrin toxicity to fish. Restricted Use Pesticides may be purchased and used only by certified applicators. Cypermethrin is classified toxicity class II - moderately toxic. Some formulations are toxicity class III - slightly toxic. Pesticides containing cypermethrin bear the Signal Word WARNING or CAUTION on the product label, depending on the particular formulation (WHO, 2013).

## 2.5 Mechanism of Cypermethrin Function

- Cypermethrin kills insects that eat or come into contact with it (WHO, 2013).
- Cypermethrin works by quickly affecting the insect's central nervous system (WHO, 2013).

## 2.6 Chemical Structure of Cypermethrin

Cypermethrin (Plate 3) is a synthetic pyrethroid insecticide used to control many pests, including moth pests of cotton, fruit, and vegetable crops. It is also used for crack, crevice, and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories, and on ships, railcars, buses, trucks, and aircraft. It may also be used in non-food areas in schools, nursing homes, hospitals, restaurants, hotels, in food processing plants, and as a barrier treatment insect repellent for horses. Technical cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties. Cypermethrin is light stable. It is available as an emulsifiable concentrate or wet table powder (WHO, 2013).

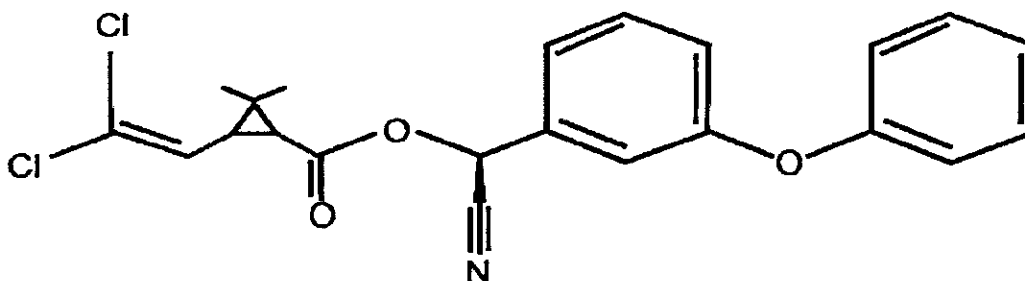
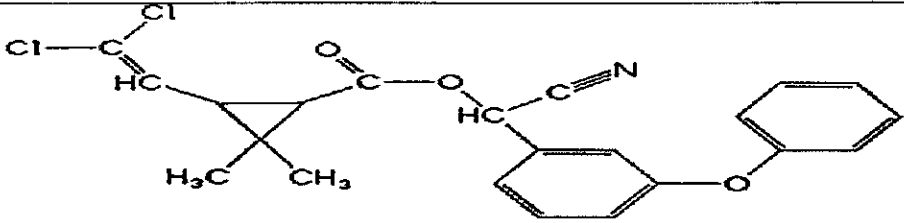


Plate 3: Structural Formula of Cypermethrin; Molecular formula:  $C_{22}H_{19}Cl_2NO_3$ ; Molecular Weight: 416.3

## 2.7 Chemical Properties of Cypermethrin at a Glance

Information on various aspects of chemical properties of cypermethrin is furnished below (WHO, 2013)

|                               |                                                                                                                                                                                                                                                                                      |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IUPAC:                        | ( <i>RS</i> )- $\alpha$ -cyano-3-phenoxybenzyl ( <i>1RS,3RS;1RS,3SR</i> )-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate<br>or ( <i>RS</i> )- $\alpha$ -cyano-3-phenoxybenzyl ( <i>1RS</i> )- <i>cis-trans</i> -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate |
| Appearance:                   | Pure isomers of cypermethrin form colorless crystals. When mixed isomers are present, cypermethrin is a viscous semi-solid or a viscous, yellow liquid                                                                                                                               |
| CAS:                          | Cyano (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate                                                                                                                                                                                            |
| Formula:                      | $C_{22}H_{19}Cl_2NO_3$                                                                                                                                                                                                                                                               |
| Activity:                     | acaricides (pyrethroid ester acaricides)<br>insecticides (pyrethroid ester insecticides)                                                                                                                                                                                             |
| Molecular Structure:          |                                                                                                                                                                                                   |
| Molecular Weight:             | 416.3                                                                                                                                                                                                                                                                                |
| Water Solubility:             | In water at 21°C, 0.01-0.2 mg/l; in hexane at 20°C, 10 g/l; soluble in acetone, cyclohexane, ethanol, xylene and chloroform.                                                                                                                                                         |
| Solubility in Other Solvents: | Methanol versus acetone                                                                                                                                                                                                                                                              |
| Melting Point:                | 60-80°C (pure isomers)                                                                                                                                                                                                                                                               |
| Stability                     | Cypermethrin is quite stable at temperatures under 220°C; photodecomposition has been observed in field tests, with no reduction in biological performance. The optimum stability occurs at pH 4 while decomposition occurs under alkaline conditions.                               |
| Vapor Pressure:               | $5.1 \times 10^{-7}$ nPa at 70°C                                                                                                                                                                                                                                                     |
| Partition Coefficient:        | 6.6020                                                                                                                                                                                                                                                                               |
| Adsorption Coefficient:       | 100,000                                                                                                                                                                                                                                                                              |

|                                                                     |                                                                                                                                                        |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Half-life is the time required for half of the compound to degrade. | 1 half-life = 50% degraded<br>2 half-lives = 75% degraded<br>3 half-lives = 88% degraded<br>4 half-lives = 94% degraded<br>5 half-lives = 97% degraded |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|

## 2.8 Review of the Previous Research Findings

### 2.8.1 Mortality Pattern

Cypermethrin is a composite pyrethroid; a broad spectrum, non-cumulative insecticide; and, a fast-acting neurotoxin with good contact and stomach action. It is of moderately high toxicity to mammals and readily metabolized with immediate loss of activity. (Manna *et al.*, 2004) reported that LD<sub>50</sub> of  $\alpha$  cypermethrin is 145 mg/kg body weight and 100% mortality in the highest dose group agrees with this finding. The group treated with 120 mg/kg body weight of cypermethrin showed 100% mortality within three to seven days (Nair *et al.*, 2013). The medium-dose group, which was given 80 mg/kg body weight of cypermethrin, showed 75% mortality, and most of the deaths occurred within six to ten days. Animals of the lowest-dose group and the control groups did not show any mortality, indicating that mortality was due to cypermethrin toxicity.

### 2.8.2 Clinical Signs/Behavioural Changes

The clinical sign observed in the low-dose group was loss of body weight during the first few days of intoxication. Later the animals adapted and gradually their body condition improved. Another sign noticed during the early days of intoxication was gradual development of hind limb extensor tone followed by recovery. Other signs noticed were increased startle response, salivation, and somnolence. The medium-dose group exhibited signs such as burrowing behavior, increased startle response, salivation, somnolence, seizures, and gradual

development of hind limb extensor tone. The highest-dose group animals exhibited increased startle response, seizures, somnolence, and salivation initially, but later showed loss of righting reflex, lateral recumbency, labored breathing, gasping, and death. Similar signs were observed by Aldana *et al.* (1998) and Manna *et al.* (2004) in cypermethrin toxicity studies in rats, at different dose levels.

Cypermethrin administered at repeated oral doses of 5 and 20 mg/kg/day for 30 days produced varying degree of mild to moderate toxic symptoms and behavioral changes in both male and female rats. The lower dose produced very mild toxicosis characterized by intermittent diarrhea, decreased feed intake, and thick eye discharge, whereas higher dose displayed mild to moderate toxicosis with diarrhea, decreased feed intake, loss of body weight, dyspnoea, ataxia, eye discharge, and salivation. Repeated administration of cypermethrin also produced hemorrhages within myocardium, disruption of branching structure, and loss of striation of cardiac tissue; thickening of alveolar septa in lungs, partial to extensive loss of various stages of spermatogenesis in testes, and loss of follicular cells and oocytes in ovaries (Grewal *et al.*, 2012).

### 2.8.3 Feed Consumption

Dahamna *et al.* (2011) reported that cypermethrin mixed diet at 1500 mg per kg for 13 weeks showed diminished feed intake in beagle hound dogs with weight loss, diarrhoea, anorexia, licking and chewing of paws. The experimental rats were fed cypermethrin at 50 mg/kg b. wt. continuously for a period of 2 and 4 weeks. Feed and water intake was also noted daily for control, vehicle treated and cypermethrin-treated rats. It was observed that there was no effect on feed and water intake in treated rats as compared to the control group. Chronic exposure to cypermethrin for 4 weeks resulted in loose fecal pellets and hyperirritability in the treated rats. Treatment related mortality also occurred at the 4<sup>th</sup> week of treatment. Significant changes in body weight and various organ weights due to cypermethrin were observed along

with disruption of estrous cycle in rats. The body weight gain in treated rats was lower at both 2 and 4 weeks as compared to the control rats. The weight of liver and spleen decreased, while that of kidneys increased as compared to the control rats. Thyroid and adrenal showed increase in weight at both 2 and 4 weeks of treatments (Sangha *et al.*, 2011).

#### **2.8.4 Body Weight Gain**

There was significant decrease in body weight gain in the mid dose and high dose groups of both male and female animals when compared to respective control groups from I to IV week. The decrease in body weight gain was in accordance with Clark *et al.* (1989) who reported a decreased growth which correlated with decreased feed intake in both male and female Wistar rats fed with 540 mg/kg cypermethrin. The growth in rats was reduced at 750 mg of cypermethrin per kg for five weeks. Dahamna *et al.* (2011) had reported reduction in body weight gain in rats fed 1600 mg cypermethrin per kg for three months.

#### **2.8.5 Histological Analysis**

Manna *et al.* (2004) observed bloat, haemorrhages in the stomach, intestine, and lungs, in both single dose and repeated dose toxicity studies of cypermethrin in rats. In crossbred calves, cypermethrin intoxication produced congestion and/or haemorrhages on the auriculoventricular groove of the heart, lungs, mucosal surface of the intestine, cortex of the kidney, and brain (Patel *et al.*, 2006). The histopathological changes in the lung and liver tissues of cypermethrin exposed mice have shown significant changes. Exposure of the mice to cypermethrin through inhalation induces significant time dependent changes in the histopathology of lung as well as liver tissue. Inhalation is a major route of exposure to air born pollutants such as the pesticides (Richards, 2008). Lungs and liver are the organs which are at highest risk to the environmental pollutants especially the air born chemicals (Dixon *et al.*, 2008). Cypermethrin has shown the carcinogenic effect in the lung tissues of mice. Exposure to cypermethrin caused a gradual distortion of normal structure of alveoli in the

lung. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long-lasting trains of repetitive impulses in sense organs (Rodriguez *et al.*, 2008). Toxicants may also pass into the systemic circulation from the lungs and affect the other body parts especially the liver because the hepatocytes directly receive the chemicals from the blood (Richards, 2008). Haemorrhages in the liver, kidneys, lungs, ventricles, and endocardium of male dwarf goats treated with high doses (0.8 and 1.6%) of cypermethrin were reported by Khan *et al.* (2009).

The most consistent lesions in the liver of animals, of all treatment groups, were varying degrees of degenerative changes and vascular changes. In some areas, the degenerative changes extended up to necrotic changes. In the livers of the group III animals, Kupffer cell hyperplasia was pronounced. These findings were in agreement with those observed in cypermethrin toxicity in rats by Manna *et al.* (2004), Yavasoglu *et al.* (2006), Luty *et al.* (2006), Muthuviveganandavel *et al.* (2008) and Grewal *et al.* (2010). All had conducted experiments in albino rats, but the dosage and route of administration of cypermethrin were different. In crossbred cow calves, cypermethrin intoxication produced moderate congestion of the sinusoids and hepatic vasculature, and the hepatocytes showed tiny vacuoles along with increased granularity in the cytoplasm of the liver. In male dwarf goats, necrosis, condensed/pyknotic nucleus, and regeneration of the hepatocytes was observed by Khan *et al.* (2009) cypermethrin metabolized in the liver via the hydrolytic ester cleavage and an oxidative pathway by the cytochrome P-450 microsomal enzyme system, which caused oxidative stress by reducing the activity of superoxide dismutase and glycogen level, leading to hepatic degeneration and necrosis. After 15 days the total body, testis, and epididymis weights were decreased more in the cypermethrin-treated mice than in the NaF-treated mice, but after 30 days the decrement was greater in the NaF-treated mice than in the cypermethrin-treated mice. The reduction in weight of accessory organs indicates atrophy of glandular

tissue and also a reduction in secretory ability. In the combined treatment, the decrement in testicular weight was greater in both 15 and 30 days compared to individual exposures. These decreases in the weights of the whole body, testis, and epididymis indicate that both cypermethrin and NaF exert toxic effects on these organs. Similar weight loss results have been reported by Wang *et al.* (2009) for moderate (10 mg/kg bw/day) and high doses (20 mg/kg bw/day) of beta-cypermethrin in male mice (Wang *et al.*, 2009). NaF treatment caused a decrease in the body weight of male mice. Similar results have been reported by others in mice fed different concentrations of Fluoride. Thus, Long *et al.* (2009) found a decrease in body weight and caput and cauda epididymis in male albino mice treated with NaF. Moreover, Long *et al.* (2009) observed fluoride toxicity in the male reproductive system, and noted negative fertility effects of sodium fluoride in male mice.

Nair *et al.*, 2010 reported elevation in serum aspartate aminotransferase (AST) and alanine amino transferase (ALT) levels in rats treated with cypermethrin and the pathological changes in liver can be correlated with the elevation in serum enzyme levels.

Cypermethrin induces time dependent changes in the liver as well as in lungs. Cypermethrin also damaged the normal organization of liver tissues causing liver injury shown by necrosis, significant decrease in number of cells, widening of sinusoids and fibrosis. It possesses carcinogenic potential as it induced carcinogenesis in the lung tissues of mice, observed as significant hyperplasia, clumping of cells and necrosis. It also induced pulmonary edema, alveolitis and pulmonary fibrosis by the deposition of collagen. Exposure to cypermethrin for long time resulted in the development of skin tumor in the epidermis of mice. From these findings, we may conclude that cypermethrin and other pyrethroids cause hazardous effects in non-target organisms through inhalation exposure. So, the use of this insecticide should be controlled seriously. Synergistic effects were noticed when male albino mice were treated at 48h intervals for 15 and 30 days with cypermethrin (4.25 and 8.5 mg/kg bw) and NaF (2.8

and 5.6 mg/kg bw) by oral gavages. Significant decrease in body, testis and epididymis weights along with histopathological changes in the testis after 30 days was recorded (Sekhar *et al.*, 2011). The cypermtherin group showed a decrease in alveolar cavities as a result of capillary congestion, an increase in the number of pneumocyte type 2 and increased thickness of the basement membrane barriers between alveolar spaces and capillaries were observed. These findings are in agreement with the study of (Ulaiwi *et al.*, 2011) who fed 24 rabbits cypermtherin for 21 days and his results showed histopathological alteration such as engorgement of pulmonary capillary and congestion of pulmonary alveoli, thickening and congestion of the alveolar septa and swollen and tortuous of smaller veins. Cypermethrin administered at repeated oral doses of 5 and 20 mg/kg/day for 30 days produced thickening of alveolar septa in lungs. Husseina *et al.* (2011) reported that Cypermethrin caused different histopathological changes in rat organs including ischemia and pyknosis of the cytoplasm of the neurons in the brain tissue.

Long-term exposure to cypermethrin during adulthood is found to induce dopaminergic neurodegeneration in rats, and postnatal exposure enhances the susceptibility of animals to dopaminergic neurodegeneration if rechallenged during adulthood (Singh *et al.*, 2012). Cypermethrin induced necrosis, degeneration, and loss of striation in hearts, necrosis of hepatic cells, with degeneration, dilatation of sinusoids and dissociated remark cordons in livers, sloughing off epithelial cell, shrinkage of glomeruli, and necrosis of renal tubules in kidneys of mice (Ince *et al.*, 2012). A Study done by Al-Azizz (2012) demonstrated also lung toxicity in pigeons upon exposure to different doses of cypermethrin.

### **2.8.6 Haematological Profiles**

Alteration in the haematological profiles can be affected by sex, altitude, pesticides, drugs, and metals, food additives, dyes, industrial effluent chemicals and parasitic infestation. The circulatory system serves as the primary target site for most pesticides. The aim of this study

is to study the effect of cypermethrin on the haematological profiles in frogs over a 7-day and 30-day cypermethrin exposure period. It is well known that farmers, pesticide applicators, industrial workers, and other pesticide users are exposed to pesticides such as cypermethrin repeatedly (Sharma *et al.*, 2007).

Blood disorders (anemia, defective blood coagulation), brain and nerve damage, paralysis, jaundice and hepatic fibrosis, allergenic sensitization, emphysema, asthma, kidney problems, cancer, genetic disorders, birth defects, miscarriage, impotence, and infertility or sterility are often associated with low residual level of pesticides. In animals, interrupted estrous cycle, decreased weight gain, decreased appetite, decreased milk production, or reproductive problems can be correlated with continues low-level exposure of pesticides (Cigankova *et al.*, 2006). The obtained data reveal a marked decrease in the percentage of body weight gain, red blood cells (R.B.Cs) counts, Hemoglobin (Hb) content mean corpuscular hemoglobin concentration (MCHC), serum total lipids and serum total cholesterol of rats treated with the high dose of amaranth. On the other hand, a noticeable increase in hematocrit (Hct) value, Mean corpuscular volume (MCV), activities of Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose level, serum total protein and globulin were found in rats treated with the high dose of amaranth. In general, there was appreciable improvement after the recovery period (Cigankova *et al.*, 2006).

The lymphocyte, neutrophil, RBC counts and haemoglobin content also remained fairly constant in the treated mice of the present study as compared to the control group. Breton *et al.*, (2006) and Gupta and Flora (2007) found that the RBC and hemoglobin level is decreased with increased concentration of arsenic. This could be due to binding ability of arsenic to hemoglobin that leads to inhibition of heme synthesis pathway. Juruli and Katsitadze (2007) used a single dose of arsenic trioxide (3 mg/kg) to rats and found that arsenic was completely removed from the blood after seven days. This may be because in the

process of arsenic metabolism, inorganic arsenic is methylated to monomethyl arsonic acid (MMA) and finally to dimethyl arsinic acid (DMA) followed by a renal excretion. Liver is the most important site of arsenic methylation but most organs show methylating activity.

A significant reduction in the platelet count was found in the mice treated with garlic in the present study. Alhamami *et al.*, (2006) also found a lowering of platelet count in Sprague Dawley rats due to garlic. A possible explanation for the above results is that garlic inhibits adenosine diphosphate (ADP), collagen, arachidonate, epinephrine, calcium ionophore as well as inhibits the formation of thromboxane, phospholipase and lipooxygenase formed in the platelets.

Substantial decrease in TEC and hemoglobin could possibly be due to suppression of erythropoiesis and heme synthesis, and also to devastation of erythrocyte in hemopoietic tissue (Fetoui *et al.*, 2008). Erythrocyte lysis is produced by agents those injure the red cell membrane, leading to oxidative damage to hemoglobin or may be suppressing the anti-oxidative protective mechanism. Augmented hemolysis usually lead to reduction in Hb, TEC and Hct and are escorted by elevated reticulocytes counts, amplified anisocytosis, increased red cell dissemination width and volumes.

Significantly increased mean corpuscular volume (MCV) after pyrethroid treatment was reported in various animals (Sayim *et al.*, 2005; Shah *et al.*, 2007) at different doses. Significantly decreased and increased MCH after pyrethroid treatment was reported in rats, but MCH was either unaffected (Shah *et al.*, 2007) or increased (Basir *et al.*, 2011) by the pyrethroid treatment in rabbits. Significantly decreased mean corpuscular Hb concentration (MCHC) after pyrethroid treatment was reported (Basir *et al.*, 2011).

#### **2.8.7. Normal Blood Values from Birth to Two Months**

The typical erythrocyte number for newborn normal mice ranges from  $3.6$  to  $5.6 \times 10^6$  RBC/mm<sup>3</sup>, with differences between animal stocks and between observers (Table 2). Within

1 month this number rises to  $6.5$  to  $8.6 \times 10^6$  cells, while the body weight increases by a factor of 10 and the total blood volume increases nearly as much, so that the circulating red-cell mass has increased 15 to 20 times. The mean cell volume of erythrocytes decreases sharply during the same interval from  $100$  to  $110 \mu^3$  at birth to  $48$  to  $63 \mu^3$  at 28 days and continues to drop slightly until stable values for erythrocyte number and mean cell volume appear at the age of 8 to 10 weeks. The change in erythrocyte mean cell volume comes from a combination of two factors. The "intermediate cells" formed in liver hematopoiesis gradually drop out of circulation and are replaced by younger and smaller cells. The size of newly formed erythrocytes also decreases, at least during the first week after birth (Wikipedia, 2012).

Table 2: Normal Range of different blood parameters of Albino Mice and Human Beings (Murray *et al.*, 2007):

| Blood Parameters                                      | Normal Range in Albino Mice | Normal Range in Human Beings   |
|-------------------------------------------------------|-----------------------------|--------------------------------|
| TC, RBC ( $\times 10^6$ Cells/mm <sup>3</sup> )       | 8.48-15.15                  | Male: 4.5-5.5; Female: 3.8-5.8 |
| TC, WBC ( $\times 10^3$ Cells/mm <sup>3</sup> )       | 9.1-28.7                    | 4.0-11.0                       |
| TC, Platelets ( $\times 10^4$ Cells/mm <sup>3</sup> ) | 66.8-154.3                  | 15-45                          |
| Neutrophils (%)                                       | 1.9-11.5                    | 40-70                          |
| Eosinophils (%)                                       | 0.05-0.51                   | 1-6                            |
| Lymphocytes (%)                                       | 40-70                       | 20-50                          |
| Monocytes (%)                                         | 0.3-1.4                     | 2-8                            |
| Basophils (%)                                         | 0-2                         | 0-1                            |
| Hb (g/dl)                                             | 14.8-18.3                   | Male: 13-18; Female: 11.5-16.0 |
| ESR (mm/hr.)                                          | 3.5-8.0                     | Male: 0-8; Female: 0-10        |

### 2.8.8 Human exposure

Excessive exposure can cause nausea, headache, muscle weakness, salivation, shortness of breath and seizures. In humans, cypermethrin is deactivated by enzymatic hydrolysis to

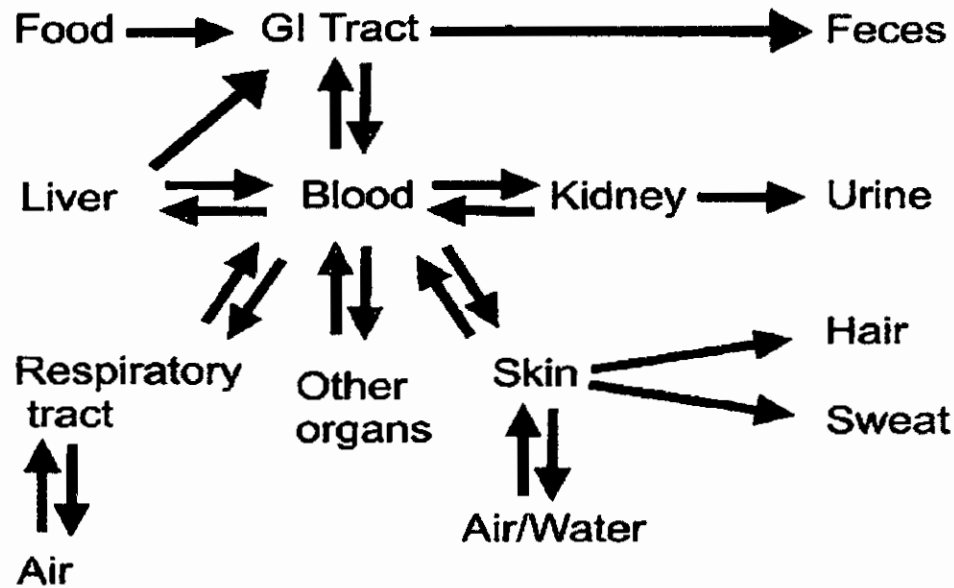


Plate 4: Absorption and distribution of chemicals in animals (Apostoli, 2002).

