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(RESEARCH ARTICLE)

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Preventive effects of selenium against cypermethrin induced haematological toxicity in Sprague-Dawley rats

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Abstract

Purpose: Cypermethrin (class II) belongs to pyrethroid insecticides which have been in wide use to control various pests including moth pests of cotton, fruit and vegetable crops. This study was aimed to investigate the effects of cypermethrin on haematology of female rats and their modulation by co-administration of sodium selenite.

Methodology: Four equal groups of female rats were made (n=20). Group 1 received 1 ml corn oil, Group 2 was administered 55 mg/kg/b.w of cypermethrin, Group 3 was provided with 1 ppm of sodium selenite and Group 4 was given both cypermethrin and sodium selenite. Haematological analysis of blood samples was performed employing Sesmex Kx-21 (Japan).

Results: Red blood cell (RBC) count, haemoglobin concentration (Hb) and hematocrit (Hct) were significantly reduced ($P<0.05$) due to cypermethrin treatment which indicates that rats were suffering from anaemia. While, the rest of the hematological parameters i.e. platelet count (PLT), number of lymphocytes, white blood cell (WBC) count, platelet larger cell ratio (P-LCR), RBC distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), lymphocyte percentage (LYM), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were non-significantly affected. Selenium coadministration with cypermethrin was found to restore the levels of above-mentioned parameters to those found in control group.

Conclusion: Hence, it can be concluded from this study that cypermethrin administration can cause hematological alterations in rats. Co-administration of selenium provided protection against cypermethrin toxicity to some extent.

Keywords: Cypermethrin; Selenium; Pyrethroid; Haematology

1. Introduction

A pesticide is a substance or a mixture of substances which is used against pests [1]. It might include a chemical substance, antimicrobial, biological agent (such as bacteria or a virus), device or disinfectant used to kill any pest. Many insects, weeds, plant pathogens, molluscs, nematodes, fish, birds and mammals are included in pests that destroy property, spread diseases, cause a nuisance and compete with humans for food [2].

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Pyrethroid insecticides have been employed in veterinary, home and agricultural formulations for many decades and comprises one-fourth (approx.) of the worldwide insecticide market. They are synthetically derived from pyrethrins which are toxic components present in the flowers of *Chrysanthemum cinerariaefolium* [3].

They are topically applied to control a vast majority of agricultural insects, veterinary pests and the insect vectors of disease [4]. In the developed world, severe pyrethroid insecticide poisonings are not common. But these are commonplace in the developing countries due to extensive use of pyrethroids in agriculture [5].

The racemic mixture of eight isomers including four cis and four trans isomers constitutes the technical grade cypermethrin. The most active are the two stereoisomers that are termed α−isomer of cypermethrin or α−cypermethrin [6]. Cypermethrin was effective in controlling many species of pests in animal breeding, household and agriculture, hence it was allowed for turnover in 1977 as a very active synthetic pyrethrin insecticide [7, 8].

Cypermethrin persists on furniture, walls and in air for about three months after household treatments [9]. It is not devoid of side effects despite the wide range of effectiveness. Cypermethrin causes toxic effects not only in insects but also in mammals [10, 11]. There are many signs reported in animals after ingestion of large doses of cypermethrin including ataxia, muscular tremors, coma, weakness of limbs, convulsions and death from respiratory depression. Its dermal contact may cause a subjective sensation of numbness or tingling in facial area [12]. It also causes irritation in skin and eyes. The rabbits treated with cypermethrin have been observed to show skin irritation (slight to severe) as well as decline in food consumption, body weight, relative and absolute gonad weights [13].

A Swedish chemist, Jons Jacob Berzelius, discovered selenium in 1817, while investigating a red deposit on the wall of lead chambers used in sulphuric acid production. Since then, fundamental importance of different forms of selenium to human health and toxicity have been extensively studied [14]. It is obtained from different dietary sources such as grain products, cereals, seafood, vegetables, meat and richly present in Brazil nuts [15-17]. Selenium is a powerful catalytic element and the active center of about twenty eukaryotic proteins, some of them possess redox regulatory (predominantly antioxidant) potentials [18]. Being a component of these selenoproteins, it exerts metabolic functions associated with maintaining defenses and integrity of organism and as a chemoprotective agent having carcinostatic ability [19].

Selenium is an indispensable nutrient for domestic animals [18]. Its intake should be enough to fulfill physiological needs as indicated by classical selenium-dependent biochemical functions. In animal feed, the range of optimum safe level of selenium is 0.25–0.85 ppm. However, the beneficial effect of selenium on several components of the immune system may be essential in disease prevention at higher nutritional intake [20-24].

Hematological values are generally employed to define physiological adaptations and systemic relationship including assessment of animal's general health condition. Indeed, clinical chemistry and hematology results add essential point of view to identify target organs and may help in understanding mechanism of action. This information is important for the assessment of risk before the commence of microscopic evaluation [25].

