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# DETRIMENTAL INFLUENCE OF CYPERMETHRIN EXPOSURE ON BLOOD INDICES AND BIOCHEMICAL PARAMETERS IN ALBINO RAT

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## ABSTRACT

Cypermethrin is a synthetic pyrethroid insecticide, which is effective against a wide variety of pests. It is widely used in agriculture, public health, and household pest control. However, cypermethrin has many harmful impacts on non-target organisms. This investigation aimed to examine the deleterious impacts which may result from exposure to sub-lethal doses of cypermethrin on body weight, haematological and biochemical parameters, liver and kidney function. The experimental animals were divided into four groups (n=40). The first one served as healthy control animals, while groups II, III, and IV received sub-lethal doses of cypermethrin in drinking water for 60 days. Blood was collected and used for haematological and biochemical analysis. The results revealed that animals received cypermethrin indicated a significant decrease in TEC, PCV, Hb, MCV, MCH and MCHC. While there was remarkable elevation in TLC and platelets. The data also revealed a considerable increase in serum levels of ALT, AST, GGT, ALP, and LDH indicating liver impairment. Moreover, the serum concentration of BUN and creatinine was also elevated, indicating nephropathy. Treatment with cypermethrin has deleterious impacts on experimental animals and induced haematological and biochemical changes. Consequently, this investigation recommends people to intercept any exposure to this toxic chemical to avoid its harmful effects on health and environment.

**KEYWORDS:** Cypermethrin, toxicity, blood indices, biochemical parameters, liver, kidney.

## INTRODUCTION

Globally, pesticides are arguably considered the best means of pest control. Yet, their use has reached worrying levels because of a multitude of negative effects on non-target organisms.[1-3] In the last few decades, pyrethroid pesticides have become well established and are used for numerous purposes, such as the use in agriculture, public health, and household pest control. Nevertheless, this increased dependency is having a greater impact on non-target species.[4,5] This problem has been made worse by the ongoing growth of chemicals, pesticides and associated industry.[6,7]

Cypermethrin is one of the most common insecticides in the world. As a kind of fourth generation synthetic pyrethroid, cypermethrin belongs to the group insecticide which is used in agriculture for pest control and prevention of crop loss. However, its widespread use can have various toxic effects on non-target organisms.[8]

Cypermethrin is hazardous because it is highly toxic and can be inhaled, ingested or absorbed via the skin. Even mild exposure can lead to skin reaction, numbness, tingling, itchy burning sensation of the eye, and irritation. Additionally, loss of control over bladder, convulsions, and death in extreme cases. Owing to cypermethrin's lipophilic characteristics, it results in accumulation in the fatty tissues, kidneys, liver, skin, brain, ovaries, and adrenal glands. The nervous and muscular systems are among the first to be affected. In mammals, Partial or total temporary impairment of the nervous system is caused by over-activation of the central nervous system, and this can also result from over stimulation of gamma-aminobutyric acid levels. In addition, increased free radical formation causing

actions.[9,11] Therefore, this study aimed to evaluate the deleterious impacts which may result from exposure to different doses of cypermethrin on body weight, blood parameters, liver and kidney function.

## MATERIALS AND METHODS

### Animals and Experimental design

The study was performed on twenty male and twenty female albino rat, *Rattus norvegicus albinus*, aged four month. The experimental animals were housed in standard compartmented rectangular and well-ventilated cages. They maintained under typical laboratory conditions, with temperature range of 18-24°C and light-dark cycle of twelve hours. Rats were acclimatized to their new environment for fourteen days prior to study commencement. All animals received humane care in accordance with the guidelines of the national institute of health, USA, for ethical treatment of laboratory animals. All rats had unrestricted access to drinking water and food, *ad libitum*, throughout the experimental duration. They were fed with a standard pellet diet (LabDiet, Missouri, USA) composed of 60% starch, 20% casein, 10% cotton seed oil, 4% salt mixture, 5% cellulose, and 1% vitamin mixture.