There are many histopathologic types of lung cancer and World Health Organization (WHO, 2013) classification, recommended originally in 1977 is usually accepted as the definitive classification of this cancer. Major types of lung cancer are adenocarcinoma, squamous cell carcinoma and large cell carcinoma collectively known as non-small-cell lung cancer (NSCLC) accounting for 75-80% of all lung cancers and small-cell lung cancer (SCLC) comprises the remaining 20-25% of lung cancer cases.

A recent study at Xuzhou Medical College in China showed that, in male rats, cypermethrin can exhibit a toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced. These data suggested that cypermethrin can induce impairments of the structure of seminiferous tubules and spermatogenesis in male rats (Hu *et al.*, 2011).

# **CHAPTER THREE**

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## **MATERIALS AND METHODS**

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#### **3.1 Site and Period of the Study**

The present study was carried out at the Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh during the period from February 2013 to June 2013.

#### **3.2 Experimental Animals**

Swiss albino mice *Mus musculus* L. (Rodentia: Muridae) of 1-2 weeks of age, each weighing  $30 \pm 3$  g, were collected from Jahangirnagar University, Bangladesh. They were kept in iron cages (45 cm  $\times$  30 cm  $\times$  30 cm) with sawdust bedding, which was replaced once a week. Water and poultry feed were supplied. Mice were maintained at laboratory conditions ( $28 \pm 4^\circ$  C;  $75 \pm 11\%$  RH and 8:16 hrs light: dark regime) throughout the course of the present study.

#### **3.3 Test Chemical**

Cypermethrin (Trade Name: CAUGHT-10 EC) technical (99% purity; *cis:trans* isomers ratio 40:60) were purchased from a local pesticide shop situated at Kantakhali, Rajshahi, Bangladesh. The chemical was registered, formulated and repacked by The ACI Formulations Ltd and manufactured by Tag rose Chemicals India Ltd.

#### **3.4 Experimental Design**

Healthy adult male albino mice were divided into five groups having five animals each. Mice in group I served as normal control while those in groups II, III, IV and V were injected caught-100 intraperitoneally. Various doses of Cypermethrin were injected intraperitoneally at 5, 7.5, 10 and 12.5 ml/kg body weight of the experimental mice for four (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup>) consecutive weeks which are further elaborated in Section 3.7.

At the end of the experiment, the animals were weighed and then sacrificed. Albino mice were placed in anesthetic jar containing cotton wool soaked in chloroform. Complete anaesthesia was considered accomplished when the pedal movements and eyelid reflex disappeared and the animal becomes recumbent while still breathing. The belly of the mice was opened up and blood was collected by cardiac puncture. Detailed postmortem examinations were conducted and gross lesions were noted. Brain, stomach, intestine, liver, kidney, lungs, heart and gonad were collected for histopathology. The brain and spinal cord were fixed in neutral buffered formalin and the other tissues were fixed in 10% formalin.

### **3.5 Analyses of the samples**

A total 25 (5 groups  $\times$  5 replicates per group) samples were analyzed in this study. The following observations were made on the samples collected:

- Behavioural/clinical signs
  1. Decrease Feed intake
  2. Loss of body weight
  3. Hind limb extensor tone
  4. Salivation
  5. Eye discharge
  6. Startle response
  7. Seizures
  8. Laboured breathing
  9. Death
  10. Haemorrhage
  11. Lesions
  12. Necrosis
  13. Edema

- **Morphometric parameters**
  1. Initial body weight (gm)
  2. Body weight gain/loss (gm)
  3. Daily body weight gain/loss (gm)
  4. Daily feed intake (gm)
  5. Total feed intake (gm)
  6. Feed conversion ratio (FCR)
  7. Mortality (%)
- **Organ/somatic indices**
  1. Brain weight
  2. Liver weight
  3. Kidney weight
  4. Lung weight
  5. Heart weight
  6. Visceral weight
  7. Gonadal weight
- **Haematological profile**
  1. TC, RBC
  2. TC, WBC
  3. TC, Platelets
  4. Neutrophils
  5. Eosinophils
  6. Lymphocytes
  7. Monocytes
  8. Basophils

9. Haemoglobin (g/dl)

10. Erythrocyte Sedimentation Rate (ESR) (mm/hr)

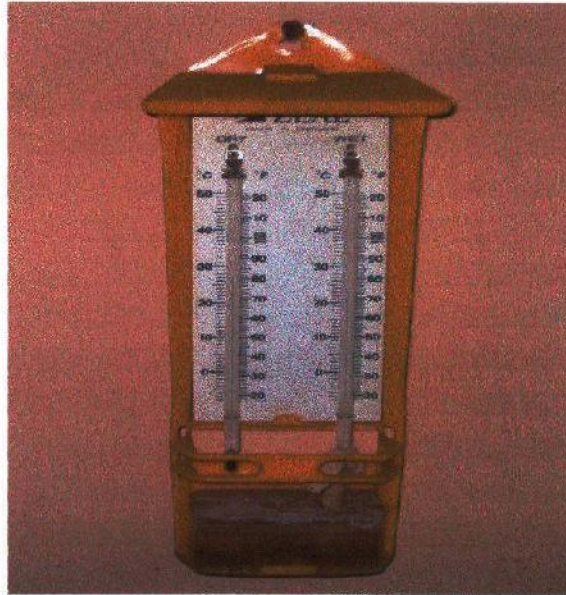


Plate 5: Dry and weight bulb hygrometer for measuring of daily Relative Humidity (RH) in the lab.

### 3.6 Materials

#### 3.6.1 Glass Ware

Petri dishes, Conical Flasks, Standard Flasks, Pasture Pipette, Droppers, Beaker, Test tube, Vial, Slide, Cover Slip and Spirit Lamp.

#### 3.6.2 Equipments

Insulin Syringe, Dry and weight bulb hygrometer (Plate 5), Distilled Water Plant, Dissecting Tray, Forceps, Scissors, Razor Blade, Electronic Balance, Knife, Light Microscope, Photographic Microscope, Spatula, Gloves.

#### 3.6.3 Reagents

Mayer's Albumen, Saline Solution, Aqueous Eosin, Alcoholic Eosin, Chloroform, Absolute Alcohol, Methanol, Ether, Bouin's Fluid, Xylene, Glycerine, Wax, Haematoxylene Dye, Ethylene Diamine Tetra Acetic Acid (EDTA)

### 3.7 Brief Protocol

- (a) 25 albino mice (All Males) were selected and their ages were recorded (in weeks) and initial body weights (in g); then they were divided into five groups (I-V); five replicates per group;
- (b) Technical grade (~99% pure) Cypermethrin (CP, commercially called *Caught-100*) were injected intraperitoneally at 5, 7.5, 10 and 12.5 ml/kg body wt of the experimental mice for four (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup>) consecutive weeks as follows:

| Groups | Treatment                                                          | Duration<br>(wk) | Day of<br>sacrifice |
|--------|--------------------------------------------------------------------|------------------|---------------------|
| I      | Control (0.10, 0.15, 0.20 and 0.25 ml DH <sub>2</sub> O/200g mice) | 4                | 29 <sup>th</sup>    |
| II     | 5 ml CP/kg bw/wk (=0.10 ml/20g mice)                               | 4                | 29 <sup>th</sup>    |
| III    | 7.5 ml CP/kg bw/wk (=0.15 ml/20g mice)                             | 4                | 29 <sup>th</sup>    |
| IV     | 10 ml CP/kg bw/wk (=0.20 ml/20g mice)                              | 4                | 29 <sup>th</sup>    |
| V      | 12.5 ml CP/kg bw/wk (=0.25 ml/20g mice)                            | 4                | 29 <sup>th</sup>    |

A group of control (Group I) was maintained for comparison.

- (c) Behavioural/clinical signs following each treatment viz. decreased feed intake, loss of body wt, hind limb extensor tone, salivation, eye discharge, startle response, seizures, laboured breathing, grasping, death etc. were noted carefully;
- (d) The following morphometric parameters: (i) initial body wt (bw in g) before treatment, (ii) bw gain/mouse, (iii) daily bw gain/mouse, (iv) daily feed intake/mouse, (v) total (4 week) feed intake/mouse, (vi) feed conversion ratio (FCR)/mouse and (vii) mortality (%) was recorded carefully;
- (e) Estimation of organ/somatic indices: At the end of CP treatments and upon sacrifice, the following organ/somatic indices were recorded for brain, liver, kidneys, lungs, heart, viscera and gonads using the formula of Sekhar *et al.*, (2011): Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100;

- (f) The following haematological profiles were estimated at the end of treatment but before sacrificing the animals: (i) total counts (TC) of RBC, WBC, and platelets; (ii) differential counts (DC) of WBC including neutrophils, eosinophils, lymphocytes, monocytes and basophils; (iii) haemoglobin (Hb%) and (iv) erythrocyte sedimentation rate (ESR) of the mice will be estimated using standard procedures (Jayakumar *et al.*, 2008; Prakash *et al.*, 2009).
- (g) At the end of the 4<sup>th</sup> week, all mice were sacrificed by treating them with chloroform and recorded (i) abnormalities *e.g.* haemorrhage, lesions, necrosis, edema etc. of the internal organs like stomach, intestine, lungs, kidneys and brain), if any (Nair *et al.*, 2013).
- (h) The tissues from brain, liver, kidney, stomach, intestine and gonad in 70% alcohol were preserved just after sacrifice and processed them for histological slides for observation (Inayat *et al.*, 2007; Sekhar *et al.*, 2010; Sekhar *et al.*, 2011; Nair *et al.*, 2013).
- (i) Supported by the behavioural/clinical signs, abnormalities and histological findings, the morphometric and haematological data were analyzed statistically to interpret the results.

### **3.8 Behavioural/clinical signs**

#### **3.8.1 Toxic Signs**

After injecting the control group with distilled water and remaining four groups injected with cypermethrin 5 groups of mice were operated separately for 29 consecutive days. All the control and treated mice were observed carefully for appearance of any toxic signs such as decrease feed intake, hind limb extension tone, loss of body weight, salivation, eye discharge, seizures, startle response, death, etc.

### 3.8.2 Haemorrhage, Necrosis and Edema

On 29<sup>th</sup> days the mice of control group and treatment group were dissected and carefully observed the haemorrhage, necrosis and edema of internal organs like brain, kidney, lung, liver and gonad.

## 3.9 Morphometric Parameters

### 3.9.1 Body weight

Body weight of the control and treated mice were taken before starting of experiment and at 7 days interval during 29 days of feeding. Compared to the control and the CYP-treated mice, the body weights of the mice were reduced significantly ( $p < 0.05$ ) with no significant difference shown among the groups.

### 3.9.2 Feed Conversion Ratio

In animal husbandry, feed conversion ratio (FCR), feed conversion rate, or feed conversion efficiency (FCE), is a measure of an animal's efficiency in converting feed mass into increased body mass. Specifically FCR is the mass of the food eaten divided by the body mass gain, all over a specified period. FCR is dimensionless, *i.e.* there is no measurement units associated with FCR. The feed conversion Ratio (FCR) was measured by using the following formula (Gull *et al.*, 2006):

$$\frac{\text{Total feed consumed by the mice}}{\text{Final weight-initial weight}}$$

### 3.10 Tissue Somatic Index

From these values the organo-somatic index (OSI) of different organ was calculated dividing organ weight with body weight and quotient being multiple by 100 (Krishnaiah and Reddy, 2007)

The body weight of the mice was taken before they were sacrificed by cervical dislocation. Afterward the testes and accessory sex organs were dissected and then, after removing the

blood and other adhering tissue mass, they were weighed to the nearest milligram on a Shimadzu electronic balance (model No: BL-220H). The tissue indices were calculated using the formula Sekhar *et al.*, (2011):

$$\text{Tissue Index} = \frac{\text{Weight of the tissue in grams}}{\text{Weight of the body in grams}} \times 100$$

### **3.11 Histological Analysis**

#### **3.11.1 Dissection of Mice**

Following the method of (Humason, 1972) histological examination of the tissues was conducted after removal from the mice. To observe the postmortem changes at the end of the experimental period at 29<sup>th</sup> days, all the mice were sacrificed and examined. The tissues were gently rinsed with a physiological saline solution (0.9% NaCl) to remove blood and adhering debris. Sections were cut by a microtome to a thickness of 6 µm and stained with hematoxylin and counter-stained with eosin dissolved in 95% ethanol (H&E). After dehydration and clearing, sections were mounted with DPX (digital picture exchange) and observed under a microscope.

#### **3.11.2 Preparation of Slides**

Air-dried slides were prepared by dropping one or two drops of the final suspension on the grease-free, pre-chilled slides with the Pasteur pipette. The slides were dried immediately by air-drying method, coded and stained. After routine processing, paraffin sections of each tissue were cut into 4 µm thickness and stained with haematoxylin and eosin

### **3.12 Haematological Studies**

#### **3.12.1 Collection of Blood Samples and Measurement of Hematological Parameters**

At the end of the 4-week study period, all mice from each of the five groups were sacrificed. Blood samples were collected from the retro-orbital sinus of the mice with heparinised 70ml

microhaematocrit capillary tubes into a vial containing 0.5ml Ethylene Diamine Tetra Acid (EDTA). White blood cell (WBC) counts, RBC counts, PCV, platelets and hemoglobin (Hb) content of the blood obtained from the mice were analyzed at the hematology unit of Islami Bank Hospital, Rajshahi, Bangladesh using Automated Mythic-22 Haematology Analyzer (Made in China).

### **3.12.2 Methods of Blood Collection in the Mouse**

Collecting blood from mice is necessary for a wide variety of scientific studies, and there are a number of efficient methods available. It is important to remember that blood collection, because it can stress the animals, may have an impact on the outcome of research data. In addition, it is extremely important that those who collect blood become skilled in the techniques they employ, and seek to stress the mice as little as possible.

### **3.12.3 Use of Anaesthetics**

To minimize discomfort to the animal, anesthesia is recommended when collecting blood. Anesthesia is also recommended when collecting blood from the jugular vein to ensure correct positioning of the mouse (Plate 6). When collecting blood from the saphenous or dorsal pedal veins, anesthesia is not necessary.

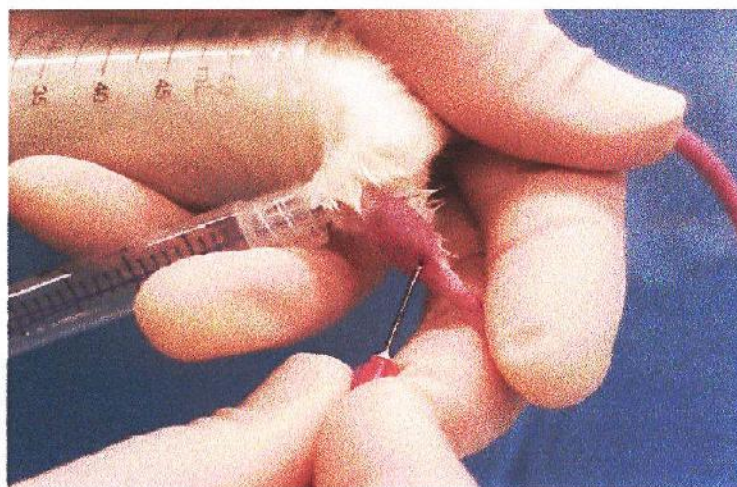


Plate 6: Blood collection from the saphenous vein.

#### **3.12.4 Aseptic Blood Collection**

The optimal blood collection method will also depend on whether the sample must be taken aseptically. Collection from the orbital sinus and jugular vein are good nonterminal choices when an aseptic sample is needed. Care must be taken to make sure that the blood does not come in contact with the mouse's skin or fur. Cardiac puncture or blood collection from the posterior vena cava are good methods of terminal blood collection when an aseptic sample is needed.

#### **3.12.5 Volume Required**

On average, the total blood volume in the mouse is 6-8% of its body weight, or 6-8 ml of blood per 100 g of body weight. Thinner animals will have relatively larger blood volumes per body mass, due to greater surface area. Younger animals, especially newborns, have proportionately larger blood volumes than older animals (Bannerman, 1983). If nonterminal blood collection is planned, the orbital sinus, jugular vein, or saphenous vein will typically yield more blood (0.2-0.3 ml in the average size adult mouse) than the tail or dorsal pedal vein. Terminal blood collection from the orbital sinus or by cardiac puncture will yield 0.8-1.0 ml. The posterior vena cava and axillary vessels typically provide a smaller blood volume.

#### **3.12.6 Red blood corpuscle (RBC) count**

RBC count was made Automated Mythic-22 Haematology Analyzer (Made in China). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant (the dilution is 1:200). The solution was mixed well by shaking gently. The number of RBC  $10^6$  cells per cumm were calculated accordingly.

#### **3.12.7 White blood corpuscles (WBC) count**

WBC count was made Automated Mythic-22 Haematology Analyzer (Made in China). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an

anticoagulant (the dilution is 1:200). The solution was mixed well by shaking gently. The number of WBC  $10^3$  cells per cumm were calculated accordingly.

### 3.12.8 Differential Leukocyte Count

Differential Leukocyte e.g. Neutrophils, Eosinophils, Lymphocytes, Monocytes and Basophils Count was made Automated Mythic-22 Haematology Analyzer (Made in China). Leucocytes were identified and the movement was repeated till a total 100 cells were counted. The values of different morphological types are expressed as the percentage.

### 3.12.9 Platelets Count

Platelets count was made Automated Mythic-22 Haematology Analyzer (Made in China). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant (the dilution is 1:200). The solution was mixed well by shaking gently. The number of Platelets  $10^4$  cells per cumm was calculated accordingly.

### 3.12.10 Estimation of Haemoglobin Concentration (Hb)

The hemoglobin concentration was estimated by Automated Mythic-22 Haematology Analyzer (Made in China). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant (the dilution is 1:200). The solution was mixed well by shaking gently. The Hb concentration was expressed in g/dl.

### 3.12.11 Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) was determined by Westergren method. The Westergren method was performed according to ICSH specifications on undiluted blood samples anticoagulated with  $K_3EDTA$  using glass pipettes (Greiner Bio-One, Kremsmuenster, Austria). During sedimentation, the pipettes were mounted vertically on appropriate supporting racks and kept at room temperature, which never exceeded 25°C. The ESR was expressed in mm/hour.

### 3.13 Basic Procedures in Microtomy

Different steps of activity in the process of preparation of histological slides have been described as follows:

#### a) Washing

Careful washing of fixing fluid is necessary prior to dehydration and infiltration. After fixation, the excess fixative must be washed out of the tissue to prevent interference with subsequent processes. Often washing is done with running water. Alcoholic fixatives are washed out with the aid of alcohol. Washing can be conducted as follows:

- Transfer the fixed tissue in a beaker or in a suitable glass container. Cover the glass container with a piece of mosquito net and fasten loosely at the top.
- Place the apparatus under a water tap and open it.
- Allow water to go inside the container through the mosquito net slowly. Excess water will come out through the side of the container.
- Let the system work at least for several hours to remove every traces of fixative from the tissue. In case of Bouin's fluid continue the process until the color of the picric acid is completely removed.

After washing the tissue may be store for several weeks or months in 70% alcohol.

#### b) Dehydration

Dehydration is achieved in a series of gradually increasing concentrations of alcohol. Gradual changes through 30%, 50%, 70%, 80%, 95% and absolute alcohol is said to reduce some of the shrinkage occurring in the tissue. The flow chart of dehydration is given at Appendix 13.

#### c) Dealcoholization or Clearing

Different steps in "clearing" can be shown in the Appendix 14.

d) Infiltration

The process of keeping tissue in hot molten wax is called infiltration (Appendix 15), which facilitate the penetration of wax to the available spaces inside the tissue.

e) Embedding with paraffin

As soon as the tissue thoroughly infiltrated with paraffin, it is ready for embedding. This process is known as block making or embedding (Appendix 16). For the purpose of embedding cavity block is required, which can be conveniently prepared with a stiff art paper.

f) Trimming

The block to be used for mounting is trimmed to the correct shape and size and then is attached to the holder of the microtome. The block should be trimmed in such a way as to get the tissue exactly at the center perfectly. The axis of the tissue should exactly the parallel to the length of the block.

g) Cutting Paraffin Ribbon or Cutting Sections:

Before cutting sections, all the screws of the microtome are tightened. Adjust the microtome for section thickness of 5-6 $\mu$ . Affixing small pieces of ribbon on the slides (Stretching) are given at Appendix 17.

h) Staining

For staining the tissues certain standard principles are applied for the processing of the tissues after affixing on the slides (Appendix 18).

**3.14 Statistical Analysis**

The experimental data on morphometric, histological and haematological analyses were analyzed using the Statistical Package for Social Sciences (SPSS for Windows, version 15.0). Comparisons were made between experimental groups using one-way analysis of variance (ANOVA) followed by LSD (least significant differences) tests. P-Values of less than 0.05 were regarded as statistically significant.

## **CHAPTER FOUR**

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# **RESULTS AND DISCUSSION**

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Clinical and Behavioural Signs

The very early clinically signs included irritability, scratching of nostrils and head shaking. The mice exposed to high doses of the cypermethrin exhibited more pronounced signs. Increased passage of watery and mucus rich stool with diarrhea, abdominal tenderness (manifested in squeaking if the side of abdomen is pressed and a tendency to stay in fetal position), fall of body temperature (as the tail temperature is decreased, piling, shivering and piloerection increased) decreased food and water intake, bald patches and skin lesions, haemorrhages, edema (**Plate 7**) were noted in treatment groups. Control rats remained highly energetic with voracious appetite and furry. No mortality was recorded in the control and test groups. The effects of cypermethrin on behavioral and clinical signs are detailed in **Table 3**.

**Table 3:** Effects of cypermethrin on behavioural/clinical signs in the albino mice *Mus musculus* L.

| Behavioural/clinical signs | Group I | Group II | Group III | Group IV | Group V |
|----------------------------|---------|----------|-----------|----------|---------|
| Decrease Feed intake       | ×       | √        | √         | √        | √       |
| Loss of body weight        | ×       | √        | √         | √        | √       |
| Hind limb                  | ×       | √        | √         | √        | √       |
| Salivation                 | ×       | ×        | ×         | ×        | ×       |
| Eye discharge              | ×       | ×        | ×         | ×        | √       |
| Startle response           | ×       | √        | √         | √        | √       |
| Seizures                   | ×       | ×        | ×         | ×        | ×       |
| Laboured breathing         | ×       | √        | √         | √        | √       |
| Death                      | ×       | ×        | ×         | ×        | ×       |
| Haemorrhage                | ×       | ×        | ×         | √        | √       |
| Lesions                    | ×       | √        | √         | √        | √       |
| Necrosis                   | ×       | √        | √         | √        | √       |
| Edema                      | ×       | ×        | ×         | ×        | √       |

Group I= control; Group II= 0.10  $\mu\text{L/kg}$  body wt; Group III=0.15  $\mu\text{L/kg}$  body wt; Group IV= 0.20  $\mu\text{L/kg}$  body wt; Group V= 0.25  $\mu\text{L/kg}$  body wt.



**Plate 7:** Edema in albino mice in treatment group V

## 4.2 Morphometric Parameters

The morphometric parameters under study were body weight, feed intake, gain/loss in body weight and feed conversion ratio (FCR). The findings on these parameters are narrated below.

**a) Body weight:** Effects of cypermethrin on the gross and week-wise body weight of normal (control) and treated mice are presented in Table 3 (**Fig. 1**) and 4, respectively. In the control group, the gross body weight was  $35.55 \pm 2.89\text{g}$  that reached to its highest value of  $38.62 \pm 0.38\text{g}$  at the end of 4 weeks. On the other hand, the gross body weights in cypermethrin-treated mice were  $27.32 \pm 1.23\text{g}$ ,  $28.71 \pm 0.96\text{g}$ ,  $34.90 \pm 0.96$  and  $35.76 \pm 1.11$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,95} = 129.22$ ;  $P < 0.001$ ). In addition, the decrease in body weights over the period of four weeks was also highly significant both in the control and treatment groups (**Table 4, Fig. 2**).