### Dosage of Cypermethrin

The experimental animals were divided into four equal groups. Each group consisting of five male and five female independently, and was labelled as group I, II, III, and IV. The first group served as the healthy control animals, while the second, third and fourth groups were treated with 10, 15, and 20 mg/kg body weight of sub-lethal doses of cypermethrin (Sigma-Aldrich Ltd., UK) respectively, mixed into their daily supply of drinking water over 60 days. The weight of each rat was recorded weekly, and their daily water consumption was monitored.

### Blood samples collection and analysis

For haematological and biochemical analysis, blood samples were obtained from each rat individually after twelve hours fasting period. All animals were anesthetized by chloroform and blood samples were drawn instantly from their hearts using heart puncture technique with the use of disposable sterile syringe and needle (Sigma). Blood sample of each rat was then placed in sterile capped tubes containing anticoagulant EDTA (Greiner Bio-One, Frickenhausen, Germany) for haematological examination. In addition, some blood was also placed in other sterile anticoagulant-free tubes and centrifuged at 3000 rpm for approximately 10 min using centrifuge 5418 R (Eppendorf, Ontario, Canada) to separate serum for biochemical analysis.

Blood cell counter URIT-2900 automated haematology analyser (Sysmex Ltd., Germany) was used to examine some haematological indices, including total erythrocyte count (TEC), total leukocyte count (TLC), Packed cell volume (PCV), Haemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT) count.

Serum biochemical parameters were analysed using auto serum analyser, Selectra ProS (Merck Ltd., Germany) and Ecoline kits (Merck Ltd.) following manufacturer's instructions. The examined parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin concentration, gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), the concentration of blood urea nitrogen (BUN), and serum creatinine concentration.

### Data analysis

The collected data from this investigation were analysed using statistical based methods, analysis of variance and student's t-test to assess the difference between parameters. Results were represented as mean values ± standard error, with significance determined at a probability level of less than 0.05 ($P<0.05$).

## RESULTS

The data obtained from this study showed that the mean body weight of the cypermethrin-treated animals was significantly declined with probability of less than 0.05 in all treated groups in both sexes after 60 days of treatment with cypermethrin (table 1). The reduction ratios were 83%, 76%, and 72% in female while 81%, 75%, and 73% in male in comparison to the healthy normal control. The detrimental effect of cypermethrin on the body weight showed a higher decrease as the dosage increased. In addition, an obvious increase in liver, kidney and heart weight of cypermethrin-treated rats was also observed relative to the healthy control Animals.

The findings of the current work also revealed significant elevation ($P<0.05$) in ALT and AST levels in male and female rats treated with cypermethrin (table 2). Their levels were increased paralleled with the increase of cypermethrin concentration. The data in table 2 also displayed significant increase of lactate dehydrogenase (LDH) level, which was gradually elevated with the increasing of cypermethrin dose in both genders of all treated animals. In addition, Serum level of alkaline phosphatase (ALP) was markedly elevated in group II, III, and IV compared to the intoxicated animals. Serum level of gamma-glutamyl transferase (GGT) was also stimulated under the influence of cypermethrin in male and female rats (table 2).

The data also showed considerable reduction ($P<0.05$) in the total erythrocyte count (TEC) following cypermethrin treatment in group II, III, and IV relative to the control animals in both genders (table 3). The results also revealed remarkable decrease in haemoglobin (Hb) level and packed cell volume (PCV). In addition, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean

corpuscular volume (MCV) were also significantly declined in intoxicated rats compared to the healthy animals. Moreover, total leukocyte count (TLC) and platelets (PLT) were remarkably increased with probability of less than 0.05 in male and female rats which were administrated cypermethrin (table 3). Serum levels of urea (BUN) and creatinine were used to assess kidney function in cypermethrin-treated animals compared to the healthy control. The result in table 2 showed significant elevation in blood concentration of BUN and creatinine in rats treated with cypermethrin.