**Table 4:** Effect of cypermethrin treatments on gross body weight, feed intake, body weight gain or loss and feed conversion ratio (FCR) in the albino mice *Mus musculus* L.

| Treatment groups<br>( $\mu\text{L/kg}$ body wt) | Body weight (g)               | Feed intake (g)               | Body weight Gain or loss (g)  | FCR <sup>1</sup>                  |
|-------------------------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------------|
| Control (0.0)                                   | 35.55 $\pm$ 2.89 <sup>a</sup> | 64.60 $\pm$ 2.75 <sup>a</sup> | 2.30 $\pm$ 0.90 <sup>a</sup>  | 35.09 $\pm$ 20.39 <sup>a</sup>    |
| 0.10                                            | 27.32 $\pm$ 1.23 <sup>b</sup> | 56.51 $\pm$ 1.94 <sup>b</sup> | -1.21 $\pm$ 0.83 <sup>b</sup> | -85.49 $\pm$ 70.02 <sup>a</sup>   |
| 0.15                                            | 28.71 $\pm$ 0.96 <sup>c</sup> | 57.60 $\pm$ 0.86 <sup>c</sup> | -0.99 $\pm$ 0.58 <sup>c</sup> | -119.73 $\pm$ 138.23 <sup>b</sup> |
| 0.20                                            | 34.90 $\pm$ 0.96 <sup>a</sup> | 57.95 $\pm$ 1.78 <sup>d</sup> | -0.94 $\pm$ 0.60 <sup>d</sup> | -115.92 $\pm$ 128.15 <sup>c</sup> |
| 0.25                                            | 35.76 $\pm$ 1.11 <sup>a</sup> | 58.28 $\pm$ 0.70 <sup>c</sup> | -1.06 $\pm$ 0.32 <sup>e</sup> | -62.99 $\pm$ 30.05 <sup>a</sup>   |
| F-values                                        | 129.22***                     | 65.182***                     | 14.083***                     | 3.073***                          |

All F-values are at 4, 95 df, \*\*\*=  $P < 0.001$ ; FCR=Total feed consumed  $\div$  final weight-initial weight; - (minus) values refer to loss in body weight; superscripts in dissimilar letters in each column differ significantly by LSD tests at  $P < 0.05$ .

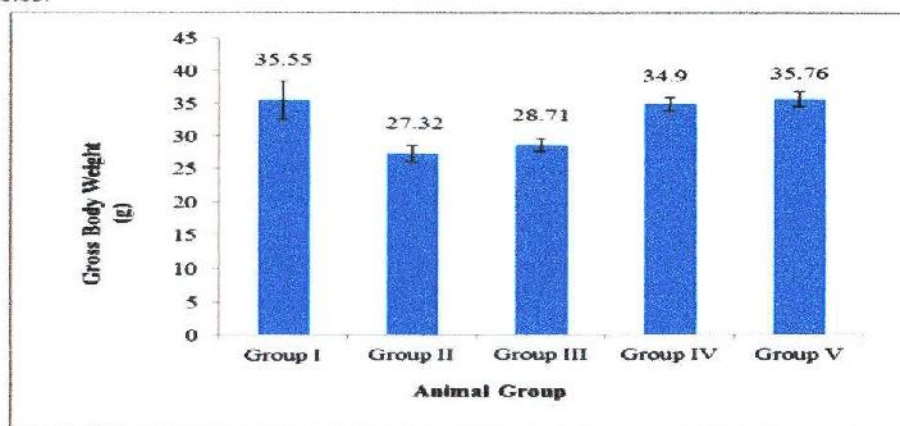


**Fig. 1** Gross body weights in the control and cypermethrin-treated albino mice *Mus musculus* L.

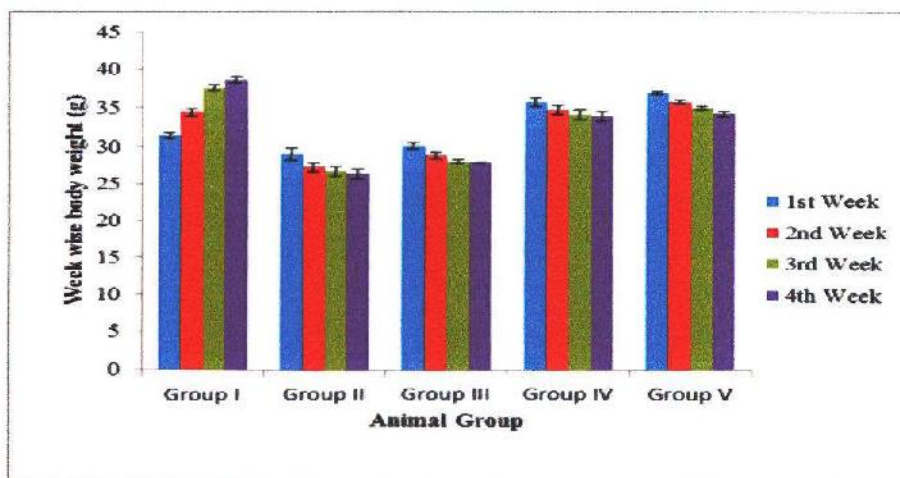


**Fig. 2** Week-wise body weights in the control and cypermethrin -treated albino mice *Mus musculus* L.

**b) Feed Intake:** Effects of cypermethrin on the gross and week-wise feed intake in the normal healthy mice and treated mice are presented in Table 3 and 4, respectively (**Fig. 3**). In the control group, the gross feed intake was  $64.60 \pm 2.75$ g that reached to its highest value of  $67.00 \pm 0.00$ g at the end of 4 weeks. On the other hand, the gross feed intake in cypermethrin-treated mice were  $56.51 \pm 1.94$ g,  $57.60 \pm 0.86$ g,  $57.95 \pm 1.78$ g and  $58.28 \pm 0.70$ g at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,95} = 65.182$ ;  $P < 0.001$ ). In addition, the decrease in body weights over the period of four weeks was also highly significant both in the control and treatment groups (**Table 5, Fig 4**).

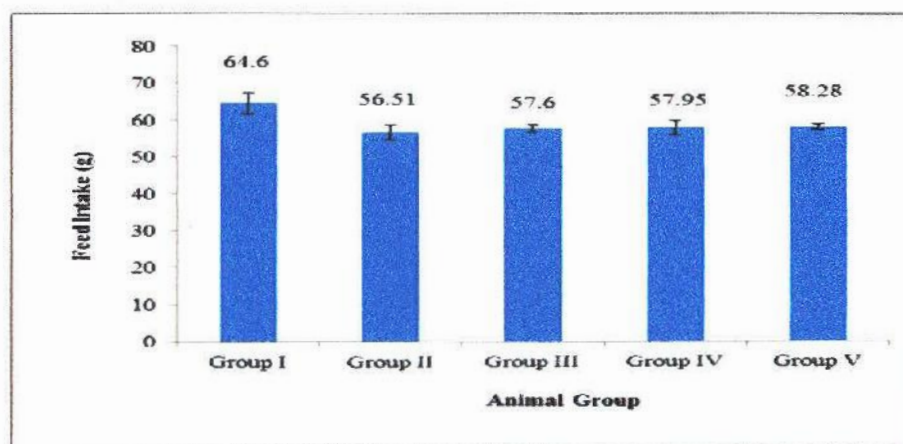


**Fig. 3** Gross feed intake in the control and cypermethrin -treated albino mice *Mus musculus* L.

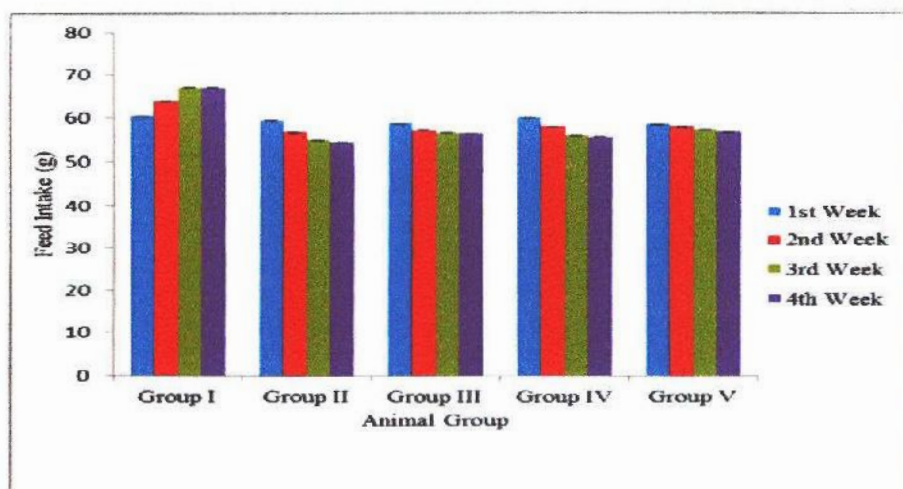


**Fig. 4** Week-wise feed intake in the control and cypermethrin -treated albino mice *Mus musculus* L.

**c) Gain/Loss in Body Weight:** Effects of cypermethrin on the gross and week wise body weight gain/loss of normal healthy mice and treated mice are presented in **Table 4** and **5**, respectively. The final weight of control (Group I) was increased significantly from their initial body weight. Members of control group gained weight progressively during the study period, reaching a maximum weight of  $38.62 \pm 0.38$ , at the end of the study period. On the other hand, the gross body weights loss in cypermethrin-treated mice were  $1.21 \pm 0.83$ g,  $0.99 \pm 0.58$ g,  $0.94 \pm 0.60$ g and  $1.06 \pm 0.32$ g at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,95} = 14.083$ ;  $P < 0.001$ ). In addition, the decrease in body weights over the period of four weeks was also highly significant both in the control and treatment groups (**Table 5**).

**d) Feed Conversion Ratio (FCR):** Effects of cypermethrin on the gross and week wise feed conversion ratio (FCR) of normal healthy mice and treated mice are presented in **Table 4** and **5**, respectively. Only group I (control) showed live weight gain and treatment group showed body weight loss. Therefore, group I showed positive FCR and treatment group showed negative FCR. In the control group, the gross FCR was  $35.09 \pm 20.39$  that reached to the value of  $67.53 \pm 12.36$  at the end of 4 weeks. On the other hand, the gross FCR in cypermethrin-treated mice were  $85.49 \pm 70.02$ ,  $119.73 \pm 138.23$ ,  $115.92 \pm 128.15$  and  $62.99 \pm 30.05$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,95} = 3.073$ ;  $P < 0.001$ ). In addition, the FCR over the period of four weeks was also highly significant both in the control and treatment groups (**Table 5**).

**Table 5:** Effect of cypermethrin treatments on week-wise gross body weight, feed intake, body weight gain or loss and feed conversion ratio (FCR) in the albino mice *Mus musculus* L.

| Treatment groups ( $\mu\text{L/kg bwt}$ ) |                      | 1 <sup>st</sup> week          | 2 <sup>nd</sup> week           | 3 <sup>rd</sup> week           | 4 <sup>th</sup> week             | F-values   |
|-------------------------------------------|----------------------|-------------------------------|--------------------------------|--------------------------------|----------------------------------|------------|
| Control (0.0)                             | Body wt (g)          | 31.50 $\pm$ 0.34 <sup>a</sup> | 34.46 $\pm$ 0.50 <sup>b</sup>  | 37.60 $\pm$ 0.35 <sup>c</sup>  | 38.62 $\pm$ 0.38 <sup>d</sup>    | 325.19***  |
|                                           | Feed intake (g)      | 60.50 $\pm$ 0.00 <sup>a</sup> | 63.90 $\pm$ 0.00 <sup>b</sup>  | 67.00 $\pm$ 0.00 <sup>c</sup>  | 67.00 $\pm$ 0.00 <sup>d</sup>    | 2E+032***  |
|                                           | Gain/loss in bwt (g) | 2.06 $\pm$ 0.20 <sup>a</sup>  | 2.96 $\pm$ 0.28 <sup>b</sup>   | 3.14 $\pm$ 0.33 <sup>c</sup>   | 1.02 $\pm$ 0.19 <sup>d</sup>     | 72.467***  |
|                                           | FCR                  | 29.56 $\pm$ 2.53 <sup>a</sup> | 21.76 $\pm$ 2.26 <sup>a</sup>  | 21.51 $\pm$ 2.04 <sup>a</sup>  | 67.53 $\pm$ 12.36 <sup>b</sup>   | 57.247***  |
| 0.10                                      | Body wt (g)          | 29.02 $\pm$ 0.81 <sup>a</sup> | 27.24 $\pm$ 0.61 <sup>b</sup>  | 26.66 $\pm$ 0.63 <sup>c</sup>  | 26.34 $\pm$ 0.63 <sup>d</sup>    | 15.714***  |
|                                           | Feed intake (g)      | 59.44 $\pm$ 0.00 <sup>a</sup> | 56.90 $\pm$ 0.00 <sup>b</sup>  | 55.10 $\pm$ 0.00 <sup>c</sup>  | 54.60 $\pm$ 0.00 <sup>d</sup>    | 3E+031***  |
|                                           | Gain/loss in bwt (g) | 2.16 $\pm$ 0.31 <sup>a</sup>  | 1.78 $\pm$ 0.31 <sup>b</sup>   | 0.58 $\pm$ 0.13 <sup>c</sup>   | 0.32 $\pm$ 0.08 <sup>d</sup>     | 75.277***  |
|                                           | FCR                  | 27.89 $\pm$ 3.35 <sup>a</sup> | 32.66 $\pm$ 5.02 <sup>a</sup>  | 99.44 $\pm$ 25.00 <sup>b</sup> | 182.00 $\pm$ 55.73 <sup>c</sup>  | 27.638***  |
| 0.15                                      | Body wt (g)          | 30.12 $\pm$ 0.39 <sup>a</sup> | 28.84 $\pm$ 0.39 <sup>b</sup>  | 28.04 $\pm$ 0.21 <sup>c</sup>  | 27.84 $\pm$ 0.09 <sup>d</sup>    | 60.127***  |
|                                           | Feed intake (g)      | 59.00 $\pm$ 0.00 <sup>a</sup> | 57.50 $\pm$ 0.00 <sup>b</sup>  | 57.00 $\pm$ 0.00 <sup>c</sup>  | 56.90 $\pm$ 0.00 <sup>d</sup>    | 2E+031***  |
|                                           | Gain/loss in bwt (g) | 1.60 $\pm$ 0.34 <sup>a</sup>  | 1.30 $\pm$ 0.16 <sup>a</sup>   | 0.80 $\pm$ 0.32 <sup>b</sup>   | 0.24 $\pm$ 0.15 <sup>c</sup>     | 26.535***  |
|                                           | FCR                  | 38.24 $\pm$ 8.12 <sup>a</sup> | 44.76 $\pm$ 5.51 <sup>a</sup>  | 88.67 $\pm$ 57.47 <sup>a</sup> | 307.26 $\pm$ 163.92 <sup>b</sup> | 10.658***  |
| 0.20                                      | Body wt (g)          | 36.08 $\pm$ 0.53 <sup>a</sup> | 35.00 $\pm$ 0.59 <sup>b</sup>  | 34.38 $\pm$ 0.64 <sup>c</sup>  | 34.12 $\pm$ 0.68 <sup>d</sup>    | 10.165***  |
|                                           | Feed intake (g)      | 60.50 $\pm$ 0.00 <sup>a</sup> | 58.60 $\pm$ 0.00 <sup>b</sup>  | 56.50 $\pm$ 0.00 <sup>c</sup>  | 56.20 $\pm$ 0.00 <sup>d</sup>    | 2E+031***  |
|                                           | Gain/loss in bwt (g) | 1.78 $\pm$ 0.13 <sup>a</sup>  | 1.08 $\pm$ 0.24 <sup>b</sup>   | 0.62 $\pm$ 0.13 <sup>c</sup>   | 0.26 $\pm$ 0.13 <sup>d</sup>     | 78.893***  |
|                                           | FCR                  | 34.13 $\pm$ 2.57 <sup>a</sup> | 54.26 $\pm$ 16.26 <sup>a</sup> | 94.30 $\pm$ 19.00 <sup>a</sup> | 281.00 $\pm$ 172.08 <sup>b</sup> | 8.423***   |
| 0.25                                      | Body wt (g)          | 37.30 $\pm$ 0.19 <sup>a</sup> | 36.06 $\pm$ 0.21 <sup>b</sup>  | 35.24 $\pm$ 0.24 <sup>c</sup>  | 34.44 $\pm$ 0.40 <sup>d</sup>    | 101.460*** |
|                                           | Feed intake (g)      | 59.20 $\pm$ 0.00 <sup>a</sup> | 58.60 $\pm$ 0.00 <sup>b</sup>  | 57.90 $\pm$ 0.00 <sup>c</sup>  | 57.40 $\pm$ 0.00 <sup>d</sup>    | 2E+030***  |
|                                           | Gain/loss in bwt (g) | 1.38 $\pm$ 0.11 <sup>a</sup>  | 1.24 $\pm$ 0.09 <sup>a</sup>   | 0.82 $\pm$ 0.08 <sup>b</sup>   | 0.80 $\pm$ 0.38 <sup>c</sup>     | 10.078***  |
|                                           | FCR                  | 43.11 $\pm$ 3.32 <sup>a</sup> | 47.47 $\pm$ 3.63 <sup>a</sup>  | 71.23 $\pm$ 7.58 <sup>a</sup>  | 90.16 $\pm$ 49.04 <sup>b</sup>   | 3.866***   |

All F-values are at 3, 16 df; bwt= body weight; \*\*\*=  $P<0.001$ ; FCR=Total feed consumed  $\div$  final weight-initial weight; superscripts in dissimilar letters in each row differ significantly by LSD tests at  $P<0.05$ .

### 4.3 Weights of Various Organs

Effects of cypermethrin on the organ weight of normal healthy mice and treated mice are presented in Table 5. Weights of all vital organs, viz., brain, lungs, heart, liver, kidneys,

viscera and gonad were determined on the day of sacrifice. Compared to the control and the CYP-treated mice, the organ weights of the CYP-treated mice were reduced significantly ( $p < 0.001$ ) (Fig. 3-9).

**Table 6:** Effect of cypermethrin treatments on different organ weights of control and treatment group in the albino mice *Mus musculus* L.

| Organs  | Animal Groups           |                         |                         |                         |                         | F-values  |
|---------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-----------|
|         | Group-I                 | Group-II                | Group-III               | Group-IV                | Group-V                 |           |
| Brain   | 0.30± 0.07 <sup>a</sup> | 0.18± 0.04 <sup>b</sup> | 0.30± 0.07 <sup>c</sup> | 0.20± 0.05 <sup>d</sup> | 0.22± 0.04 <sup>e</sup> | 5.699***  |
| Liver   | 2.60± 0.07 <sup>a</sup> | 2.42± 0.08 <sup>b</sup> | 2.50± 0.07 <sup>a</sup> | 2.42± 0.10 <sup>c</sup> | 2.36± 0.05 <sup>d</sup> | 5.625***  |
| Kidney  | 0.76± 0.05 <sup>a</sup> | 0.56± 0.08 <sup>b</sup> | 0.58± 0.04 <sup>c</sup> | 0.64± 0.09 <sup>d</sup> | 0.54± 0.05 <sup>e</sup> | 6.283***  |
| Lung    | 0.42± 0.04 <sup>a</sup> | 0.22± 0.04 <sup>b</sup> | 0.26± 0.09 <sup>c</sup> | 0.28± 0.08 <sup>d</sup> | 0.40± 0.07 <sup>a</sup> | 7.035***  |
| Heart   | 0.34± 0.05 <sup>a</sup> | 0.18± 0.04 <sup>b</sup> | 0.16± 0.05 <sup>c</sup> | 0.16± 0.05 <sup>d</sup> | 0.16± 0.05 <sup>e</sup> | 8.009***  |
| Viscera | 3.16± 0.18 <sup>a</sup> | 2.50± 0.07 <sup>b</sup> | 2.46± 0.05 <sup>c</sup> | 2.96± 0.05 <sup>d</sup> | 2.48± 0.08 <sup>e</sup> | 61.222*** |
| Gonad   | 0.34± 0.05 <sup>a</sup> | 0.18± 0.04 <sup>b</sup> | 0.18± 0.04 <sup>c</sup> | 0.18± 0.04 <sup>d</sup> | 0.16± 0.05 <sup>e</sup> | 10.696*** |

All F-values are at 4, 19 df; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ ; superscripts in dissimilar letters in each column differ significantly by LSD tests at  $P < 0.05$ .

**a) Brain Weight:** Mean values with SD of brain weight of mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in Table 6 and Fig. 5. In the control group, the brain weight was 0.30± 0.07g. On the other hand, the brain weights in cypermethrin-treated mice were 0.18± 0.04g, 0.30± 0.07g, 0.20± 0.05g and 0.22± 0.04g at 0.10, 0.15, 0.20 and 0.25 µL/kg body weights respectively, thus causing a significant decrease in this organ weight ( $F_{4,19} = 5.699$ ;  $P < 0.001$ ).

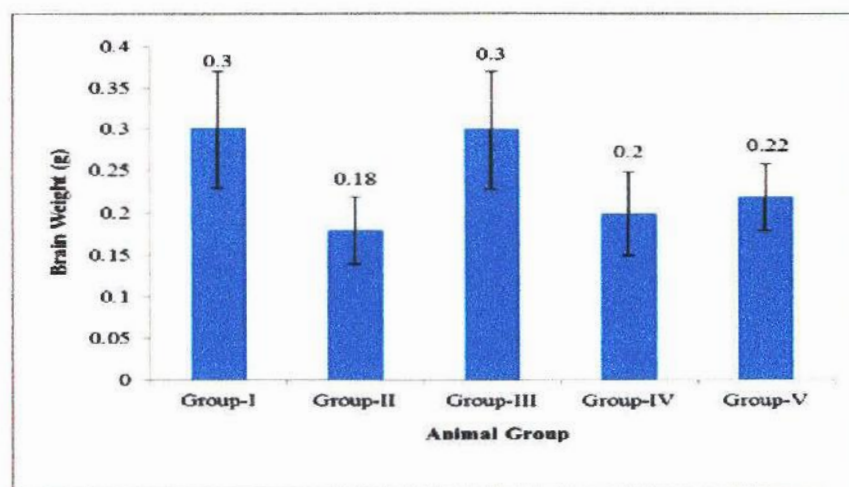


Fig. 5 Comparison of brain weight in different treatment groups of albino mice *Mus musculus*

**b) Liver Weight:** Effects of cypermethrin on the on the liver weight with mean values and SD of mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 6** and **Fig 6**. In the control group, the liver weight was  $2.60 \pm 0.07$ g. On the other hand, the liver weights in cypermethrin-treated mice were  $2.42 \pm 0.08$ g,  $2.50 \pm 0.07$ g,  $2.42 \pm 0.10$ g and  $2.36 \pm 0.05$ g at 0.10, 0.15, 0.20 and 0.25  $\mu$ L/kg body weights respectively, thus causing a significant decrease in this organ ( $F_{4,19} = 5.625$ ;  $P < 0.001$ ) (**Fig. 6**).

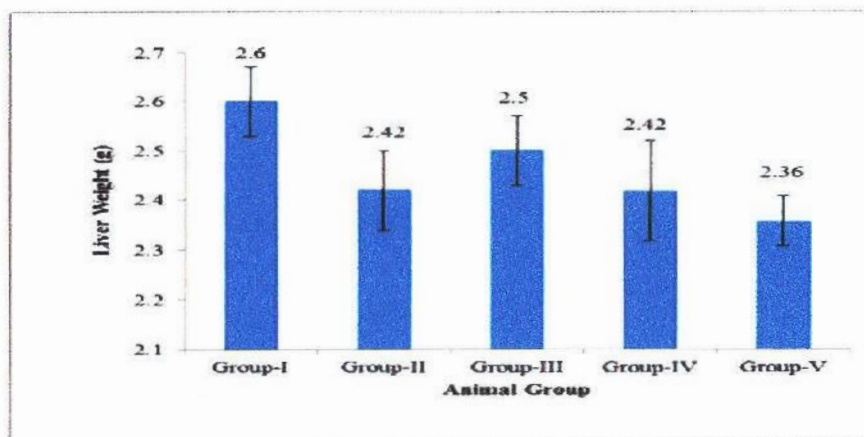


Fig. 6 Variation in the liver weight in the experimental albino mice where Group I refers to the control and Groups II-V refer to the treatment groups.

**c) Kidney Weight:** Mean values with SD of kidney weight of mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been

shown in **Table 6** and **Fig. 7**. In the control group, the kidney weight was  $0.76 \pm 0.05\text{g}$ . On the other hand, the kidney weights in cypermethrin-treated mice were  $0.56 \pm 0.08\text{g}$ ,  $0.58 \pm 0.04\text{g}$ ,  $0.64 \pm 0.09\text{g}$  and  $0.54 \pm 0.05\text{g}$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,19} = 6.283$ ;  $P < 0.001$ ).