**Table 1: Effect of cypermethrin on body and organs weight of rats after 60 days of treatment**.

| Cypermethrin concentration | gender | Body weight (g) | Liver weight (g) | Heart weight (g) | Kidney weight (g) |
|---|---|---|---|---|---|
| Group I (control) 0 mg/kg b. wt. | ♀<br>♂ | 142.21 ± 1.42<br>146.05 ± 1.31 | 5.59 ± 0.37<br>5.76 ± 0.64 | 0.60 ± 0.38<br>0.62 ± 0.45 | 0.61 ± 0.16<br>0.64 ± 0.20 |
| Group II 10 mg/kg b. wt. | ♀<br>♂ | 127.81 ± 1.87*<br>130.73 ± 2.09* | 6.02 ± 0.78*<br>6.18 ± 0.62* | 0.67 ± 0.48*<br>0.69 ± 0.05* | 0.80 ± 0.25*<br>0.78 ± 0.36* |
| Group III 15 mg/kg b. wt. | ♀<br>♂ | 124.79± 2.28*<br>128.83 ± 2.16* | 6.23 ± 0.41*<br>6.57 ± 0.63* | 0.79 ± 0.37*<br>0.86 ± 0.18* | 0.86 ± 0.41*<br>0.89 ± 0.19* |
| Group IV 20 mg/kg b. wt. | ♀<br>♂ | 112.37 ± 2.53*<br>110.08 ± 1.17* | 6.89 ± 0.67*<br>6.98 ± 0.71* | 0.91 ± 0.08<br>0.93 ± 0.19 | 0.95 ± 0.32*<br>0.99 ± 0.28* |

Values are expressed as mean±SE, n=5, *P<0.05

**Table 2: Biochemical changes in cypermethrin-treated rats after 60 days of exposure.**

| Cypermethrin concentration | Group I (control) 0.0 mg/kg b. wt. | | Group II 10 mg/kg b. wt. | | Group III 15 mg/kg b. wt. | | Group IV 20 mg/kg b. wt. | |
|---|---|---|---|---|---|---|---|---|
| Gender | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | |
| ALT (U/L) | 20.6 ± 1.3 | 22.1 ± 1.83 | 35.3 ± 1.33* | 43.0 ± 1.47* | 45.7 ± 1.35* | 58.4 ± 2.23* | 59.9 ± 1.40* | 73.1 ± 2.06* |
| AST (U/L) | 24.7 ± 1.03 | 27.8 ± 0.53 | 38.4 ± 1.74* | 36.3 ± 1.28* | 43.8 ± 2.11* | 65.5 ± 2.74* | 64.7 ± 2.41* | 69.4 ± 2.01* |
| GGT (U/L) | 41.20 ± 1.05 | 43.10 ± 1.20 | 54.10 ± 2.23* | 51.90 ± 2.48* | 60.87 ± 2.09* | 66.06 ± 2.82* | 77.89 ± 3.36* | 81.69 ± 3.11* |
| LDH (U/L) | 100.4 ± 2.25 | 99.03 ± 2.01 | 109.6 ± 4.31* | 113.2 ± 2.15* | 125.09 ± 2.60* | 130.8 ± 3.11* | 140.7 ± 2.94* | 143.5 ± 3.63* |
| ALP (U/L) | 68.80 ± 1.76 | 67.9 ± 1.08 | 76.8 ± 1.45* | 70.5 ± 1.01* | 88.4 ± 2.20* | 84.6 ± 2.13* | 118.6 ± 3.74* | 114.9 ± 3.26* |
| Creatinine (mg/dl) | 0.86 ± 0.12 | 0.93 ± 0.17 | 1.44 ± 0.38* | 1.51 ± 0.49* | 2.82 ± 0.62* | 3.10 ± 0.29* | 3.67 ± 0.14* | 4.03 ± 0.79* |
| BUN (mg/dl) | 18.75 ± 1.56 | 18.53 ± 1.82 | 19.71 ± 1.65* | 20.09 ± 1.21* | 24.68 ± 1.93* | 23.93 ± 1.28* | 31.44 ± 1.60* | 30.80 ± 1.17* |
| Bilirubin (mg/dl) | 0.84 ± 0.32 | 0.89 ± 0.21 | 2.15 ± 0.40* | 2.36 ± 0.52* | 3.29 ± 0.14* | 3.71 ± 0.76* | 3.94 ± 0.81* | 4.03 ± 0.68* |

Values are expressed as mean±SE, n=5, *P<0.05.