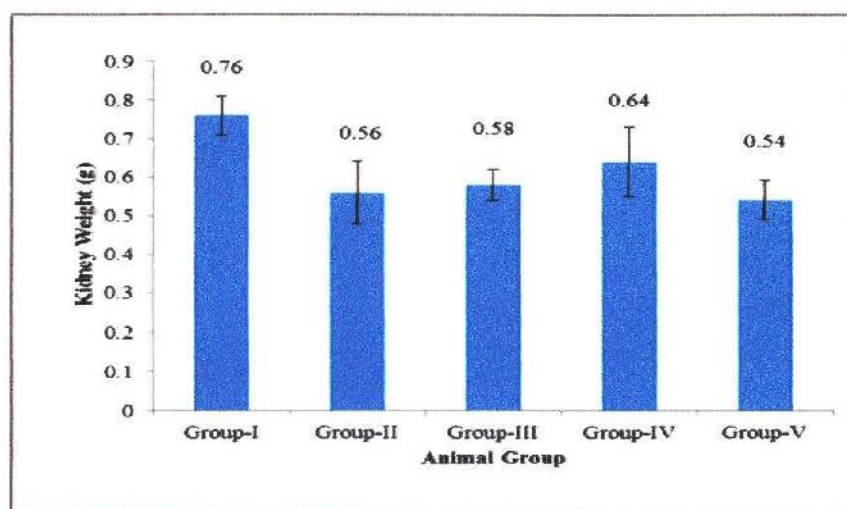


**Fig 7.** Variation in the kidney weight in the experimental albino mice where Group I refer to the control and Groups II-V refers to the treatment groups.

**d) Lung Weight:** Mean values with SD of lung weight of mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 6** and **Fig. 8**. In the control group, the lung weight was  $0.42 \pm 0.04\text{g}$ . On the other hand, the lung weights in cypermethrin-treated mice were  $0.22 \pm 0.04\text{g}$ ,  $0.26 \pm 0.09\text{g}$ ,  $0.28 \pm 0.08\text{g}$  and  $0.40 \pm 0.07\text{g}$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively. As seen in Table 4, when the mice were treated with different dosages of cypermethrin separately, they exhibited a significant persistent decrease ( $F_{4,19} = 7.035$ ;  $P < 0.001$ ) in lung weight, when compared to the controls.

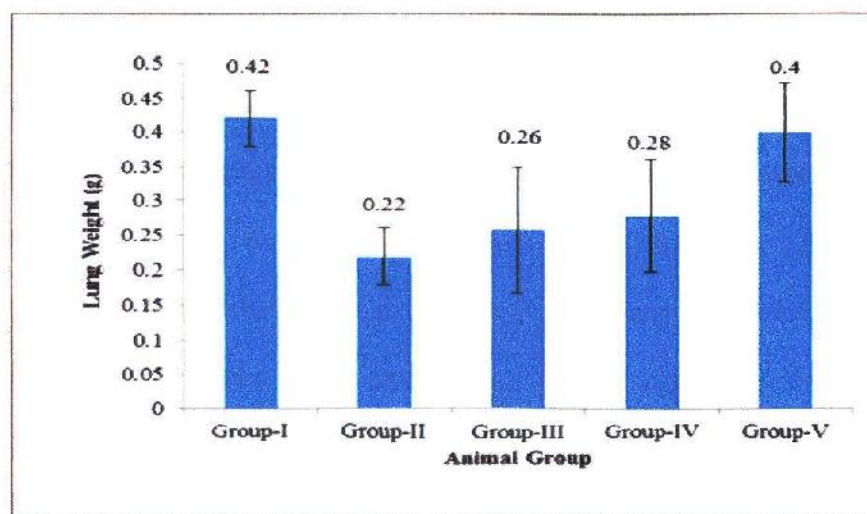


Fig. 8 Variation in the lung weight in the experimental albino mice where Group I refers to the control and Groups II-V refer to the treatment groups.

**e) Heart Weight:** Mean values with SD of heart weight of mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 6** and **Fig. 9**. In the control group, the heart weight was  $0.34 \pm 0.05$ g. On the other hand, the heart weights in cypermethrin-treated mice were  $0.18 \pm 0.04$ g,  $0.16 \pm 0.05$ g,  $0.16 \pm 0.05$ g and  $0.16 \pm 0.05$ g at 0.10, 0.15, 0.20 and 0.25  $\mu$ L/kg body weights respectively. As seen in Table 4, when the mice were treated with different dosages of cypermethrin separately, they exhibited a significant persistent decrease ( $F_{4,19} = 8.009$ ;  $P < 0.001$ ) in heart weight, when compared to the controls.

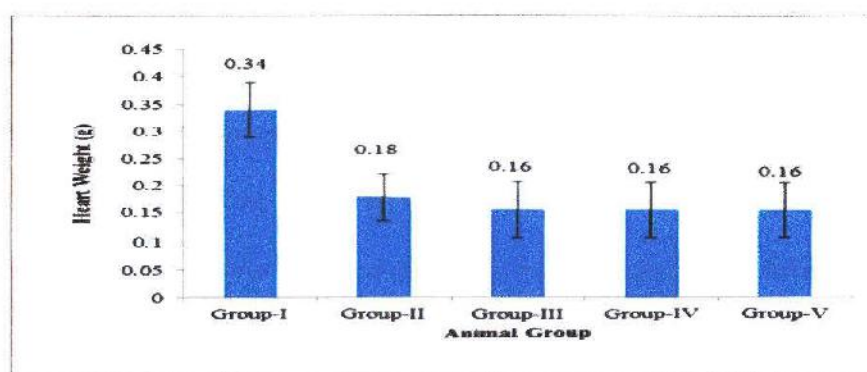


Fig. 9 Variation in the liver weight in the experimental albino mice where Group I refers to the control and Groups II-V refer to the treatment groups.

**Table 7:** Effect of cypermethrin treatments on somatic index (SI) in the albino mice *Mus musculus* L.

| Organs  | SI in Animal Groups      |                          |                          |                          |                           | F-Values            |
|---------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------|
|         | Group-I                  | Group-II                 | Group-III                | Group-IV                 | Group-V                   |                     |
| Brain   | 0.7756±0.18 <sup>a</sup> | 0.6821±0.17 <sup>a</sup> | 1.0763±0.25 <sup>b</sup> | 0.4665±0.1 <sup>c</sup>  | 0.6392± 0.13 <sup>a</sup> | 7.732***            |
| Liver   | 6.7333±0.2 <sup>a</sup>  | 9.1864±0.16 <sup>b</sup> | 8.9735±0.26 <sup>c</sup> | 7.0929±0.3 <sup>d</sup>  | 6.8540± 0.21 <sup>a</sup> | 132.178***          |
| Kidney  | 1.9156±0.1 <sup>a</sup>  | 2.1257±0.34 <sup>a</sup> | 2.0814±0.15 <sup>a</sup> | 1.8740±0.2 <sup>a</sup>  | 1.5674± 0.15 <sup>b</sup> | 5.194***            |
| Lung    | 1.0872±0.1 <sup>a</sup>  | 0.8362±0.18 <sup>a</sup> | 0.9329±0.32 <sup>a</sup> | 0.8188±0.2 <sup>a</sup>  | 1.1620± 0.21 <sup>a</sup> | 2.366 <sup>NS</sup> |
| Heart   | .8809± 0.15 <sup>a</sup> | .6830± 0.17 <sup>a</sup> | 0.5744±0.20 <sup>b</sup> | .4704± 0.17 <sup>c</sup> | .4654± 0.16 <sup>d</sup>  | 5.289***            |
| Viscera | 8.1827±0.4 <sup>a</sup>  | 9.4926±0.23 <sup>b</sup> | 8.8296±0.18 <sup>c</sup> | 8.6755±0.0 <sup>d</sup>  | 7.2019± 0.27 <sup>e</sup> | 47.559***           |
| Gonad   | .8809± 0.15 <sup>a</sup> | .6830± 0.17 <sup>a</sup> | 0.6456±0.16 <sup>b</sup> | .5300± 0.13 <sup>c</sup> | .4653± 0.16 <sup>d</sup>  | 5.382***            |

All F-values are at 4, 20 df; \*\*\*= P<0.001; NS= Not-Significant; SI (somatic index) = weight of the organ in g ÷ weight of the body in g ×100; superscripts in dissimilar letters in each column differ significantly by LSD tests at P<0.05.

#### 4.5 Histopathological Observations

Toxic effects of cypermethrin pesticide on the liver and kidney were observed in this study. Sections from the liver and kidney were prepared from both control and treated mice and were examined under the light microscope. The microscopic observations were reported for liver and kidney.

**a) Liver:** Sections of control mice liver revealed that the hepatocytes arranged in strands with one or two spherical nuclei and eosinophilic cytoplasm. The sinusoids are occupied by Kupffer cells (**Plate 8a**). Examination of liver of CYP-treated mice displayed apparent signs of degenerative changes. The normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost. In addition, severe inflammatory leucocytic infiltrations were abundant. Such inflammatory

infiltration is spread over several liver areas and around the blood vessels (**Plate 8b**).

Enlargement and congestion of blood vessels, especially veins were observed. The hepatocytes were clearly manifested by marked cytoplasmic vacuolization (**Plate 8c**) and most cells showed nuclei with signs of karyolysis and pyknosis.

After 28 days of treatment with cypermethrin histopathological examination in liver revealed nucleus to be normal but with congested sinusoidal spaces (**Plate 8a**). High dose treated liver showed increased sinusoidal spaces, condensed nuclei and enlarged hepatocytes with hydropic degeneration together with cytoplasmic vacuolization (**Plate 8**).

Nuclear hypertrophy and binucleated hepatocytes were observed at some places. The liver sections from treated mice showed moderate changes when compared with those from the control mice (**Plate 8a**). These changes include the presence of more endothelial cells scattered among the hepatocytes and some vacuolation in the liver texture (**Plate8b**), showed rupture in some hepatocytes and dark stained hepatocytic nuclei indicating cell pycnosis (**Plate 8b, c, d and e**). Some hepatocytes have shrunk nuclei (**Plate 8d**).

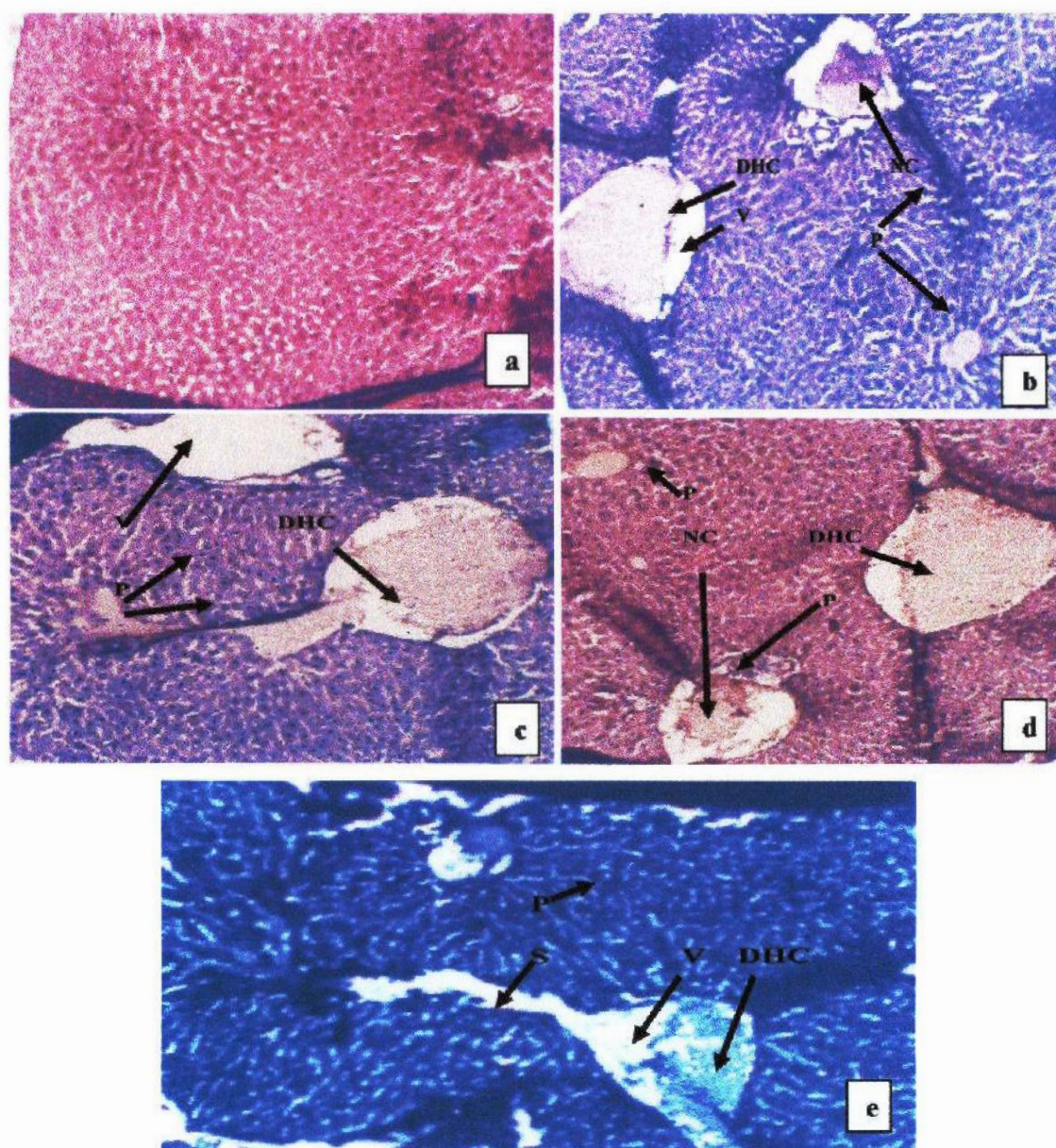


**Plate 8** Histological sections of liver of the control and treated albino mice. Histological sections showing normal appearance of liver of control group. Treated group showing necrosis of hepatic cells with pyknotic nuclei, disorganization of hepatic laminae and dilation of sinusoids: (a) a normal histological structure of liver and (b) (0.10 ml CYP/ 20g of bw). (c) (0.15 ml CYP/ 20g of bw). (d) (0.20 ml CYP/ 20g of bw). (e) (0.25 ml CYP/ 20g of bw). (a, b, c, d & e: were stained with heamatoxylin and eosin). (DHC: Degenerated hepatic cells; P: pyknotic nuclei; S: sinusoids; V: Vacuole; Nc: necrotic cells). Original magnification  $\times 200$ .

**b) Kidney:** Kidneys of all the animals of the control as well as the experimental groups were paired structures located on the posterior abdominal wall, one on either side of the midline. The kidneys were reddish brown in colour and were covered by a thin glistening capsule, which was not adherent to the surrounding tissues.

Sections from the kidneys of mice treated with cypermethrin as well as those from the controls were also examined (**Plate 9a, b, c, d and e**). Control group showed normal renal architecture. The histological changes observed in the kidneys after damage by toxins include changes in the tubules and in the interstitium. There is necrosis predominantly in the proximal tubules. **Plate 9a** showed that kidney cortex consists of renal corpuscles and tubules. Renal corpuscles consist of Bowman 'capsule with double membrane with urinary space in between. Also, a tuft of glomerular capillaries is enclosed in Bowman's capsule. Renal tubules are of two types, proximal and distal tubules. Proximal tubules lined with low columnar epithelium and have narrow lumen. Distal tubules possess wide lumen and lined by cuboidal epithelial. Even the kidneys showed marked hemorrhage and congestion in cypermethrin-treated animals as compared to normal histological examination of renal tissue in control mice. Animals treated with cypermethrin showed degeneration and deterioration of the cortical constituents. The epithelial lining of the renal tubules appeared with cloudy swelling and vacuolated cytoplasm with pyknotic nuclei. Intertubular leucocytic infiltrations were observed (**Plate 9b, c, d and e**). A number of glomerular capillaries were suffering from severe signs of glomerular congestion, while others were completely damaged (**Plate 9 b, c, d and e**).

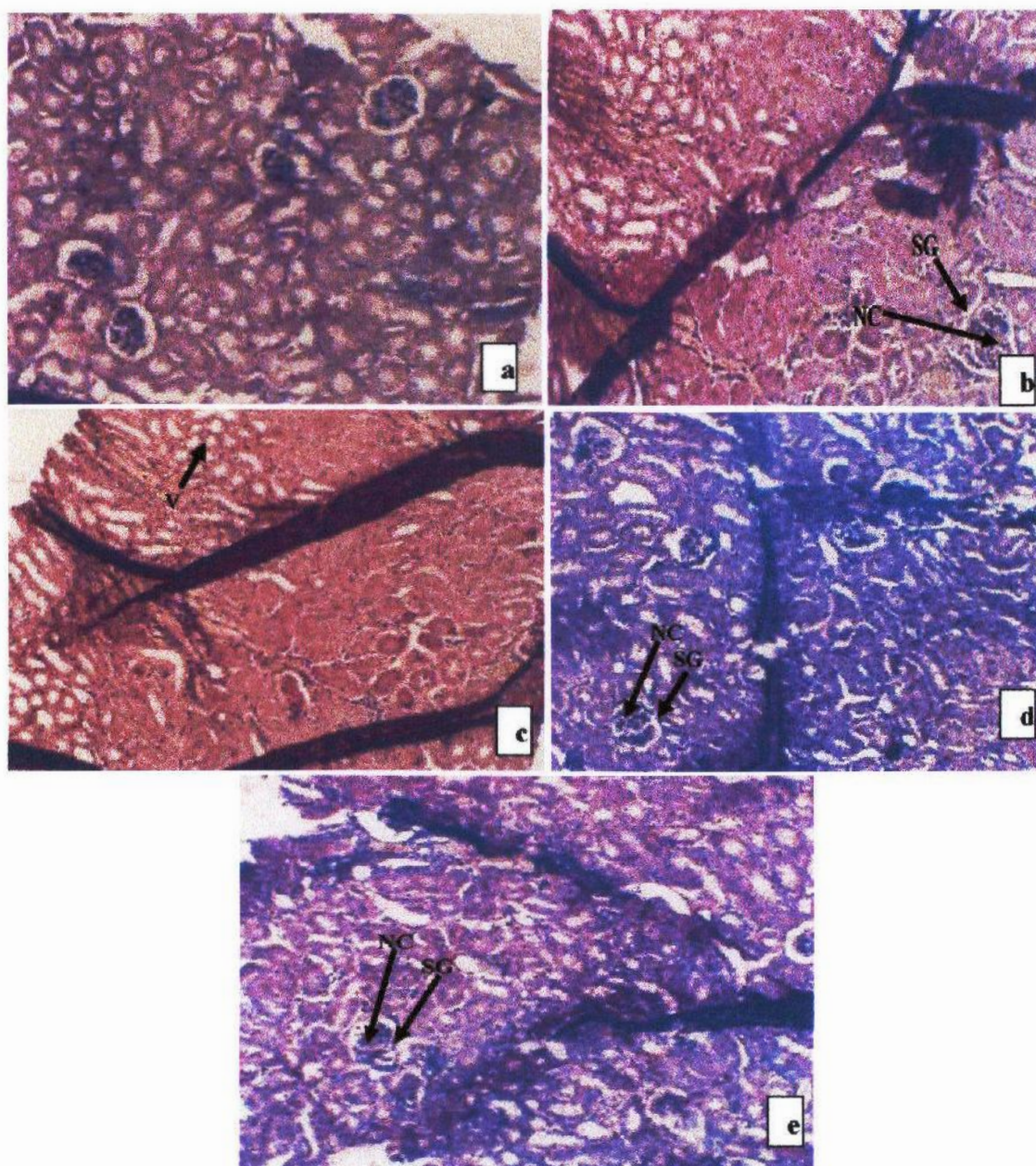


**Plate 9** Histological sections of kidney of treated and control mice. Histological section showing normal appearance of kidney of control group. Treated group showing shrinkage of glomeruli, necrosis and disruption of renal tubules (a) a normal histological structure of kidney and (b) (0.10 ml CYP/ 20g of bw). (c) (0.15 ml CYP / 20g of bw). (d) (0.20 ml CYP / 20g of bw). (e) (0.25 ml CYP / 20g of bw). (a, b, c, d & e: were stained with Hematoxylin & Eosin). (NC = necrosis; SG = shrinkage of glomeruli; V: Vacuole). Original magnification  $\times 200$ .

#### 4.6 Haematological Parameters

Total counts on erythrocytes; total and differential counts on leucocytes and level of haemoglobin determine the health situation of an animal. Treatment was accomplished through intraperitoneal route by injecting cypermethrin to albino mice. On the day of sacrifice of mice blood was collected to estimate the haematological parameters of control and treated mice.

Hematological parameters showed significant deviations from those of the control and treated groups (Table 8). CYP decreased the numbers of RBC ( $p<0.001$ ), WBC ( $p<0.001$ ), Platelets ( $p<0.001$ ) and hemoglobin ( $p<0.001$ ), but increased those of lymphocytes ( $p<0.001$ ), neutrophils ( $p<0.001$ ), monocytes ( $p<0.001$ ) and ESR ( $p<0.001$ ).

**Table 8:** Effects of Cypermethrin on Haematological profile of control and treatment groups of albino mice *Mus musculus* L. (Mean  $\pm$  SD).

| Haematological Parameters                            | Animal Groups                 |                               |                               |                               |                               | F-values    |
|------------------------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------|
|                                                      | Group-I                       | Group-II                      | Group-III                     | Group-IV                      | Group-V                       |             |
| R.B.C<br>( $\times 10^6$ Cells/mm <sup>3</sup> )     | 5.59 $\pm$ 0.24 <sup>a</sup>  | 3.74 $\pm$ 0.21 <sup>b</sup>  | 3.93 $\pm$ 0.11 <sup>c</sup>  | 2.71 $\pm$ 0.32 <sup>d</sup>  | 2.85 $\pm$ 0.08 <sup>c</sup>  | 149.837***  |
| W.B.C<br>( $\times 10^3$ Cells/mm <sup>3</sup> )     | 15.56 $\pm$ 0.38 <sup>a</sup> | 15.51 $\pm$ 0.27 <sup>a</sup> | 12.53 $\pm$ 0.20 <sup>b</sup> | 11.5 $\pm$ 0.30 <sup>c</sup>  | 15.80 $\pm$ 0.03 <sup>a</sup> | 283.763***  |
| Platelets<br>( $\times 10^4$ Cells/mm <sup>3</sup> ) | 93.16 $\pm$ 0.48 <sup>a</sup> | 73.37 $\pm$ 0.51 <sup>b</sup> | 72.80 $\pm$ 0.22 <sup>c</sup> | 73.48 $\pm$ 0.30 <sup>d</sup> | 72.92 $\pm$ 0.91 <sup>e</sup> | 1384.936*** |
| Neutrophils (%)                                      | 17.66 $\pm$ 0.22 <sup>a</sup> | 13.40 $\pm$ 0.32 <sup>b</sup> | 12.76 $\pm$ 0.48 <sup>c</sup> | 12.22 $\pm$ 0.08 <sup>d</sup> | 8.67 $\pm$ 0.10 <sup>c</sup>  | 808.488***  |
| Eosinophils (%)                                      | 1.06 $\pm$ 0.09 <sup>a</sup>  | 1.06 $\pm$ 0.09 <sup>a</sup>  | 1.16 $\pm$ 0.21 <sup>a</sup>  | 1.24 $\pm$ 0.15 <sup>b</sup>  | 0.78 $\pm$ 0.08 <sup>a</sup>  | 9.543***    |
| Lymphocytes (%)                                      | 74.00 $\pm$ 0.38 <sup>a</sup> | 84.25 $\pm$ 0.24 <sup>b</sup> | 84.90 $\pm$ 0.18 <sup>c</sup> | 85.05 $\pm$ 0.21 <sup>d</sup> | 90.38 $\pm$ 0.26 <sup>e</sup> | 2649.693*** |
| Monocytes (%)                                        | 4.44 $\pm$ 0.36 <sup>a</sup>  | 1.18 $\pm$ 0.15 <sup>b</sup>  | 1.24 $\pm$ 0.05 <sup>c</sup>  | 1.10 $\pm$ 0.34 <sup>d</sup>  | 0.84 $\pm$ 0.23 <sup>c</sup>  | 176.620***  |
| Basophils (%)                                        | 1.24 $\pm$ 0.25 <sup>a</sup>  | 0.68 $\pm$ 0.20 <sup>b</sup>  | 0.76 $\pm$ 0.18 <sup>c</sup>  | 0.94 $\pm$ 0.17 <sup>a</sup>  | 0.98 $\pm$ 0.18 <sup>a</sup>  | 5.985***    |
| Hb (g/dl)                                            | 15.80 $\pm$ 0.33 <sup>a</sup> | 12.26 $\pm$ 0.13 <sup>b</sup> | 12.52 $\pm$ 0.24 <sup>c</sup> | 11.54 $\pm$ 0.25 <sup>d</sup> | 10.56 $\pm$ 0.30 <sup>e</sup> | 290.676***  |
| ESR (mm/hr)                                          | 3.72 $\pm$ 0.22 <sup>a</sup>  | 4.59 $\pm$ 0.16 <sup>b</sup>  | 4.40 $\pm$ 0.22 <sup>c</sup>  | 3.30 $\pm$ 0.12 <sup>d</sup>  | 2.89 $\pm$ 0.11 <sup>c</sup>  | 85.839***   |

All F-values are at 4, 20 df; \*\*=  $P<0.01$ ; \*\*\*=  $P<0.001$ ; superscripts in dissimilar letters in each column differ significantly by LSD tests at  $P<0.05$ .