**Table 3: Alterations in blood indices of cypermethrin-intoxicated rats after 60 days of treatment**.

| Cypermethrin dose | Group I (control) 0 mg/kg b. wt. | | Group II 10 mg/kg b. wt. | | Group III 15 mg/kg b. wt. | | Group IV 20 mg/kg b. wt. | |
|---|---|---|---|---|---|---|---|---|
| Gender | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| TLC (× $10^3$ / µl) | 10.12 ± 0.53 | 10.05 ± 0.29 | 10.94 ± 0.68 | 10.73 ± 0.49* | 11.21 ± 0.91* | 10.99 ± 0.34* | 11.64 ± 0.57* | 12.01 ± 0.28* |
| PLT (× $10^3$ / µl) | 462 ± 3.52 | 471 ± 2.87 | 520 ± 4.41* | 539 ± 5.26* | 597 ± 3.79* | 588 ± 4.53* | 630 ± 5.66* | 674 ± 3.18* |
| TEC (× $10^3$ / µl) | 9.53 ± 0.64 | 9.21 ± 0.92 | 8.31 ± 0.80* | 8.70 ± 0.78* | 7.76 ± 0.89* | 8.06 ± 0.67* | 7.01 ± 0.12* | 7.20 ± 0.08* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HGB (g/dl) | 15.74 ± 1.62 | 16.03 ± 1.31 | 13.56 ± 1.74* | 14.21 ± 1.19* | 12.49 ± 1.09* | 13.80 ± 1.67* | 12.04 ± 1.42* | 11.29 ± 1.12* |
| PCV (%) | 49.46 ± 1.84 | 49.28 ± 2.06 | 48.40 ± 1.59* | 47.02 ± 1.60* | 37.85 ± 2.79* | 35.57 ± 1.25* | 34.21 ± 1.18* | 33.08 ± 2.06* |
| MCV (fl) | 64.54 ± 2.75 | 63.62 ± 1.39 | 62.12 ± 2.40* | 62.47 ± 2.05 | 59.73 ± 1.44* | 59.12 ± 2.78* | 57.02 ± 1.91* | 58.11 ± 2.16* |
| MCH (pg) | 21.83 ± 1.74 | 22.71 ± 1.26 | 17.54 ± 1.41 | 18.46 ± 1.53* | 15.73 ± 1.78* | 16.19 ± 1.62* | 14.03 ± 1.51* | 14.62 ± 1.09* |
| MCHC (%) | 33.68 ± 1.35 | 34.11 ± 2.21 | 27.73 ± 1.68* | 27.59 ± 2.60 | 25.71 ± 2.71* | 24.48 ± 1.48* | 23.34 ± 2.26* | 23.09 ± 2.13* |

Values are expressed as mean±SE, n=5, *P<0.05.

## DISCUSSION

Cypermethrin is a synthetic insecticide used in agriculture, public health, household and veterinary applications to control a wide range of pests. However, its widespread use can have adverse toxic effects on non-target species. The current study investigated the harmful impacts of cypermethrin on some haematological and biochemical parameters in male and female rats which received sub-lethal doses of cypermethrin for 60 days of treatment.

### Influence on body and organs weight

The results of this study showed that the mean body weight of the cypermethrin-treated animals was significantly reduced in both genders. The negative effect of cypermethrin on body weight showed a higher decrease as the dosage increased. These findings concur with the observations of earlier studies which stated that cypermethrin induced a reduction in the growth rate of experimental animals which received cypermethrin as part of their diet.[9,10] It has been observed that there is weight loss associated with cypermethrin induced toxicity in rats.[12,13] A reduction in weight gain in both male and female rats has also been observed when lambda cyhalothrin administered orally to the animals[14] The body weight reduction may refer to the effects of cypermethrin on gastrointestinal tract leading to reduced appetite and disrupting the absorption and metabolism of vital nutrients necessary for proper health.[15] or perhaps due to the direct poisoning effects caused by cypermethrin.[16]