**a) RBC:** Effects of cypermethrin on the RBC count of control and albino mice poisoned with cypermethrin are shown in Table 8 (Fig. 12). In the control group, RBC count was 5.59

$\pm 0.24$ . On the other hand, the RBC count in cypermethrin-treated mice were  $27.32 \pm 1.23$ ,  $3.74 \pm 0.21$ ,  $3.93 \pm 0.11$ ,  $2.71 \pm 0.32$  and  $2.85 \pm 0.08$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively. The total count of RBC of mice poisoned with cypermethrin significantly decreases ( $F_{4,20} = 149.837$ ;  $p < 0.001$ ) compared to their control groups (Fig. 12).

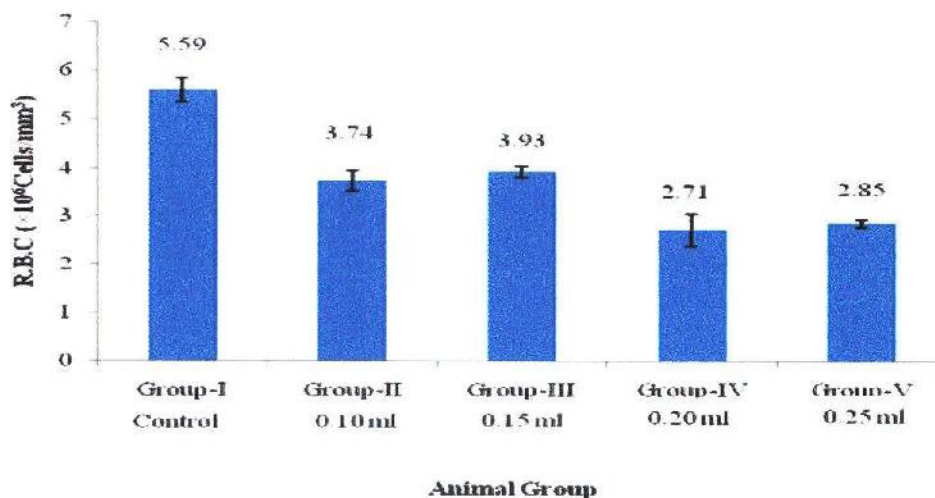


Fig. 12 Bar diagram showing variation of RBC counts in the control and treated mice *Mus musculus* L.

**b) WBC:** Mean values with SD of WBC of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in Table 8 and Fig. 13. In the present experimental mice, decrease in the number of leukocytes (WBC - white blood cells) in peripheral blood occurred on 28 days after administration of cypermethrin, compared to their control groups. Cypermethrin poisoning in animals, resulted in a statistically decrease ( $F_{4,20} = 283.763$ ;  $p < 0.001$ ) (Table 7) in the number of leukocytes in peripheral blood, compared to the control groups and animals poisoned with the lower dose of the pesticide.

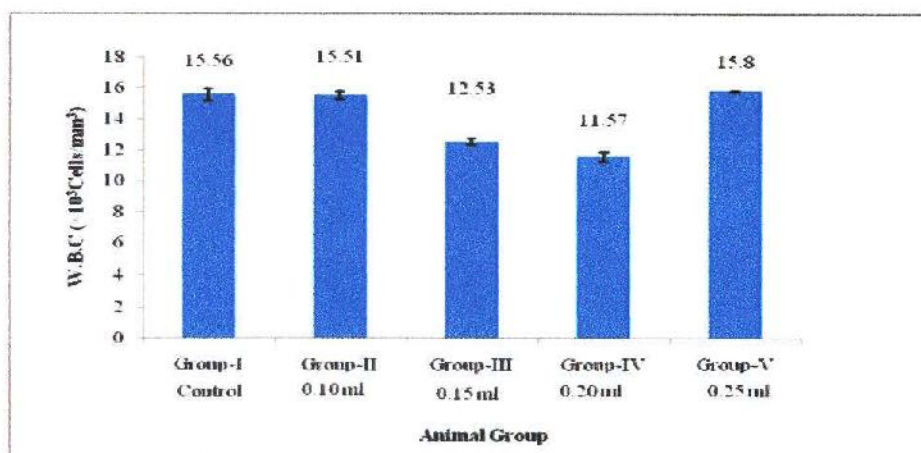


**Fig. 13** Bar diagram showing variation in WBC counts in the control and treated mice *Mus musculus* L

**c) Platelets:** Mean values with SD of total platelets count of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 14**. Statistically significant ( $F_{4,20} = 1384.936$ ;  $p < 0.001$ ) decreases of total platelets of mice poisoned with cypermethrin compared to the level noted in the control groups (**Table 7**). Platelets counts was statistically decrease after treatment with cypermethrin and accounted to  $(72.80 \pm 0.22)$  compared to control value  $(93.16 \pm 0.48)$ .

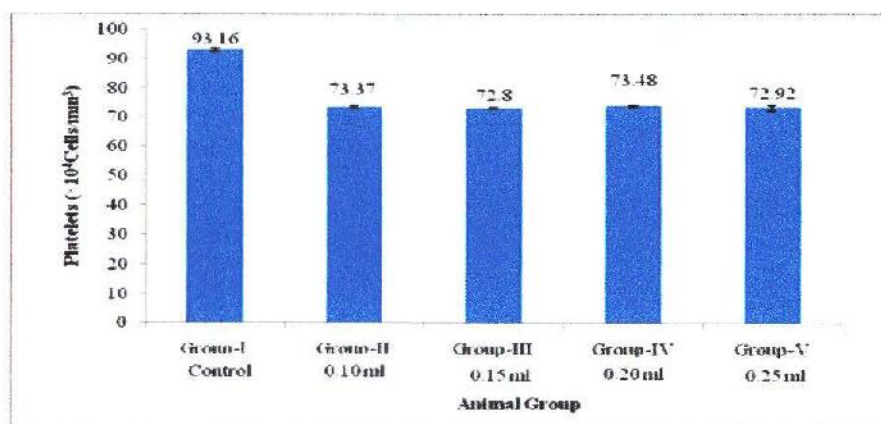


**Fig. 14** Variation in total platelets counts in the control and treated albino mice *Mus musculus* L.

#### d) Differential Leucocyte Count (DLC)

**Neutrophils:** Mean values of neutrophils percentage of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 15**. Neutrophils counts was statistically decrease ( $F_{4,20} = 808.488$ ;  $P < 0.001$ ) after treatment with cypermethrin and accounted to ( $8.67 \pm 0.10$ ) compared to their control value ( $17.66 \pm 0.22$ ).

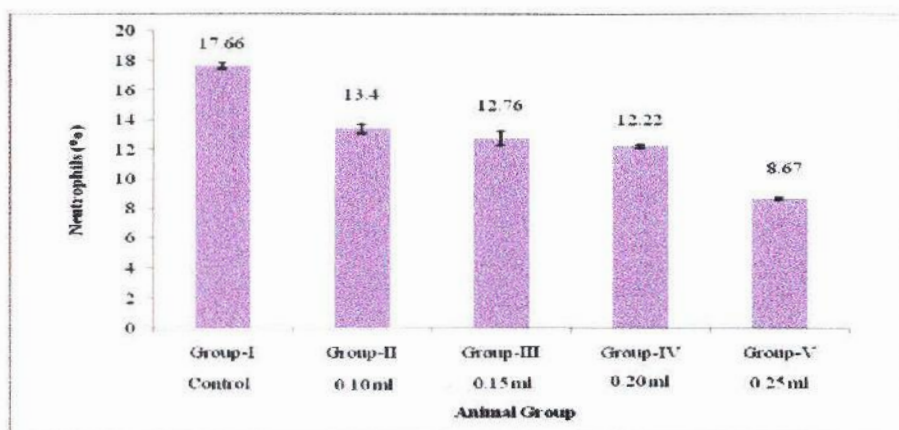


**Fig. 15** Bar diagram showing neutrophils percentage of control and treated mice *Mus musculus* L.

**Eosinophils:** Mean values with SD of Eosinophils percentage of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 16**. As seen in **Table 7**, when the mice were treated with different dosages of cypermethrin separately, they exhibited a significant persistent decrease ( $F_{4,20} = 9.543$ ;  $P < 0.001$ ) in eosinophils percentage, when compared to the controls. Eosinophils counts was statistically decrease ( $p < 0.001$ ) after treatment with cypermethrin and accounted to ( $0.78 \pm 0.08$ ) compared to control value ( $1.06 \pm 0.21$ ).

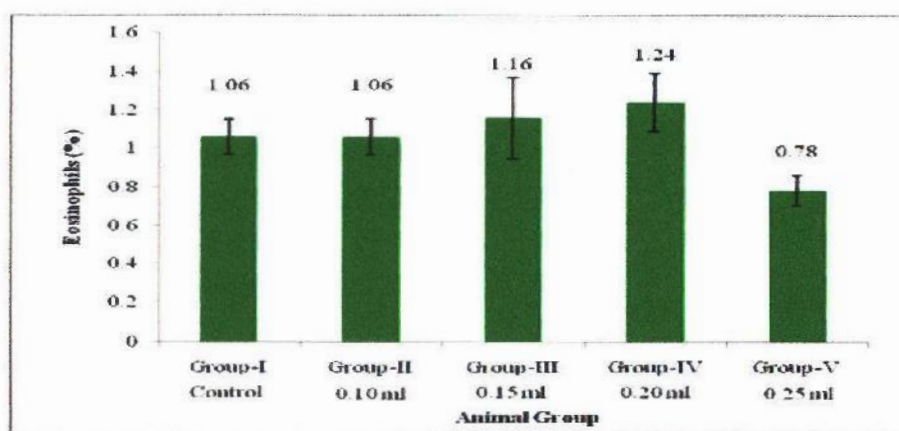


**Fig. 16** Bar diagram showing eosinophil percentage in the control and treated mice *Mus musculus* L.

**Lymphocytes:** Mean values with SD of Lymphocyte percentage of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 17**. From Table 6 we can see statistically significant ( $F_{4,20} = 2649.693$ ;  $P < 0.001$ ) differences in the percentage of lymphocytes in peripheral blood of mice poisoned with cypermethrin compared to the level noted in the control groups. The total count of WBC were slightly decreases but in case of differential count of WBC, number of eosinophils increased and the number of lymphocytes were very high (**Table 8**). Lymphocytes counts was statistically significant increases ( $F_{4,20} = 2649.693$ ;  $p < 0.001$ ) after treatment with cypermethrin and accounted to  $(90.38 \pm 0.26)$  compared to control value  $(74.00 \pm 0.38)$ .

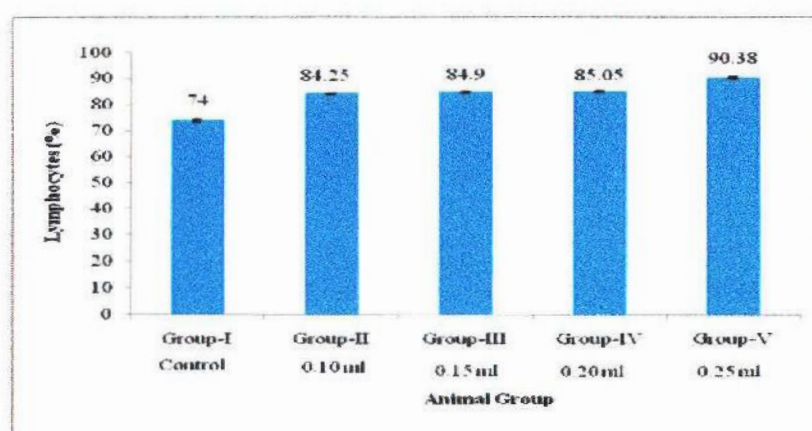


**Fig. 17** Bar diagram showing lymphocytes percentage of control and treated mice *Mus musculus* L.

**Monocytes:** Mean values with SD of Monocytes percentage of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 18**. From the **Table 8**, statistically significant ( $F_{4,20} = 176.620$ ;  $p < 0.001$ ) differences were noted in the percentage of monocytes in peripheral blood of mice poisoned with cypermethrin compared to the level noted in the control groups. Monocytes counts was statistically decrease ( $F_{4,20} = 176.620$ ;  $p < 0.001$ ) after treatment with cypermethrin and accounted to ( $0.84 \pm 0.23$ ) compared to control value ( $4.44 \pm 0.36$ ).

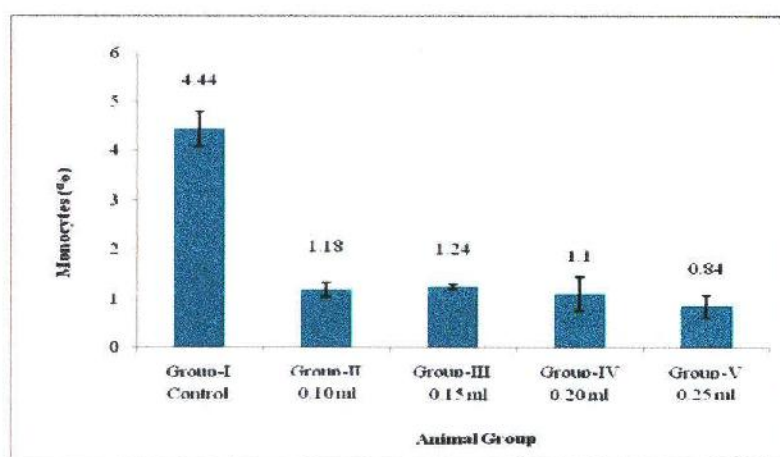


**Fig. 18** Bar diagram showing monocytes percentage of control and treated mice *Mus musculus* L.

**Basophils:** Basophils percentage of blood in mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 19**. As shown in **Table 8**, statistically significant ( $F_{4,20} = 5.985$ ;  $p < 0.001$ ) differences were noted in the percentage of basophils in peripheral blood of mice poisoned with cypermethrin compared to the control groups. In the control group, the basophils percentage was  $1.24 \pm 0.25$ . On the other hand, the basophils percentage in cypermethrin-treated mice were  $0.68 \pm 0.20$ ,  $0.76 \pm 0.18$ ,  $0.94 \pm 0.17$  and  $0.98 \pm 0.18$  at 0.10, 0.15, 0.20 and 0.25  $\mu\text{L/kg}$  body weights respectively, thus causing a significant decrease in this morphometric parameter ( $F_{4,95} = 5.985$ ;  $P < 0.001$ ).

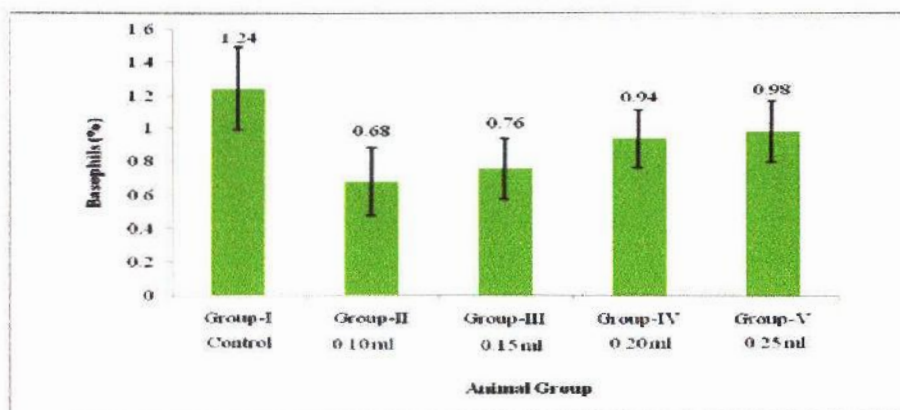


**Figure-19:** Bar diagram showing basophils percentage of control and treated mice *Mus musculus* L.

**e) Haemoglobin** In the case of cypermethrin administration, significant differences in the level of hemoglobin were observed between the control and experimental groups in albino mice. The mean values of haemoglobin content of blood in albino mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig. 20**. In the control group, the haemoglobin content was  $15.80 \pm 0.33$ g/dl. On the other hand, the haemoglobin content in cypermethrin-treated mice were  $12.26 \pm 0.13$ g/dl,  $12.52 \pm 0.24$ g/dl,  $11.54 \pm 0.25$ g/dl and  $10.56 \pm 0.30$ g/dl at 0.10, 0.15, 0.20 and 0.25  $\mu$ L/kg body weights respectively. Haemoglobin counts was statistically decrease ( $F_{4,20} = 290.676$ ;  $P < 0.001$ ) after treatment with cypermethrin and accounted to  $(10.56 \pm 0.30)$  compared to control value .

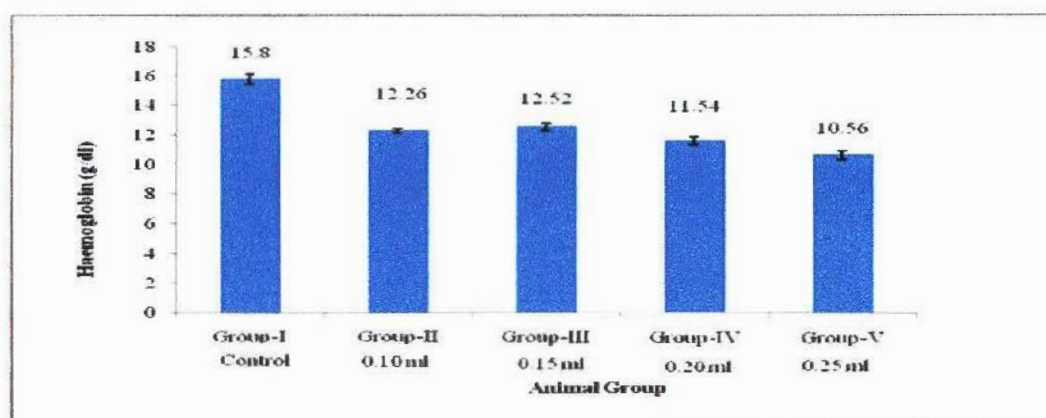


**Fig. 20** Bar diagram showing haemoglobin concentration of control and treated mice *Mus musculus* L.

**f) Erythrocyte Sedimentation Rate (ESR):** Mean values of ESR of blood in albino mice of group I (control) and treatment groups on day 29 following four weeks administration of cypermethrin have been shown in **Table 8** and **Fig 21**. In the case of cypermethrin administration, significant differences ( $F_{4,20} = 85.839$ ;  $P < 0.001$ ) in the level of ESR were observed between the control and experimental groups in albino mice. In treatment group II and III, the ESR level increased significantly ( $F_{4,20} = 85.839$ ;  $p < 0.001$ ) compared to the control group. In treatment group IV and V, the ESR level decreased significantly ( $F_{4,20} = 85.839$ ;  $p < 0.001$ ) compared to the control group. ESR counts was statistically decrease ( $p < 0.001$ ) after treatment with cypermethrin and accounted to ( $2.89 \pm 0.11$ ) compared to control value ( $3.72 \pm 0.22$ ).

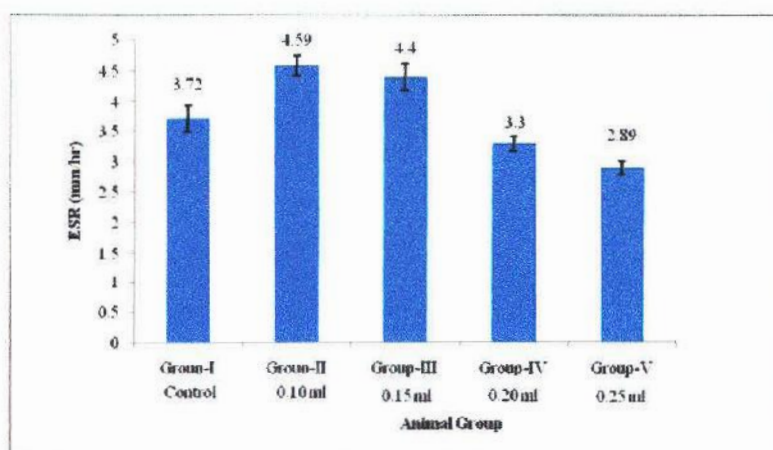


**Fig 21** Bar diagram showing variation in ESR in the control and treated albino mice *Mus musculus* L.

## Discussion

### 4.7 Cypermethrin Toxicity

In the present study, intraperitoneal administration of cypermethrin over a period of four consecutive weeks showed a number of effects which are crystallized in the following paragraphs.

### 4.8 Clinical and Behavioural Symptoms

Cypermethrin has moderate effects on clinical and behavioural signs in albino mice. The clinical signs observed in the present low-dose groups were loss of body weight during the first few days of intoxication. Later the animals adapted and their body condition improved gradually. Another sign noticed during the early days of intoxication was gradual development of hind limb extensor tone followed by recovery. Other signs recorded were increased startle response, salivation, and somnolence. The medium-dose group exhibited signs such as burrowing behavior, increased startle response, salivation, somnolence, seizures, and gradual development of hind limb extensor tone. The highest-dose group animals exhibited increased startle response, seizures, somnolence, and salivation initially, but later showed loss of righting reflex, lateral recumbence, laboured breathing, gasping, and death. Similar signs were observed by Aldana *et al.* (1998) and Manna *et al.* (2004) in cypermethrin toxicity studies in rats at different dose levels.

Grewal *et al.* (2010) observed that the lower dose produced very mild toxicosis characterized by intermittent diarrhea, decreased feed intake, and thick eye discharge, whereas higher dose displayed mild to moderate toxicosis with diarrhea, decreased feed intake and loss of body weight, dyspnoea, ataxia, eye discharge, and salivation.

In my present investigation, Clinical signs of cypermethrin toxicity of lower dose produced slight nervousness, mild depression, reduced feed intake, rough hair coat, and abnormal gait. Animal in higher dose (0.25 ml/20g bw) displayed diarrhea, decreased feed intake and loss of

body weight, dyspnoea, ataxia, eye discharge, and salivation. Repeated administration of cypermethrin produced haemorrhage, necrosis, edema and lesions.

#### 4.9 Morphometric Parameters

Cypermethrin has moderate effects on morphometric parameters in albino mice. Morphometric parameters of the present study included body weight, feed intake, gain or loss of body weight and feed conversion ratio (FCR). In addition, changes in the weights of various organs such as brain, liver, kidney, lungs, heart, viscera and gonads were also observed. In the control group, the gross body weight was increased from their initial body weight and reached to its highest value at the end of 4 weeks. On the other hand, the gross body weights in cypermethrin-treated mice were decreased from their initial body weight. In addition, the decrease in body weights, feed intake and FCR over the period of four weeks was also highly significant both in the control and treatment groups. The weights of different organs of albino mice were decreased in the treatment group compared to the control group.

The decrease in body weight gain was in accordance with Clark (1982) who reported a decreased growth which correlated with decreased feed intake in both male and female Wister rats fed with 540 mg/kg cypermethrin. The growth in rats was reduced at 750 mg of cypermethrin per kg for five weeks.

The findings on weight loss following cypermethrin treatment in the present experiment were almost similar to the findings of some other researchers such as Haque *et al.* (2005) and Kamruzumman *et al.* (2006) who reported reduced weight gain in mice after intoxication with cypermethrin. The reduction of body weight might be due to the interruption in absorption and metabolism of feed nutrients essential for health (Marchlewicz *et al.*, 2006). Similar weight loss results were reported by Wang *et al.* (2009) for moderate (10 mg/kg body weight/day) and high doses (20 mg/kg body weight/day) of  $\beta$ -cypermethrin in male mice.