In addition, at the end of the experimental period, marked increase was observed in some organs weight including liver, kidney, and heart in male and female treated rats. It has been reported there was an increase in kidney weight following exposure to cypermethrin.[17] Furthermore, this suggests that the probable congestion of vessels as well as lymphocytic infiltration may explain the increase in kidney size.[18] In addition, these observations concur with the findings of previous study reported alterations in body and organ weight in rats treated with cypermethrin.[19,11] and in rabbits also.[20] The detected increased in organs weight under the effect of cypermethrin might be due to the necrosis and apoptosis which accompanied by the accumulation of lipids in the examined organs.

### Effects on biochemical parameters

Liver is one of the most important organs in the body, particularly in detoxification of endogenous and exogenous compounds. It performs crucial functions in metabolism and bioconversion of toxic compounds, which may lead to xenobiotic stress. Loss of liver homeostasis or liver constancy under such stress is enough to disrupt the body physiological functions.[21] Liver also acts as protein synthesis hub orchestrating several cellular functions like providing structural integrity to cells, controlling materials' flow through cell membranes, performing a huge number of chemical reactions, regulating the metabolites channels, controlling metabolic concentration, and spatial regulating of nuclear materials to fine-tune gene expression.[22,23] Liver malfunction could lead to hepatotoxicity which represents a serious health problem.

To evaluate the effect of cypermethrin on liver, serum concentration of AST and ALT were investigated. Liver biomarker enzymes including AST, ALT and ALP have been associated with liver impairment and injury. They are widely used to assess liver function. Liver abnormality was indicated by the increase in serum concentration of aminotransferases which are crucial in amino acids biosynthesis and catabolism.[24,13] The observations of the current study showed significant increase in serum level of ALT and AST in male and female of the intoxicated rats. The levels of these enzymes were elevated paralleled with the increase of cypermethrin doses. This increase might be due to increasing of cell membrane permeability or cell membrane damage of hepatocytes under the influence of cypermethrin.

Raised concentration of serum ALT and AST, through the monitored levels, indicated stimulating hydrolysis of amino acids in energy-yielding metabolic pathways like gluconeogenesis. Hepatic transaminases, such as AST and ALT, are very sensitive indicators of hepatocellular injury under the condition of xenobiotic metabolism which induced oxidative stress.[25,26] High levels of serum transaminases are attached by high liver microsomal membrane fluidity, production of free radicals, and alteration in hepatocytes. The elevated activity of hepatic AST and ALT caused by cypermethrin detoxifying pathways, refers to genetic

abnormality for overproduction of these enzymes to cope with the pyrethroid-induced oxidative stress.[27,31]

The present study also investigated the changes in serum level of ALP under the influence of cypermethrin. The examined values of ALP were significantly elevated compared to healthy animals. The effect was raised paralleled with the increase in cypermethrin dose. ALP is an important enzyme which catalyzes the hydrolysis of large number of phosphoric acid esters in alkaline medium. It is a lysosomal enzyme which plays pivotal function in metabolism and biosynthesis of energetic macromolecules, which are essential for many physiological processes in the body.[32] Cell membrane damage could result in releasing ALP from liver cells into the blood stream. Therefore, this led to interrupting and ceasing the normal processes within hepatocytes, which caused abnormal alterations, including pyknosis and necrosis.[33,35] It has been reported an increasing in serum ALP activity might be resulting from liver, kidney, and bone damage leading to leakage of ALP.[36] These results are accorded with other findings.[37,38] in which stimulation of ALP had been noted in rats under the effect of cypermethrin.