Grewal *et al.* (2010) repeated oral doses of cypermethrin for 30 days and recorded enhanced relative weight of liver and heart, but significantly decreased that of brain, kidneys and testes. The study suggested that repeated oral exposure of cypermethrin had considerable harmful effects on body organs in the albino rat *Rattus norvegicus*. Dahamna *et al.* (2011) also reported reduction in body weight gain in rats fed 1600 mg cypermethrin per kg body weight for three months. Thyroid and adrenal glands showed increase in weight at both 2 and 4 weeks of treatments (Sangha *et al.*, 2011).

The lower the value of FCR in the present study indicated the highest consumption capacity of feed by the experimental mice. Similar to the present results, Enami and Safafar (2010) in a 6-week feeding trial of Wister rat with graded level of canola also reported that adding canola meal did not change daily weight gained but reduced feed intake. Partial substitution of soybean meal by canola meal caused an increase in FCR. It was observed that the rats on 10% untreated CBC went off feed and probably died out of starvation. The animals appeared to have sensed the irritating factors in the diets. The high oil content that characterized castor bean cake prompted another study to assess the effect of residual oil on cake quality, performance and nutrient digestibility of albino rats. The higher value of FCR indicates the reduced feed intake of albino mice and this caused decreases body weight. On the contrary, Dahamna *et al.* (2011) reported that cypermethrin mixed diet at 1500 mg per kg for 13 weeks showed diminished feed intake in beagle hound dogs with weight loss, diarrhoea, anorexia, licking and chewing of paws.

Nair *et al.* (2011) used technical grade cypermethrin in 2 ml gingerly oil produced toxic effects in male Sprague Dawley rats (200±3g body wt) at 40, 80 and 120 mg/kg body weight administered daily for 21 days. Gross lesions, congestion of lungs, heart, pulmonary hemorrhage and degenerative changes in the liver and kidneys were observed. In agreement with the above reports, chronic exposure to cypermethrin for 4 weeks resulted in decreased

feed intake that causes loss of body weight in albino mice in the treatment group compared to the control group. The decreased in feed intake in Group V (0.25 ml/20g bw; highest dose) was high as compared to the treatment group I (0.10 ml/20g bw; lowest dose). The body weight loss in treated mice was lower at both 3 and 4 weeks as compared to the 1 and 2 week. Body weight gain resulted only in control group as compared to the treatment group II-V; this means the final body weight of treatment group decreased as compared to their initial body weight. Therefore, the FCR value of treatment group was high. The higher value of FCR indicates the reduced feed intake of albino mice and this causes decreased body weight.

#### 4.10 Tissue/somatic indices

Cypermethrin has less effect on tissue/somatic indices in albino mice. In previous studies, the weight of liver and spleen decreased, while that of kidneys increased as compared to the control rats. Thyroid and adrenal showed increase in weight at both 2 and 4 weeks of treatments (Sangha *et al.*, 2011). Moreover, synergistic effects were noticed when male albino mice were treated at 48h intervals for 15 and 30 days with cypermethrin (4.25 and 8.5 mg/kg body weight) and sodium fluoride (2.8 and 5.6 mg/kg body weight) by oral gavages. Significant decrease in body, testis and epididymis weights along with histopathological changes in the testis after 30 days was recorded (Sekhar *et al.*, 2011).

In agreements with the most findings discussed above, the present investigation reports decrease in the total body weight, brain, lungs, heart, liver, kidney, viscera and gonads in the cypermethrin-treated mice after 28 days owing to toxic effects of the synthetic pyrethroid used. The reduction in weight of accessory organs indicated atrophy of glandular tissue and also a reduction in secretory ability. As the decline in different organs weight may also be a result of a decrease in tissue/somatic indices of the experimental mice. The result of the present study clearly shows significant alterations in different organs due to intoxication of cypermethrin and oxidative stress of cypermethrin in albino mice. Finally it can be stated that

long term exposure to sub lethal doses of pyrethroid pesticides can result in cell metabolism toxicosis. Cypermethrin might have synergistic effects on muscle and kidney functions leading to physiological impairment.

#### 4.11 Histopathological Alterations

Cypermethrin has severe effects on histology in albino mice. The present investigation reported the findings on histopathological changes of liver and kidneys in cypermethrin-treated groups of albino mice. Moreover, the liver and kidney are the most important organs in which chemicals are structurally altered and resulting metabolites are biologically toxic (Saha *et al.*, 2006). The effects of cypermethrin in mice showed much histopathological alterations in liver. Sections of control mice liver revealed that the hepatocytes arranged in strands with one or two spherical nuclei and eosinophilic cytoplasm (Plate 11a). Examination of liver of cypermethrin treated mice displayed apparent signs of degenerative changes. After 28 days of treatment with cypermethrin, histopathological examination in liver revealed nucleus to be normal but with congested sinusoidal spaces (Plate 11e). High dose treated liver showed increased sinusoidal spaces, condensed nuclei and enlarged hepatocytes with hydrophobic degeneration together with cytoplasmic vacuolization (Plate 11e). Most cells showed nuclei with signs of karyolysis and pyknosis. Nuclear hypertrophy and binucleated hepatocytes were observed at some places.

Sections from the kidneys of mice treated with cypermethrin as well as those from the controls were also examined (Plate 12-a, b, c, d and e). Microscopic observation of the kidney of control group showed no histopathological and the kidney consisted of normal arrangement (Plate 12a). Control group showed normal renal architecture. But in case of the treatment group, the kidney showed distinct histological changes. The histological changes observed in the kidneys after damage by toxins include changes in the tubules and in the interstitium. There is necrosis predominantly in the proximal tubules. Plate 12a showed that

kidney cortex consists of renal corpuscles and tubules. Renal corpuscles consisted of Bowman's capsule with double membrane with urinary space in between. Even the kidneys showed marked haemorrhage and congestion in cypermethrin-treated animals as compared to normal histological examination of renal tissue in control mice. Animals treated with cypermethrin showed degeneration and deterioration of the cortical constituents.

Present investigation showed marked histological alterations in cypermethrin treated mice and it may be possibly due to oxidative stress induced by cypermethrin. Pyrethroids compounds have the ability to cause oxidative stress by stimulating the production of ROS and this mechanism has been implicated in the hepatotoxicity and carcinogenicity of these chemicals. Cypermethrin metabolism in the body generates some Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (ROS) which induced oxidative stress causing severe damage to cell membrane. However, further depth investigation are required.

Khalequzzaman *et al.* (2004) and Saha *et al.* (2006) reported many histopathological alterations in the kidney after the inhalation of cypermethrin and chloropyrifos. Cypermethrin intoxication produced congestion and/or haemorrhages on the auriculoventricular groove of the heart, lungs, mucosal surface of the intestine, cortex of the kidney, and brain (Patel *et al.*, 2006).

Inayat *et al.* (2007) used dermal administration of cypermethrin at 30 mg/kg body weight for a period of 6 weeks which resulted in congestion of vessels and marked lymphocytic infiltration in the kidneys. Similarly, Sakr *et al.* (2009) reported that cypermethrin caused different histological and biochemical alterations in liver of mice. On the other hand, haemorrhages in the liver, kidneys, lungs, ventricles, and endocardium of male dwarf goats treated with high doses of cypermethrin (0.8 and 1.6%) were reported by Khan *et al.* (2009). Sheikh and Javed (2009) observed that long time exposure to cypermethrin resulted in the development of skin tumor in the epidermis of the experimental animals.

Repeated administration of cypermethrin also produced hemorrhages within myocardium, disruption of branching structure, and loss of striation of cardiac tissue; thickening of alveolar septa in lungs, partial to extensive loss of various stages of spermatogenesis in testes, and loss of follicular cells and oocytes in ovaries (Grewal *et al.*, 2010).

The results of Dahamna *et al.* (2011) indicated a significant increase in transaminases GOT, GPT, and ALP. Cypermethrin treatment exhibited severe histopathological changes, especially in the liver and kidney accompanied by weight loss of some organs, suggesting that cypermethrin induces oxidative stress and modifies biochemical parameters and histological aspects of liver and kidney. Whereas Muthuviveganandavel *et al.* (2011) studied the low-dose exposure of cypermethrin (5, 10, 25 and 50 mM for 12 and 24hrs) on the biochemical and histological effects on the brain tissues of the male albino rats, suggesting degenerative tissue morphology.

Similar findings were reported by Hussien *et al.* (2011) who reported that cypermethrin caused different histopathological changes in rat organs including ischemia and pyknosis of the cytoplasm of the neurons in the brain tissue. Repeated administration of cypermethrin also produced hemorrhages within myocardium, disruption of branching structure, and loss of striation of cardiac tissue; thickening of alveolar septa in lungs, partial to extensive loss of various stages of spermatogenesis in testes, and loss of follicular cells and oocytes in ovaries (Ince *et al.*, 2012). Sekhar *et al.* (2012) observed histopathological changes in liver tissues; moreover, total carbohydrate, glycogen and protein decreased but free amino acid contents increased.

#### **4.12 Haematological Profiles**

Cypermethrin has severe effects on haematological profiles in albino mice. In the present investigation, it was found that exposure of mice to cypermethrin showed remarkable haematological alterations. Cypermethrin is said to have two modes of action on blood cells.

It induces oxidative stress and as a hydrophobic compound, it accumulates in cell membranes and disturbs the membrane structure. Intraperitoneal administration of cypermethrin at 0.10, 0.15, 0.20 and 0.25 ml per 20g body weight for 28 days showed dose-dependent decrease in some haematological parameters such as RBC counts, neutrophils, Hb and ESR. In this study the RBC count, platelets, Hb (g/dl) decreased significantly while lymphocytes, monocytes and ESR increased. This means cypermethrin increased in the number of leukocytes in peripheral blood of the treated albino mice. The decline in RBC count, haemoglobin concentration, presumably reflected erythrocyte haemolysis and/or decreased synthesis of RBC. The decrease in Hb concentration may be because of either an increase in the rate at which Hb is destroyed, or a decrease in the rate of Hb synthesis. Moreover, an increase in lymphocytes and monocytes were noted in cypermethrin-treated mice was an indication cypermethrin poisoning of blood, owing to the stimulation of the process of lymphopoiesis. Like-wise, a decreased percentage of neutrophils in the peripheral blood observed in animals poisoned with cypermethrin suggested that neutrophils were involved in phagocytosis during xenobiotic intoxication, as a result of which, some of the neutrophils were destroyed. Cypermethrin intoxication in mice in the present study was therefore manifested by the neutrophil count consistently being decreased over a period of 28 days.

In earlier reports Heratym (2002) showed that cypermethrin increased in the number of leukocytes in peripheral blood where the females were affected more than the male mice. Subsequently, Sayim *et al.* (2005) using oral administration of 60, 150 and 300 mg cypermethrin per kg body weight for 30 days showed dose-dependent decrease in some haematological parameters such as RBC counts, plus significant increase in final body weight but significant decrease in brain-body weight ratio; brain tissues showed deformation areas due to ischemia and pyknosis of the cytoplasm of the neurons. Further, the decline in RBC count, haemoglobin concentration, and haematocrit presumably reflects erythrocyte

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## APPENDICES

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## Data Collection Sheet

Sheet No.:01

Control/Treatment group: Group-I (Control Treated with distilled water)

Replication No.: I

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | x        | x        | x        | x        |
| Loss of bw                 | x        | x        | x        | x        |
| Hind limb                  | x        | x        | x        | x        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | x        | x        | x        | x        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | x        | x        | x        | x        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | x        | x        | x        | x        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before Treatment- 29.9 g

| Morphometric parameters    | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Initial bw (g)             | 31.9     | 35.0     | 37.9     | 38.8     |
| Bw gain/loss(g)            | 2.0      | 3.1      | 2.9      | 0.9      |
| Daily body wt gain/loss(g) | 0.286    | 0.443    | 0.415    | 0.129    |
| Daily feed intake (g)      | 8.65     | 9.13     | 9.58     | 9.58     |
| Total feed intake (g)      | 60.5     | 63.9     | 67.0     | 67.0     |
| Feed conversion ratio      | 30.25    | 20.61    | 23.10    | 74.44    |
| Mortality (%)              | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.3         | 0.7732                 |
| Liver wt    | 2.6         | 6.7010                 |
| Kidney wt   | 0.8         | 2.0619                 |
| Lung wt     | 0.4         | 1.0309                 |
| Heart wt    | 0.3         | 0.7732                 |
| Visceral wt | 2.9         | 7.4742                 |
| Gonadal wt  | 0.3         | 0.7732                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-I       | 5.32                                           | 15.80                                          | 93.47                                                | 17.9            | 1.0             | 74.10           | 4.6           | 1             | 15.5      | 3.50        |

Note/comments: Lung was found normal pink color in Group-I (Control) and other organs was found normal.

## Data Collection Sheet

Sheet No.:02

Control/Treatment group: Group-I (Control Treated with distilled water)

Replication No.: II

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | x        | x        | x        | x        |
| Loss of bw                 | x        | x        | x        | x        |
| Hind limb                  | x        | x        | x        | x        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | x        | x        | x        | x        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | x        | x        | x        | x        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | x        | x        | x        | x        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 29.0 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 31.0     | 33.9     | 37.0     | 38.0     |
| Bw gain/loss (g)            | 2.0      | 2.9      | 3.1      | 1.0      |
| Daily body wt gain/loss (g) | 0.286    | 0.415    | 0.443    | 0.143    |
| Daily feed intake (g)       | 8.65     | 9.13     | 9.58     | 9.58     |
| Total feed intake (g)       | 60.5     | 63.9     | 67.0     | 67.0     |
| Feed conversion ratio       | 30.25    | 22.03    | 21.61    | 67       |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5263                 |
| Liver wt    | 2.6         | 6.8421                 |
| Kidney wt   | 0.7         | 1.8421                 |
| Lung wt     | 0.4         | 1.0526                 |
| Heart wt    | 0.4         | 1.0526                 |
| Visceral wt | 3.2         | 8.4211                 |
| Gonadal wt  | 0.4         | 1.0526                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-I       | 5.70                                           | 15.45                                          | 92.80                                                | 17.5            | 1.0             | 73.5            | 3.8           | 1             | 15.8      | 3.91        |

Note/comments: Lung was found normal pink color in Group-I (Control) and other organs was found normal.

## Data Collection Sheet

Sheet No.:03

Control/Treatment group: Group-I (Control Treated with distilled water)

Replication No.: III

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | x        | x        | x        | x        |
| Loss of bw                 | x        | x        | x        | x        |
| Hind limb                  | x        | x        | x        | x        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | x        | x        | x        | x        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | x        | x        | x        | x        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | x        | x        | x        | x        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 29.1 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 31.5     | 34.0     | 37.7     | 38.5     |
| Bw gain/loss (g)            | 2.4      | 2.5      | 3.7      | 0.8      |
| Daily body wt gain/loss (g) | 0.343    | 0.358    | 0.529    | 0.115    |
| Daily feed intake (g)       | 8.65     | 9.13     | 9.58     | 9.58     |
| Total feed intake (g)       | 60.5     | 63.9     | 67.0     | 67.0     |
| Feed conversion ratio       | 25.21    | 25.56    | 18.11    | 83.75    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.3         | 0.7792                 |
| Liver wt    | 2.7         | 7.0130                 |
| Kidney wt   | 0.7         | 1.8182                 |
| Lung wt     | 0.4         | 1.0390                 |
| Heart wt    | 0.3         | 0.7792                 |
| Visceral wt | 3.1         | 8.0519                 |
| Gonadal wt  | 0.3         | 0.7792                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-I       | 5.72                                           | 14.93                                          | 92.50                                                | 17.5            | 1.0             | 73.8            | 4.6           | 1.5           | 16.3      | 3.95        |

Note/comments: Lung was found normal pink color in Group-I (Control) and other organs was found normal.

## Data Collection Sheet

Sheet No.:04

Control/Treatment group: Group-I (Control Treated with distilled water)

Replication No.: IV

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Feed intake                | x        | x        | x        | x        |
| Loss of bw                 | x        | x        | x        | x        |
| Hind limb                  | x        | x        | x        | x        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | x        | x        | x        | x        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | x        | x        | x        | x        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | x        | x        | x        | x        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 29.7 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 31.7     | 34.9     | 37.8     | 38.9     |
| Bw gain/loss (g)            | 2.0      | 3.2      | 2.9      | 1.1      |
| Daily body wt gain/loss (g) | 0.286    | 0.457    | 0.414    | 0.157    |
| Daily feed intake (g)       | 8.65     | 9.13     | 9.58     | 9.58     |
| Total feed intake (g)       | 60.5     | 63.9     | 67.0     | 67.0     |
| Feed conversion ratio       | 30.25    | 19.97    | 23.10    | 60.91    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.4         | 1.0283                 |
| Liver wt    | 2.5         | 6.4267                 |
| Kidney wt   | 0.8         | 2.0566                 |
| Lung wt     | 0.4         | 1.0283                 |
| Heart wt    | 0.3         | 0.7712                 |
| Visceral wt | 3.2         | 8.2262                 |
| Gonadal wt  | 0.4         | 1.0283                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-I       | 5.35                                           | 15.80                                          | 93.47                                                | 17.5            | 1.0             | 74.50           | 4.6           | 1.2           | 15.9      | 3.72        |

Note/comments: Lung was found normal pink color in Group-I (Control) and other organs was found normal.

## Data Collection Sheet

Sheet No.:02

Control/Treatment group: Group-II (Treated with Cypermethrin 0.10 ml/20 g of B. W.)  
Replication No.: II

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 31.9 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 29.8     | 27.5     | 27.1     | 26.8     |
| Bw gain/loss (g)            | 2.1      | 2.3      | 0.4      | 0.3      |
| Daily body wt gain/loss (g) | 0.300    | 0.330    | 0.060    | 0.050    |
| Daily feed intake (g)       | 8.50     | 8.13     | 7.88     | 7.80     |
| Total feed intake (g)       | 59.44    | 56.90    | 55.10    | 54.60    |
| Feed conversion ratio       | 28.3     | 24.74    | 137.75   | 182      |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.7463                 |
| Liver wt    | 2.5         | 9.3284                 |
| Kidney wt   | 0.6         | 2.2388                 |
| Lung wt     | 0.2         | 0.7463                 |
| Heart wt    | 0.2         | 0.7463                 |
| Visceral wt | 2.6         | 9.7015                 |
| Gonadal wt  | 0.2         | 0.7463                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC (×10 <sup>6</sup> Cells/mm <sup>3</sup> ) | TC, WBC (×10 <sup>3</sup> Cells/mm <sup>3</sup> ) | TC, Platelets (×10 <sup>4</sup> Cells/mm <sup>3</sup> ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-II      | 3.97                                              | 15.80                                             | 73.50                                                   | 13.5            | 1.0             | 84.14           | 1.0           | 0.7           | 12.2      | 4.50        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-II (0.10 ml/20 g body weight) due to oxidative stress.

## Data Collection Sheet

Sheet No.:03

Control/Treatment group: Group-II (Treated with Cypermethrin 0.10 ml/20 g of B. W.)  
Replication No.: III

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 31.9 g

| Morphometric parameters | 1st week | 2nd week | 3rd week | 4th week |
|-------------------------|----------|----------|----------|----------|
| Initial bw (g)          | 29.9     | 28.1     | 27.5     | 27.2     |
| Bw gain                 | 2.0      | 1.8      | 0.6      | 0.3      |
| Daily body wt gain      | 0.290    | 0.260    | 0.090    | 0.043    |
| Daily feed intake       | 8.50     | 8.13     | 7.88     | 7.80     |
| Total feed intake       | 59.44    | 56.90    | 55.10    | 54.60    |
| Feed conversion ratio   | 29.72    | 31.61    | 91.83    | 182      |
| Mortality (%)           | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.7353                 |
| Liver wt    | 2.5         | 9.1912                 |
| Kidney wt   | 0.6         | 2.2059                 |
| Lung wt     | 0.2         | 0.7353                 |
| Heart wt    | 0.2         | 0.7353                 |
| Visceral wt | 2.5         | 9.1912                 |
| Gonadal wt  | 0.2         | 0.7353                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/mm <sup>3</sup> ) | TC, WBC ( $\times 10^3$ Cells/mm <sup>3</sup> ) | TC, Platelets ( $\times 10^4$ Cells/mm <sup>3</sup> ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-II      | 3.97                                            | 15.80                                           | 74.20                                                 | 13.2            | 1.0             | 83.90           | 1.2           | 1             | 12.4      | 4.80        |

Note/comments: Lung was found red color instead of normal pink color in treatment group-II (0.10 ml/20 g body weight) due to oxidative stress.

## Data Collection Sheet

Sheet No.:04

Control/Treatment group: Group-II (Treated with Cypermethrin 0.10 ml/20 g of B. W.)  
Replication No.: IV

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 30.6 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 28.6     | 26.9     | 26.2     | 25.8     |
| Bw gain/loss (g)            | 2.0      | 1.7      | 0.7      | 0.4      |
| Daily body wt gain/loss (g) | 0.286    | 0.243    | 0.100    | 0.057    |
| Daily feed intake (g)       | 8.50     | 8.13     | 7.88     | 7.80     |
| Total feed intake (g)       | 59.44    | 56.90    | 55.10    | 54.60    |
| Feed conversion ratio       | 29.72    | 33.47    | 78.71    | 136.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.1         | 0.3876                 |
| Liver wt    | 2.3         | 8.9147                 |
| Kidney wt   | 0.6         | 2.3256                 |
| Lung wt     | 0.2         | 0.7752                 |
| Heart wt    | 0.2         | 0.7752                 |
| Visceral wt | 2.5         | 9.6899                 |
| Gonadal wt  | 0.2         | 0.7752                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-II      | 3.58                                           | 15.39                                          | 73.05                                                | 13.1            | 1.2             | 84.5            | 1.1           | 0.7           | 12.2      | 4.53        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-II (0.10 ml/20 g body weight) due to oxidative stress.

## Data Collection Sheet

Sheet No.:05

Control/Treatment group: Group-II (Treated with Cypermethrin 0.10 ml/20 g of B. W.)  
Replication No.: V

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | x        | x        | x        | x        |
| Edema                      | x        | x        | x        | x        |

## Morphometric parameters: Initial Body Weight Before treatment- 31.5 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 28.8     | 27.2     | 26.5     | 26.1     |
| Bw gain/loss (g)            | 2.7      | 1.6      | 0.7      | 0.4      |
| Daily body wt gain/loss (g) | 0.386    | 0.229    | 0.100    | 0.057    |
| Daily feed intake (g)       | 8.50     | 8.13     | 7.88     | 7.80     |
| Total feed intake (g)       | 59.44    | 56.90    | 55.10    | 54.60    |
| Feed conversion ratio       | 22.01    | 35.56    | 78.71    | 136.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.7663                 |
| Liver wt    | 2.4         | 9.1954                 |
| Kidney wt   | 0.4         | 1.5326                 |
| Lung wt     | 0.3         | 1.1494                 |
| Heart wt    | 0.1         | 0.3831                 |
| Visceral wt | 2.5         | 9.5785                 |
| Gonadal wt  | 0.1         | 0.3831                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-II      | 3.61                                           | 15.39                                          | 73.06                                                | 13.9            | 1.1             | 84.3            | 1.4           | 0.5           | 12.1      | 4.4         |

Note/comments: Lung was found red color instead of normal pink color in treatment group-II (0.10 ml/20 g body weight) due to oxidative stress.

## Data Collection Sheet

Sheet No.:01

Control/Treatment group: Group-III (Treated with Cypermethrin 0.15 ml/20 g of B. W.)  
Replication No.: I

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 31.9 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 30.0     | 28.9     | 28.0     | 27.9     |
| Bw gain/loss (g)            | 1.9      | 1.1      | 0.9      | 0.1      |
| Daily body wt gain/loss (g) | 0.280    | 0.160    | 0.130    | 0.020    |
| Daily feed intake (g)       | 8.43     | 8.22     | 8.15     | 8.13     |
| Total feed intake (g)       | 59.0     | 57.5     | 57.0     | 56.9     |
| Feed conversion ratio       | 31.05    | 52.27    | 63.33    | 569.00   |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.3         | 1.0753                 |
| Liver wt    | 2.5         | 8.9606                 |
| Kidney wt   | 0.6         | 2.1505                 |
| Lung wt     | 0.4         | 1.4337                 |
| Heart wt    | 0.2         | 0.7168                 |
| Visceral wt | 2.4         | 8.6022                 |
| Gonadal wt  | 0.2         | 0.7168                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-III     | 3.88                                           | 12.50                                          | 72.80                                                | 13.20           | 1.0             | 84.84           | 1.3           | 1             | 12.8      | 4.29        |

Note/comments: Lung was found red color instead of normal pink color in treatment group-III (0.15 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found.

## Data Collection Sheet

Sheet No.:02

Control/Treatment group: Group-III (Treated with Cypermethrin 0.15 ml/20 g of B. W.)  
Replication No.: II

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

## Morphometric parameters: Initial Body Weight Before treatment- 31.9 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 30.5     | 29.1     | 27.9     | 27.7     |
| Bw gain/loss (g)            | 1.4      | 1.4      | 1.2      | 0.2      |
| Daily body wt gain/loss (g) | 0.200    | 0.200    | 0.180    | 0.029    |
| Daily feed intake (g)       | 8.43     | 8.22     | 8.15     | 8.13     |
| Total feed intake (g)       | 59.0     | 57.5     | 57.0     | 56.9     |
| Feed conversion ratio       | 42.14    | 41.07    | 47.5     | 284.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.4         | 1.4337                 |
| Liver wt    | 2.6         | 9.3190                 |
| Kidney wt   | 0.6         | 2.1505                 |
| Lung wt     | 0.2         | 0.7168                 |
| Heart wt    | 0.1         | 0.3584                 |
| Visceral wt | 2.5         | 8.9606                 |
| Gonadal wt  | 0.2         | 0.7168                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-III     | 3.82                                           | 12.80                                          | 73.10                                                | 12.9            | 1.2             | 84.88           | 1.3           | 0.8           | 12.4      | 4.50        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-III (0.15 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found.

## Data Collection Sheet

Sheet No.:03

Control/Treatment group: Group-III (Treated with Cypermethrin 0.15 ml/20 g of B. W.)  
Replication No.: III

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

## Morphometric parameters: Initial Body Weight Before treatment- 31.8 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 30.3     | 28.8     | 28.0     | 27.8     |
| Bw gain/loss (g)            | 1.5      | 1.5      | 0.8      | 0.2      |
| Daily body wt gain/loss (g) | 0.220    | 0.220    | 0.120    | 0.030    |
| Daily feed intake (g)       | 8.43     | 8.22     | 8.15     | 8.13     |
| Total feed intake (g)       | 59.0     | 57.5     | 57.0     | 56.9     |
| Feed conversion ratio       | 39.33    | 38.33    | 71.25    | 284.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.3         | 1.0753                 |
| Liver wt    | 2.5         | 8.9606                 |
| Kidney wt   | 0.6         | 2.1505                 |
| Lung wt     | 0.2         | 0.7168                 |
| Heart wt    | 0.2         | 0.7168                 |
| Visceral wt | 2.5         | 8.9606                 |
| Gonadal wt  | 0.2         | 0.7168                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-III     | 4.10                                           | 12.25                                          | 72.50                                                | 12.3            | 1.5             | 85.20           | 1.2           | 0.5           | 12.2      | 4.75        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-III (0.15 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found.

## Data Collection Sheet

Sheet No.:04

Control/Treatment group: Group-III (Treated with Cypermethrin 0.15 ml/20 g of B. W.)  
Replication No.: IV

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Feed intake                | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

**Morphometric parameters:** Initial Body Weight Before treatment- 31.5 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 29.5     | 28.2     | 27.9     | 27.7     |
| Bw gain/loss (g)            | 2.0      | 1.3      | 0.3      | 0.2      |
| Daily body wt gain/loss (g) | 0.286    | 0.186    | 0.043    | 0.029    |
| Daily feed intake (g)       | 8.43     | 8.22     | 8.15     | 8.13     |
| Total feed intake (g)       | 59.0     | 57.5     | 57.0     | 56.9     |
| Feed conversion ratio       | 29.5     | 44.23    | 190      | 284.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.7220                 |
| Liver wt    | 2.5         | 9.0253                 |
| Kidney wt   | 0.5         | 1.8051                 |
| Lung wt     | 0.2         | 0.7220                 |
| Heart wt    | 0.2         | 0.7220                 |
| Visceral wt | 2.4         | 8.6643                 |
| Gonadal wt  | 0.1         | 0.3610                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-III     | 3.89                                           | 12.52                                          | 72.88                                                | 13.2            | 1.1             | 84.74           | 1.2           | 0.8           | 12.5      | 4.27        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-III (0.15 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found.

## Data Collection Sheet

Sheet No.:05

Control/Treatment group: Group-III (Treated with Cypermethrin 0.15 ml/20 g of B. W.)

Replication No.: V

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | x        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 31.7 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 30.3     | 29.2     | 28.4     | 27.9     |
| Bw gain/loss (g)            | 1.2      | 1.1      | 0.8      | 0.5      |
| Daily body wt gain/loss (g) | 0.171    | 0.157    | 0.114    | 0.071    |
| Daily feed intake (g)       | 8.43     | 8.22     | 8.15     | 8.13     |
| Total feed intake (g)       | 59.0     | 57.5     | 57.0     | 56.9     |
| Feed conversion ratio       | 49.17    | 47.92    | 71.25    | 113.8    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.3         | 1.0753                 |
| Liver wt    | 2.4         | 8.6022                 |
| Kidney wt   | 0.6         | 2.1505                 |
| Lung wt     | 0.3         | 1.0753                 |
| Heart wt    | 0.1         | 0.3584                 |
| Visceral wt | 2.5         | 8.9606                 |
| Gonadal wt  | 0.2         | 0.7168                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-III     | 3.94                                           | 12.57                                          | 72.74                                                | 13.2            | 1.0             | 84.82           | 1.2           | 0.7           | 12.7      | 4.20        |

Note/comments: Lung was found red color instead of normal pink color in treatment group-III (0.15 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found.

## Data Collection Sheet

Sheet No.: 01

Control/Treatment group: Group-IV (Treated with Cypermethrin 0.20 ml/20 g of B. W.)

Replication No.: I

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | x        | x        | x        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 37.1 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 35.5     | 34.1     | 33.5     | 33.3     |
| Bw gain/loss (g)            | 1.6      | 1.4      | 0.6      | 0.2      |
| Daily body wt gain/loss (g) | 0.229    | 0.200    | 0.086    | 0.029    |
| Daily feed intake (g)       | 8.65     | 8.38     | 8.08     | 8.03     |
| Total feed intake (g)       | 60.5     | 58.6     | 56.5     | 56.2     |
| Feed conversion ratio       | 37.81    | 30.84    | 94.17    | 281      |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.1         | 0.3003                 |
| Liver wt    | 2.3         | 6.9069                 |
| OKidney wt  | 0.6         | 1.8018                 |
| Lung wt     | 0.2         | 0.6006                 |
| Heart wt    | 0.2         | 0.6006                 |
| Visceral wt | 2.9         | 8.7087                 |
| Gonadal wt  | 0.2         | 0.6006                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC (×10 <sup>6</sup> Cells/mm <sup>3</sup> ) | TC, WBC (×10 <sup>3</sup> Cells/mm <sup>3</sup> ) | TC, Platelets (×10 <sup>4</sup> Cells/mm <sup>3</sup> ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-IV      | 2.93                                              | 11.38                                             | 73.50                                                   | 12.2            | 1.2             | 84.90           | 1.5           | 0.8           | 11.8      | 3.20        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-IV (0.20 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver.

## Data Collection Sheet

Sheet No.:02

Control/Treatment group: Group-IV (Treated with Cypermethrin 0.20 ml/20 g of B. W.)  
Replication No.: II

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 38.0 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 36.1     | 35.3     | 34.8     | 34.6     |
| Bw gain/loss (g)            | 1.9      | 0.8      | 0.5      | 0.2      |
| Daily body wt gain/loss (g) | 0.272    | 0.115    | 0.072    | 0.029    |
| Daily feed intake (g)       | 8.65     | 8.38     | 8.08     | 8.03     |
| Total feed intake (g)       | 60.5     | 58.6     | 56.5     | 56.2     |
| Feed conversion ratio       | 31.84    | 73.25    | 113      | 281      |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5780                 |
| Liver wt    | 2.5         | 7.2254                 |
| Kidney wt   | 0.6         | 1.7341                 |
| Lung wt     | 0.3         | 0.8671                 |
| Heart wt    | 0.1         | 0.2890                 |
| Visceral wt | 3.0         | 8.6705                 |
| Gonadal wt  | 0.2         | 0.5780                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-IV      | 2.60                                           | 11.96                                          | 73.80                                                | 12.3            | 1.5             | 85.30           | 0.8           | 0.8           | 11.3      | 3.50        |

Note/comments: Lung was found red color instead of normal pink color in treatment group-IV (0.20 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver.

## Data Collection Sheet

Sheet No.:03

Control/Treatment group: Group-IV (Treated with Cypermethrin 0.20 ml/20 g of B. W.)

Replication No.: III

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 38.2 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 36.5     | 35.4     | 34.9     | 34.8     |
| Bw gain/loss (g)            | 1.7      | 1.1      | 0.5      | 0.1      |
| Daily body wt gain/loss (g) | 0.243    | 0.158    | 0.072    | 0.015    |
| Daily feed intake (g)       | 8.65     | 8.38     | 8.08     | 8.03     |
| Total feed intake (g)       | 60.5     | 58.6     | 56.5     | 56.2     |
| Feed conversion ratio       | 35.59    | 53.27    | 113      | 562      |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5747                 |
| Liver wt    | 2.5         | 7.1839                 |
| Kidney wt   | 0.8         | 2.2989                 |
| Lung wt     | 0.4         | 1.1494                 |
| Heart wt    | 0.2         | 0.5747                 |
| Visceral wt | 3.0         | 8.6207                 |
| Gonadal wt  | 0.2         | 0.5747                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/mm <sup>3</sup> ) | TC, WBC ( $\times 10^3$ Cells/mm <sup>3</sup> ) | TC, Platelets ( $\times 10^4$ Cells/mm <sup>3</sup> ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-IV      | 2.19                                            | 11.82                                           | 72.98                                                 | 12.2            | 1.2             | 85.23           | 0.7           | 1.2           | 11.8      | 3.32        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-IV (0.20 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver.

## Data Collection Sheet

Sheet No.: 04

Control/Treatment group: Group-IV (Treated with Cypermethrin 0.20 ml/20 g of B. W.)  
Replication No.: IV

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 38.5 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 36.7     | 35.5     | 34.8     | 34.4     |
| Bw gain/loss (g)            | 1.8      | 1.2      | 0.7      | 0.4      |
| Daily body wt gain/loss (g) | 0.257    | 0.171    | 0.100    | 0.057    |
| Daily feed intake (g)       | 8.65     | 8.38     | 8.08     | 8.03     |
| Total feed intake (g)       | 60.5     | 58.6     | 56.5     | 56.2     |
| Feed conversion ratio       | 33.61    | 48.83    | 80.71    | 140.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5814                 |
| Liver wt    | 2.3         | 6.6860                 |
| Kidney wt   | 0.6         | 1.7442                 |
| Lung wt     | 0.2         | 0.5814                 |
| Heart wt    | 0.1         | 0.2907                 |
| Visceral wt | 3.0         | 8.7209                 |
| Gonadal wt  | 0.1         | 0.2907                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-IV      | 2.92                                           | 11.35                                          | 73.57                                                | 12.1            | 1.2             | 84.95           | 1.3           | 1.0           | 11.5      | 3.25        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-IV (0.20 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver.

## Data Collection Sheet

Sheet No.: 05

Control/Treatment group: Group-IV (Treated with Cypermethrin 0.20 ml/20 g of B. W.)

Replication No.: V

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | x        | x        | x        | x        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | x        | x        | x        | x        |

Morphometric parameters: Initial Body Weight Before treatment- 37.5 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 35.6     | 34.7     | 33.9     | 33.5     |
| Bw gain/loss (g)            | 1.9      | 0.9      | 0.8      | 0.4      |
| Daily body wt gain/loss (g) | 0.271    | 0.129    | 0.114    | 0.057    |
| Daily feed intake (g)       | 8.65     | 8.38     | 8.08     | 8.03     |
| Total feed intake (g)       | 60.5     | 58.6     | 56.5     | 56.2     |
| Feed conversion ratio       | 31.84    | 65.11    | 70.63    | 140.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.1         | 0.2985                 |
| Liver wt    | 2.5         | 7.4627                 |
| Kidney wt   | 0.6         | 1.7910                 |
| Lung wt     | 0.3         | 0.8955                 |
| Heart wt    | 0.2         | 0.5970                 |
| Visceral wt | 2.9         | 8.6567                 |
| Gonadal wt  | 0.2         | 0.5970                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-IV      | 2.90                                           | 11.33                                          | 73.55                                                | 12.3            | 1.1             | 84.85           | 1.2           | 0.9           | 11.3      | 3.25        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-IV (0.20 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver.

## Data Collection Sheet

Sheet No.:02

Control/Treatment group: Group- V (Treated with Cypermethrin 0.25 ml/20 g of B. W.)  
Replication No.: II

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | √        | √        | √        | √        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | -        | -        | -        | √        |

**Morphometric parameters:** Initial Body Weight Before treatment- 39.0 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 37.5     | 36.3     | 35.4     | 34.3     |
| Bw gain/loss (g)            | 1.5      | 1.2      | 0.9      | 1.1      |
| Daily body wt gain/loss (g) | 0.214    | 0.171    | 0.129    | 0.157    |
| Daily feed intake (g)       | 8.46     | 8.37     | 8.27     | 8.20     |
| Total feed intake (g)       | 59.2     | 58.6     | 57.9     | 57.4     |
| Feed conversion ratio       | 39.47    | 48.83    | 64.33    | 52.19    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5831                 |
| Liver wt    | 2.3         | 6.7055                 |
| Kidney wt   | 0.5         | 1.4577                 |
| Lung wt     | 0.4         | 1.1662                 |
| Heart wt    | 0.1         | 0.2915                 |
| Visceral wt | 2.4         | 6.9971                 |
| Gonadal wt  | 0.2         | 0.5831                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-V       | 2.75                                           | 15.80                                          | 73.47                                                | 8.55            | 0.7             | 90.32           | 1.2           | 0.8           | 10.2      | 2.70        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-V (0.25 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver. Edema or excess body fluid also found.

## Data Collection Sheet

Sheet No.:04

Control/Treatment group: Group-V (Treated with Cypermethrin 0.25 ml/20 g of B. W.)  
Replication No.: IV

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | √        | √        | √        | √        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | -        | -        | -        | √        |

Morphometric parameters: Initial Body Weight Before treatment- 38.8 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 37.5     | 36.2     | 35.5     | 35.1     |
| Bw gain/loss (g)            | 1.3      | 1.3      | 0.7      | 0.4      |
| Daily body wt gain/loss (g) | 0.186    | 0.186    | 0.100    | 0.057    |
| Daily feed intake (g)       | 8.46     | 8.37     | 8.27     | 8.20     |
| Total feed intake (g)       | 59.2     | 58.2     | 57.9     | 57.4     |
| Feed conversion ratio       | 45.54    | 45.08    | 82.71    | 143.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5698                 |
| Liver wt    | 2.3         | 6.5527                 |
| Kidney wt   | 0.6         | 1.7094                 |
| Lung wt     | 0.4         | 1.1396                 |
| Heart wt    | 0.1         | 0.2849                 |
| Visceral wt | 2.5         | 7.1225                 |
| Gonadal wt  | 0.1         | 0.2849                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g ×100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC ( $\times 10^6$ Cells/ $\text{mm}^3$ ) | TC, WBC ( $\times 10^3$ Cells/ $\text{mm}^3$ ) | TC, Platelets ( $\times 10^4$ Cells/ $\text{mm}^3$ ) | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | Monocytes (%) | Basophils (%) | Hb (g/dl) | ESR (mm/hr) |
|---------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-----------------|-----------------|-----------------|---------------|---------------|-----------|-------------|
| Group-V       | 2.91                                           | 15.75                                          | 72.57                                                | 8.80            | 0.9             | 90.25           | 0.7           | 1.1           | 10.7      | 2.95        |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-V (0.25 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver. Edema or excess body fluid also found.

## Data Collection Sheet

Sheet No.:05

Control/Treatment group: Group-V (Treated with Cypermethrin 0.25 ml/20 g of B. W.)

Replication No.: V

## Behavioural/clinical signs

| Behavioural/clinical signs | 1st week | 2nd week | 3rd week | 4th week |
|----------------------------|----------|----------|----------|----------|
| Decrease Feed intake       | √        | √        | √        | √        |
| Loss of bw                 | √        | √        | √        | √        |
| Hind limb                  | √        | √        | √        | √        |
| Salivation                 | x        | x        | x        | x        |
| Eye discharge              | √        | √        | √        | √        |
| Startle response           | √        | √        | √        | √        |
| Seizures                   | x        | x        | x        | x        |
| Laboured breathing         | √        | √        | √        | √        |
| Death                      | x        | x        | x        | x        |
| Haemorrhage                | -        | -        | -        | √        |
| Lesions                    | √        | √        | √        | √        |
| Necrosis                   | -        | -        | -        | √        |
| Edema                      | -        | -        | -        | √        |

Morphometric parameters: Initial Body Weight Before treatment- 38.6 g

| Morphometric parameters     | 1st week | 2nd week | 3rd week | 4th week |
|-----------------------------|----------|----------|----------|----------|
| Initial bw (g)              | 37.1     | 35.8     | 34.9     | 34.5     |
| Bw gain/loss (g)            | 1.5      | 1.3      | 0.9      | 0.4      |
| Daily body wt gain/loss (g) | 0.214    | 0.186    | 0.129    | 0.057    |
| Daily feed intake (g)       | 8.46     | 8.37     | 8.27     | 8.20     |
| Total feed intake (g)       | 59.2     | 58.2     | 57.9     | 57.4     |
| Feed conversion ratio       | 39.47    | 45.08    | 64.33    | 143.5    |
| Mortality (%)               | 0        | 0        | 0        | 0        |

Organ/somatic indices (on 29<sup>th</sup> day upon sacrifice)

| Organs      | Weights (g) | Organ/somatic indices* |
|-------------|-------------|------------------------|
| Brain wt    | 0.2         | 0.5797                 |
| Liver wt    | 2.4         | 6.9565                 |
| Kidney wt   | 0.5         | 1.4493                 |
| Lung wt     | 0.3         | 0.8696                 |
| Heart wt    | 0.2         | 0.5797                 |
| Visceral wt | 2.4         | 6.9565                 |
| Gonadal wt  | 0.2         | 0.5797                 |

\*Organ/somatic index= wt of the organ in g ÷ wt of the body in g × 100

Haematological profile (on 29<sup>th</sup> day upon sacrifice)

| Blood Profile | TC, RBC<br>(×10 <sup>6</sup><br>Cells/<br>mm <sup>3</sup> ) | TC, WBC<br>(×10 <sup>3</sup><br>Cells/<br>mm <sup>3</sup> ) | TC, Platelets<br>(×10 <sup>4</sup><br>Cells/<br>mm <sup>3</sup> ) | Neutrophils<br>(%) | Eosinophils<br>(%) | Lymphocytes<br>(%) | Monocytes<br>(%) | Basophils<br>(%) | Hb<br>(g/dl) | ESR<br>(mm/hr) |
|---------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------------|--------------------|--------------------|--------------------|------------------|------------------|--------------|----------------|
| Group-V       | 2.88                                                        | 15.84                                                       | 73.50                                                             | 8.65               | 0.7                | 90.30              | 0.9              | 1.2              | 10.7         | 2.91           |

**Note/comments:** Lung was found red color instead of normal pink color in treatment group-V (0.25 ml/20 g body weight) due to oxidative stress. Necrosis of muscle tissue was found and haemorrhage was found in liver. Edema or excess body fluid also found.

Appendix 3 Daily relative humidity (RH) record of the laboratory

| Date       | Dry Bulb Temperature (°C) | Wet Bulb Temperature (°C) | Depression of Bulb Degree (°C) | Relative Humidity (%) |
|------------|---------------------------|---------------------------|--------------------------------|-----------------------|
| 01.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 02.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 03.04.2013 | 30                        | 25                        | 5.0                            | 65                    |
| 04.04.2013 | 29                        | 26                        | 3.0                            | 78                    |
| 05.04.2013 | 31                        | 27                        | 4.0                            | 73                    |
| 06.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 07.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 08.04.2013 | 30                        | 25                        | 5.0                            | 65                    |
| 09.04.2013 | 28                        | 24                        | 4.0                            | 70                    |
| 10.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 11.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 12.04.2013 | 29                        | 25                        | 4.0                            | 71                    |
| 13.04.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 14.04.2013 | 30                        | 25.5                      | 4.5                            | 68                    |
| 15.04.2013 | 29                        | 24                        | 5.0                            | 64                    |
| 16.04.2013 | 29                        | 26                        | 3.0                            | 78                    |
| 17.04.2013 | 28                        | 25                        | 3.0                            | 77                    |
| 18.04.2013 | 28                        | 24                        | 4.0                            | 70                    |
| 19.04.2013 | 28                        | 25                        | 3.0                            | 77                    |
| 20.04.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 21.04.2013 | 27                        | 24                        | 3.0                            | 77                    |
| 22.04.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 23.04.2013 | 26                        | 23                        | 3.0                            | 76                    |
| 24.04.2013 | 27                        | 24                        | 3.0                            | 77                    |
| 25.04.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 26.04.2013 | 27                        | 25                        | 2.0                            | 84                    |
| 27.04.2013 | 29                        | 26.5                      | 2.5                            | 81                    |
| 28.04.2013 | 29                        | 26.5                      | 2.5                            | 81                    |
| 29.04.2013 | 27                        | 24                        | 3.0                            | 77                    |
| 30.04.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 01.05.2013 | 29                        | 26                        | 3.0                            | 78                    |
| 02.05.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 03.05.2013 | 27                        | 23                        | 4.0                            | 70                    |
| 04.05.2013 | 26                        | 23                        | 3.0                            | 76                    |
| 05.05.2013 | 28                        | 25                        | 3.0                            | 77                    |
| 06.05.2013 | 30                        | 25.5                      | 4.5                            | 68                    |
| 07.05.2013 | 29                        | 26.5                      | 2.5                            | 81                    |
| 08.05.2013 | 27                        | 25                        | 2.0                            | 84                    |
| 09.05.2013 | 29                        | 26                        | 3.0                            | 78                    |
| 10.05.2013 | 28                        | 25                        | 3.0                            | 77                    |
| 11.05.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 12.05.2013 | 30                        | 26                        | 4.0                            | 72                    |
| 13.05.2013 | 29                        | 26                        | 3.0                            | 78                    |
| 14.05.2013 | 27                        | 23                        | 4.0                            | 70                    |

Mean  $\pm$ SD of Dry Bulb Temperature = 28.52 $\pm$ 1.36 °CMean  $\pm$ SD of Wet Bulb Temperature = 24.94 $\pm$ 1.25 °CMean  $\pm$ SD of relative humidity = 73.73 $\pm$ 4.87%

**Appendix 4** ANOVA table showing the gross morphometric parameters

| Parameters        | Sources of variance | Sum of Squares | Df | Mean sum of Square | F-values  |
|-------------------|---------------------|----------------|----|--------------------|-----------|
| Week              | Between Groups      | 0.00           | 4  | 0.00               | 0.00ns    |
|                   | Within Groups       | 125.00         | 95 | 1.32               |           |
|                   | Total               | 125.00         | 99 |                    |           |
| Body wt           | Between Groups      | 1337.38        | 4  | 334.34             | 129.22*** |
|                   | Within Groups       | 245.81         | 95 | 2.59               |           |
|                   | Total               | 1583.19        | 99 |                    |           |
| Feed intake       | Between Groups      | 822.95         | 4  | 205.74             | 65.18***  |
|                   | Within Groups       | 299.85         | 95 | 3.16               |           |
|                   | Total               | 1122.80        | 99 |                    |           |
| Body wt gain/loss | Between Groups      | 25.76          | 4  | 6.44               | 14.08***  |
|                   | Within Groups       | 43.45          | 95 | 0.46               |           |
|                   | Total               | 69.21          | 99 |                    |           |
| FCR               | Between Groups      | 102638.02      | 4  | 25659.51           | 3.07*     |
|                   | Within Groups       | 793274.92      | 95 | 8350.26            |           |
|                   | Total               | 895912.94      | 99 |                    |           |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*= P&lt;0.05; \*\*\*= P&lt;0.001; ns= not significant

**Appendix 5** Analysis of Variance for morphometrics, Control Week wise

|                 | Sources of variation | Sum of Squares | Df | Mean Square | F         |
|-----------------|----------------------|----------------|----|-------------|-----------|
| BODY_W<br>T     | Between Groups       | 156.08         | 3  | 52.03       | 325.18*** |
|                 | Within Groups        | 2.56           | 16 | 0.16        |           |
|                 | Total                | 158.65         | 19 |             |           |
| Feed intake     | Between Groups       | 144.10         | 3  | 48.03       | 2E+03***  |
|                 | Within Groups        | 0.00           | 16 | 0.00        |           |
|                 | Total                | 144.10         | 19 |             |           |
| Bd wt gain/loss | Between Groups       | 14.19          | 3  | 4.72        | 72.46***  |
|                 | Within Groups        | 1.04           | 16 | 0.06        |           |
|                 | Total                | 15.23          | 19 |             |           |
| FCR             | Between Groups       | 7225.63        | 3  | 2408.54     | 57.24***  |
|                 | Within Groups        | 673.17         | 16 | 42.07       |           |
|                 | Total                | 7898.80        | 19 |             |           |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*\*\*= P&lt;0.001.