Elevated serum level of GGT in the current investigation, under the effect of cypermethrin was also observed in intoxicated animals of both genders. This increasing in serum GGT might be an indication of hepatotoxicity and oxidative damage in liver cells.[39,40] Data also revealed an increasing of LDH in male and female rats received sub-lethal doses of cypermethrin. LDH is an essential oxidative enzyme involved in carbohydrate metabolism due to its role in transformation of pyruvate into lactate. The heightened level of serum LDH under cypermethrin influence may refer to hypoxia which causes a switch from aerobic to abnormal anaerobic respiration.[5,28,41,42]

The raised level of serum bilirubin following cypermethrin exposure might be due to an induction of heme-oxygenase which plays a vital role in heme catabolism and conversion to bilirubin.[43] The treatment with cypermethrin in our study, resulted in remarkable elevation in serum levels of BUN and creatinine in male and female treated rats. It has been observed an increase in blood urea concentration accompanied by raising rate of protein breakdown in mammals.[13,44] In addition, this might result from stimulating more conversion of ammonia to urea due to increased formation of enzymes which catalyze more urea production.[44,45] Similar observations indicated increase in serum urea and creatinine concentrations in rats received acute and chronic doses of cypermethrin.[13,46,48] Creatinine is a metabolite of creatine which is released entirely into urine. Therefore, its increased concentration in serum might be due to kidney dysfunction, which is considered as functional evidence of cypermethrin induced nephrotoxicity.[49]

**Effects on blood indices**

The data obtained from the present work indicated significant decline in TEC following cypermethrin exposure. In addition, there was a marked reduction in the levels of Hb, PCV, MCV, MCH, and MCHC, in cypermethrin-treated rats. The blood content of Hb and PCV are directly associated with erythrocytes count, therefore any drop in their values suggests an inhibition in the process of erythropoiesis.[50] It has been reported declines in TEC, PCV and hemoglobin concentration in rats weighing 200g which received 14.5mg of cypermethrin orally for 30 days.[51] Other studies observed significant dose dependent reduction of erythrocytes count, haematocrit, and MCH values when rats fed cypermethrin orally.[9,39,48,52] Another report indicated that crossbred cow calves showed a significant decrease in TEC and PCV, after being administered 200mg of cypermethrin over 14 days.[53] Alterations in TEC, HB, and haematocrit levels have been described in female Swiss mice following exposure to 5mg and 25mg of cypermethrin, which resulted in a significant decrease in these parameters.[54] The reduction of TEC and associated indices in the current study, might be attributed to the toxic effect of cypermethrin on cell metabolism, interaction with some reactions where calcium is their secondary mediator, and inhibition of some enzymatic activities which play key role of heme biosynthesis, resulting in shortening erythrocyte life span and decrease the production of haemoglobin.[55] The described decrease in Hb content might be a result of increased destruction of red blood cells and/or reduced production of erythrocytes.[56] The decline in RBC count may refer either to excessive loss of erythrocytes or to factors involved in depressing RBCs production. Previous investigations illustrated the direct impact of pesticides which reported a reduction in TEC, PCV and Hb content.[26,47,57,60] It is most likely that these changes occur as a result from increase the rate of RBCs breakdown, as well as the toxic effects associated with pesticide exposure on the bone marrow.

The study also showed that TLC was also significantly elevated in male and female rats received sub-lethal doses of cypermethrin. The increase in white blood cells count may be attributed to mobilization induced by the activation of animals' defense mechanisms as well as the immune system. When male rats intoxicated with 40mg of cypermethrin in groundnut oil for 90 days, a considerable increase of TLC was reported.[56,61] A significant elevation in TLC, monocytes and lymphocytes was also observed, when Swiss mice weighing 21-24g were intoxicated orally with 5-25mg of cypermethrin.[54,62] In addition, platelets (PLT) count in the current investigation revealed a remarkable increase in cypermethrin-treated rats in both genders. This may be due to thrombocytopenia followed by thrombocytosis.

## CONCLUSION

Cypermethrin is a synthetic pyrethroid used effectively as insecticide. It is broadly used in agriculture, public

health, and domestic pest control. However, Cypermethrin toxicity is associated with number of physiological and biochemical alterations. Exposure to sub-lethal doses of cypermethrin may result in deleterious impacts on non-target organisms and induced haematological and biochemical changes. Therefore, this study advises people to prevent any direct contact to cypermethrin to avoid its harmful effects on health.

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