**Appendix 6** Analysis of Variance for morphometrics, Treatment 0.10 week-wise

|                 | Sources of variation | Sum of Squares | Df | Mean Square | F        |
|-----------------|----------------------|----------------|----|-------------|----------|
| BODY_W<br>T     | Between Groups       | 21.46          | 3  | 7.15        | 15.71*** |
|                 | Within Groups        | 7.28           | 16 | 0.45        |          |
|                 | Total                | 28.75          | 19 |             |          |
| Feed intake     | Between Groups       | 71.87          | 3  | 23.96       | 3E+03*** |
|                 | Within Groups        | 0.00           | 16 | 0.00        |          |
|                 | Total                | 71.87          | 19 |             |          |
| Bd wt gain/loss | Between Groups       | 12.08          | 3  | 4.03        | 75.27*** |
|                 | Within Groups        | 0.86           | 16 | 0.05        |          |
|                 | Total                | 12.94          | 19 |             |          |
| FCR             | Between Groups       | 78084.34       | 3  | 26028.13    | 27.63*** |
|                 | Within Groups        | 15067.77       | 16 | 941.73      |          |
|                 | Total                | 93152.16       | 19 |             |          |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*\*\*= P<0.001.

**Appendix 7** Analysis of Variance for morphometrics, Treatment 0.15 week-wise

|                 | Sources of variation | Sum of Squares | Df | Mean Square | F        |
|-----------------|----------------------|----------------|----|-------------|----------|
| BODY_WT         | Between Groups       | 16.05          | 3  | 5.35        | 60.12*** |
|                 | Within Groups        | 1.42           | 16 | 0.08        |          |
|                 | Total                | 17.48          | 19 |             |          |
| Feed intake     | Between Groups       | 14.10          | 3  | 4.70        | 2E+03*** |
|                 | Within Groups        | 0.00           | 16 | .000        |          |
|                 | Total                | 14.10          | 19 |             |          |
| Bd wt gain/loss | Between Groups       | 5.33           | 3  | 1.78        | 26.54*** |
|                 | Within Groups        | 1.07           | 16 | 0.07        |          |
|                 | Total                | 6.41           | 19 |             |          |
| FCR             | Between Groups       | 241966.60      | 3  | 80655.53    | 10.66*** |
|                 | Within Groups        | 121085.99      | 16 | 7567.88     |          |
|                 | Total                | 363052.59      | 19 |             |          |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*\*\*= P<0.001.

**Appendix 8** Analysis of Variance for morphometrics, Treatment 0.20 week-wise

|                 | Sources of variation | Sum of Squares | Df | Mean Square | F        |
|-----------------|----------------------|----------------|----|-------------|----------|
| BODY_WT         | Between Groups       | 11.41          | 3  | 3.80        | 10.16*** |
|                 | Within Groups        | 5.98           | 16 | 0.37        |          |
|                 | Total                | 17.39          | 19 |             |          |
| Feed intake     | Between Groups       | 60.45          | 3  | 20.15       | 2E+03*** |
|                 | Within Groups        | 0.00           | 16 | 0.00        |          |
|                 | Total                | 60.45          | 19 |             |          |
| Bd wt gain/loss | Between Groups       | 6.45           | 3  | 2.150       | 78.89*** |
|                 | Within Groups        | 0.43           | 16 | 0.03        |          |
|                 | Total                | 6.89           | 19 |             |          |
| FCR             | Between Groups       | 191044.98      | 3  | 63681.66    | 8.42***  |
|                 | Within Groups        | 120969.59      | 16 | 7560.60     |          |
|                 | Total                | 312014.57      | 19 |             |          |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*\*\*= P<0.001.

**Appendix 9** Analysis of Variance for morphometrics, Treatment 0.25 week-wise

|                 | Sources of variation | Sum of Squares | Df | Mean Square | F         |
|-----------------|----------------------|----------------|----|-------------|-----------|
| BODY_WT         | Between Groups       | 22.37          | 3  | 7.45        | 101.46*** |
|                 | Within Groups        | 1.17           | 16 | 0.07        |           |
|                 | Total                | 23.55          | 19 |             |           |
| Feed intake     | Between Groups       | 9.34           | 3  | 3.11        | 2E+03***  |
|                 | Within Groups        | 0.00           | 16 | 0.00        |           |
|                 | Total                | 9.34           | 19 |             |           |
| Bd wt gain/loss | Between Groups       | 1.30           | 3  | 0.43        | 10.08***  |
|                 | Within Groups        | 0.69           | 16 | 0.04        |           |
|                 | Total                | 1.99           | 19 |             |           |
| FCR             | Between Groups       | 7210.54        | 3  | 2403.51     | 3.87***   |
|                 | Within Groups        | 9946.26        | 16 | 621.64      |           |
|                 | Total                | 17156.80       | 19 |             |           |

Df= Degrees of freedom; FCR = Feed conversion ratio; \*\*\*= P<0.001.

**Appendix 10** Analysis of Variance for different organs of mice

|              | Sources of variation | Sum of Squares | Df | Mean Square | F         |
|--------------|----------------------|----------------|----|-------------|-----------|
| Body wt.     | Between Groups       | 470.55         | 4  | 117.64      | 488.66*** |
|              | Within Groups        | 4.57           | 19 | 0.24        |           |
|              | Total                | 475.13         | 23 |             |           |
| Brain wt.    | Between Groups       | 0.08           | 4  | 0.02        | 5.70***   |
|              | Within Groups        | 0.06           | 19 | 0.00        |           |
|              | Total                | 0.15           | 23 |             |           |
| Liver wt.    | Between Groups       | 0.15           | 4  | 0.04        | 5.63***   |
|              | Within Groups        | 0.13           | 19 | 0.01        |           |
|              | Total                | 0.28           | 23 |             |           |
| Kidney wt.   | Between Groups       | 0.12           | 4  | 0.03        | 6.28***   |
|              | Within Groups        | 0.09           | 19 | 0.01        |           |
|              | Total                | 0.22           | 23 |             |           |
| Lung wt.     | Between Groups       | 0.13           | 4  | 0.03        | 7.04***   |
|              | Within Groups        | 0.09           | 19 | 0.01        |           |
|              | Total                | 0.22           | 23 |             |           |
| Heart wt.    | Between Groups       | 0.09           | 4  | 0.02        | 8.01***   |
|              | Within Groups        | 0.05           | 19 | 0.00        |           |
|              | Total                | 0.14           | 23 |             |           |
| Visceral wt. | Between Groups       | 1.70           | 4  | 0.43        | 61.22***  |
|              | Within Groups        | 0.13           | 19 | 0.01        |           |
|              | Total                | 1.83           | 23 |             |           |
| Gonadal wt.  | Between Groups       | 0.10           | 4  | 0.03        | 10.70***  |
|              | Within Groups        | 0.05           | 19 | 0.00        |           |
|              | Total                | 0.15           | 23 |             |           |

Df= Degrees of freedom; \*\*\*= P&lt;0.001

**Appendix 11** Analysis of Variance for tissue/somatic indices of mice

|         | Sources of variation | Sum of Squares | Df | Mean Square | F         |
|---------|----------------------|----------------|----|-------------|-----------|
| BRAIN   | Between Groups       | 1.01           | 4  | 0.25        | 7.73***   |
|         | Within Groups        | 0.65           | 20 | 0.03        |           |
|         | Total                | 1.66           | 24 |             |           |
| LIVER   | Between Groups       | 29.13          | 4  | 7.28        | 132.19*** |
|         | Within Groups        | 1.10           | 20 | 0.05        |           |
|         | Total                | 30.23          | 24 |             |           |
| KIDNEY  | Between Groups       | 0.97           | 4  | 0.24        | 5.19***   |
|         | Within Groups        | 0.93           | 20 | 0.04        |           |
|         | Total                | 1.90           | 24 |             |           |
| LUNG    | Between Groups       | 0.46           | 4  | 0.12        | 2.36ns    |
|         | Within Groups        | 0.98           | 20 | 0.05        |           |
|         | Total                | 1.44           | 24 |             |           |
| HEART   | Between Groups       | 0.60           | 4  | 0.15        | 5.29***   |
|         | Within Groups        | 0.56           | 20 | 0.03        |           |
|         | Total                | 1.17           | 24 |             |           |
| VISCERA | Between Groups       | 14.54          | 4  | 3.64        | 47.56***  |
|         | Within Groups        | 1.53           | 20 | 0.08        |           |
|         | Total                | 16.07          | 24 |             |           |
| GONAD   | Between Groups       | 0.51           | 4  | 0.13        | 5.38***   |
|         | Within Groups        | 0.48           | 20 | 0.02        |           |
|         | Total                | 0.989          | 24 |             |           |

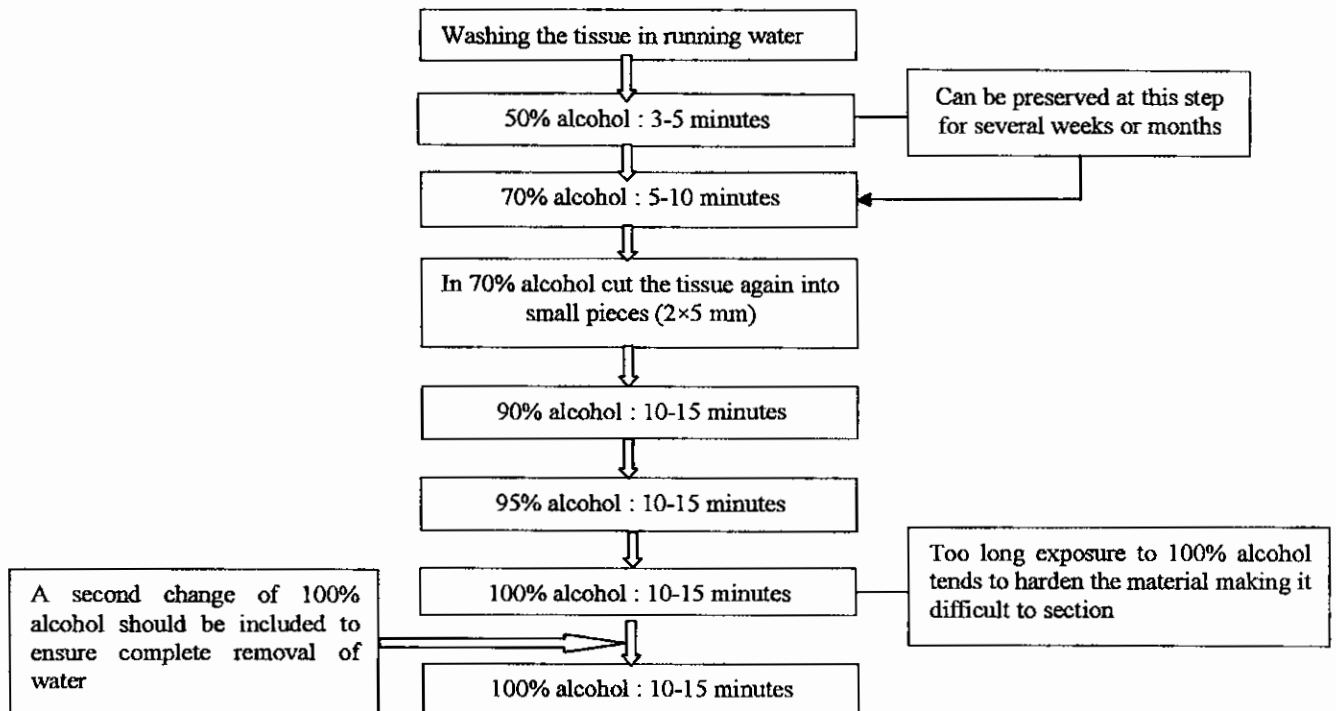
Df= Degrees of freedom; \*\*\*= P&lt;0.001; ns= not significant

**Appendix 12** Analysis of Variance for Haematology Profile of mice

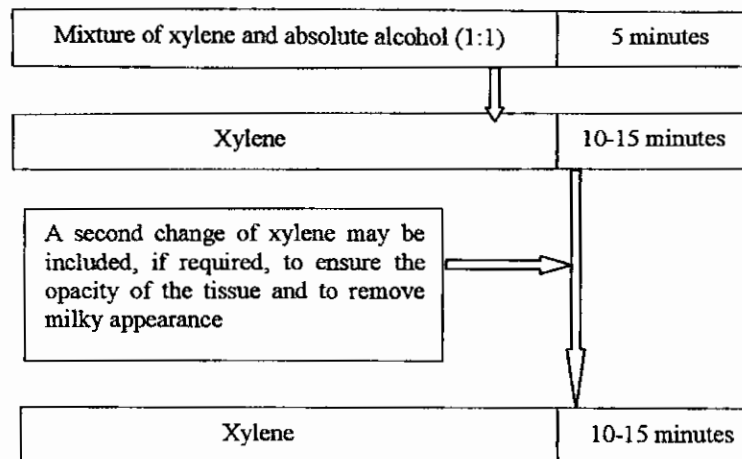
|             | Sources of variation | Sum of Squares | Df | Mean Square | F          |
|-------------|----------------------|----------------|----|-------------|------------|
| RBC         | Between Groups       | 26.48          | 4  | 6.62        | 149.83***  |
|             | Within Groups        | 0.88           | 20 | 0.04        |            |
|             | Total                | 27.36          | 24 |             |            |
| WBC         | Between Groups       | 79.21          | 4  | 19.80       | 283.76***  |
|             | Within Groups        | 1.39           | 20 | 0.07        |            |
|             | Total                | 80.60          | 24 |             |            |
| Platelets   | Between Groups       | 1604.93        | 4  | 401.23      | 1384.94*** |
|             | Within Groups        | 5.79           | 20 | 0.29        |            |
|             | Total                | 1610.73        | 24 |             |            |
| Neutrophils | Between Groups       | 206.16         | 4  | 51.54       | 808.49***  |
|             | Within Groups        | 1.27           | 20 | 0.06        |            |
|             | Total                | 207.43         | 24 |             |            |
| Eosinophils | Between Groups       | 0.62           | 4  | 0.15        | 9.54***    |
|             | Within Groups        | 0.32           | 20 | 0.02        |            |
|             | Total                | 0.94           | 24 |             |            |
| Lymphocytes | Between Groups       | 710.88         | 4  | 177.72      | 2649.69*** |
|             | Within Groups        | 1.34           | 20 | 0.06        |            |
|             | Total                | 712.22         | 24 |             |            |
| Monocytes   | Between Groups       | 45.36          | 4  | 11.33       | 176.62***  |
|             | Within Groups        | 1.28           | 20 | 0.06        |            |
|             | Total                | 46.64          | 24 |             |            |
| Basophils   | Between Groups       | 0.95           | 4  | 0.23        | 5.99***    |
|             | Within Groups        | 0.79           | 20 | 0.04        |            |
|             | Total                | 1.74           | 24 |             |            |
| Hb          | Between Groups       | 78.13          | 4  | 19.53       | 290.67***  |
|             | Within Groups        | 1.34           | 20 | 0.06        |            |
|             | Total                | 79.48          | 24 |             |            |
| ESR         | Between Groups       | 10.28          | 4  | 2.57        | 85.84***   |
|             | Within Groups        | 0.59           | 20 | 0.03        |            |
|             | Total                | 10.888         | 24 |             |            |

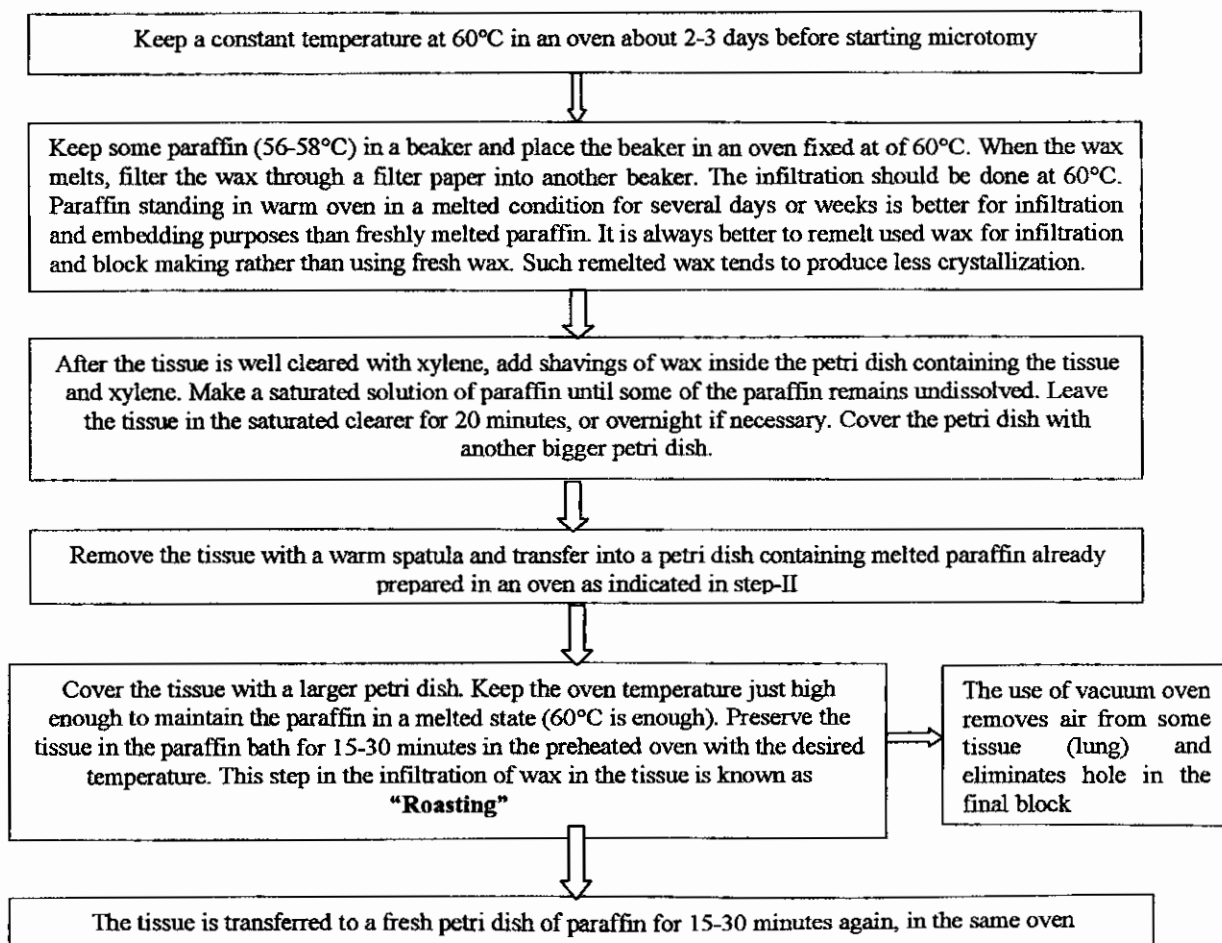
Df= Degrees of freedom; \*\*\*= P&lt;0.001;

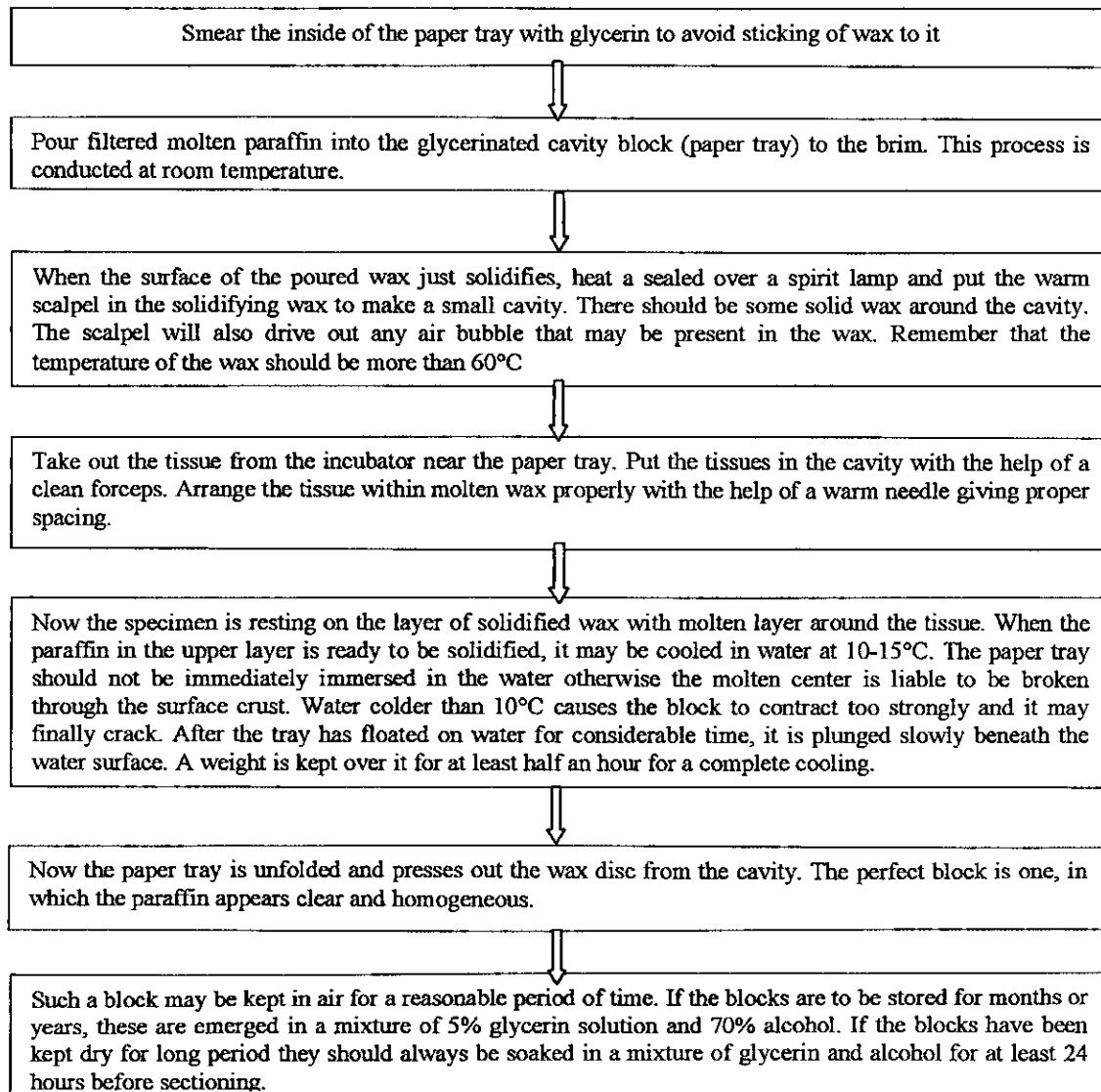
**Appendix 13: Flow chart showing different steps in the dehydration of fixed tissue**



**Appendix 14: Flow chart showing different steps in dealcoholization**



**Appendix 15:** Flow chart showing different steps in the process of infiltration and roasting

**Appendix 16: Flow chart showing different steps in the preparation of paraffin block**

**Appendix 17:** Flow chart showing different Method of stretching and affixing ribbon on the slide