2. Material and methods

2.1. Animal maintenance

The present study was performed on 20 adult female (8-10 weeks old) Sprague-Dawley rats with an average weight of approximately 180 g. The animals were purchased from National Institute of Health Islamabad (NIH), and maintain in the Animal House facility of Quaid-i-Azam University under standard laboratory conditions where they acclimatized for a week, and fed standard rat chow and water *ad libitum*. The temperature was maintained at an average of 25°C with air condition system during the experimental period where light-dark photoperiod was 12 hrs. Stress that might have occurred due to crowding was avoided by grouping five animals per cage. Good health status was also monitored. The animal handling and subsequent killings were performed in accordance with the guidelines provided by the ethical committee of the Department of Zoology, Quaid-i-Azam University, Islamabad.

2.2. Chemicals

Alpha Cypermethrin [(RS)-cyano-(3-phenoxyphenyl)methyl-(IRS)-cis-trans-3-(2,2 dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate] and sodium selenite Na_2SeO_3 (Surechem, England) were used in the experiment.

Commercial pyrethroid product Ripcord procured from the local market containing 10% cypermethrin was used as test compound. Ripcord (Basf Aktiengesellschaft, Germany) contained cypermethrin (100 g/l) and a mixture of petrol (820 g/l) and xylene. It was in the emulsion form out of which adequate dilutions were made in corn oil to obtain test concentration (55 mg/kg). The percentage of active ingredient of commercial formulation of cypermethrin was used to calculate the test concentration of cypermethrin. The solutions were prepared freshly immediately before usage. Rest of the reagents used in the study were obtained from Sigma and of analytical reagent grade (St. Louise, U.S.A).

2.3. Experimental design and treatment

The effects of cypermethrin and selenium on the hematological parameters were determined by using 20 mature female rats which were randomly split into four equal groups of each having 5 rats.

Group one was taken as control. Cypermethrin (55 mg/kg body weight), sodium selenite (1 ppm) or their combination were given to groups two, three and four respectively.

The oral LD_{50} in male and female rats were found to be 187 to 326 and 150 to 500 mg/kg b.w [26, 27] respectively. Therefore, a low dose of 55 mg/kg body weight of rats was used for this sub-chronic study for 21 days.

Cypermethrin was orally given in corn oil (1 ml) by gavage, and control rats received a corresponding amount of corn oil in the same manner for 21 days. Similarly, selenium was given in drinking water as sodium selenite (Na_2SeO_3) daily.

The doses were given to the non-fasted rats daily in the morning. The actual volume of the doses given was according to the body weight which was recorded daily during the dosing period. The rats were observed daily and the behavior of the cypermethrin treated rats was compared with those of control animals which were kept under the same conditions as the experimental and received corn oil as treatment.

2.4. Dissections

The animals were sacrificed after the desired experimental time period i.e. 21 days. Animals were anesthetized with an overdose of chloroform according to the guidelines provided by National Institute of Health Islamabad, on human use of animal for basic and medical research. Abdomen was cut opened and blood was aspirated through cardiac puncture using 10 ml sterile syringes. Blood was collected in K_3 EDTA vacutainers for hematological analysis.

2.5. Hematological analysis

The haematological analysis of blood samples was performed using Sesmex Kx-21 (Japan) to study haematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) concentration, haematocrit (Ht), platelet count (PLT), number of lymphocytes, mean platelet volume (MPV), platelet larger cell ratio (P-LCR), RBC distribution width (RDW), platelet distribution width (PDW) and lymphocyte percentage (LYM).

Erythrocyte indices including mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were also computed.

2.6. Statistical analysis

Data are expressed as mean ± SEM (standard error of mean). Statistical analysis was done using GraphPad Prism 5. The statistical significance of differences between the means was assessed by single factor analysis of variance (ANOVA). Dunnett test was used to compare the parameters of treatment groups with those of control. Tukey's test was applied to carry out the comparison among different treatment groups. A difference at $P < 0.05$ was considered significant.

3. Results

3.1. Hematological Parameters

The results of hematological analysis in control and treatment groups of rats are given in table no. 1 and 2.

3.2. Cypermethrin treatment

Cypermethrin caused a significant ($P<0.05$) decline in red blood cells number (RBCs), haemoglobin concentration (Hb) and hematocrit (Hct) (Fig 1 & 2). The values of mean corpuscular haemoglobin (MCH) and mean red blood cell volume (MCV) decreased while mean corpuscular hemoglobin concentration (MCHC) increases (Table 1).

There was no statistically significant increase in white blood cell (WBC) count, percentage of lymphocytes, mean platelet volume (MPV) and platelet larger cell ratio (P-LCR) (Fig 3 & 4). A decline in platelet count and RBC distribution width (RDW) (non-significant) was observed. The value of platelet distribution width (PDW) increases significantly ($P < 0.05$) (Fig 1, 3 & 6; Table 2).

3.3. Sodium selenite treatment

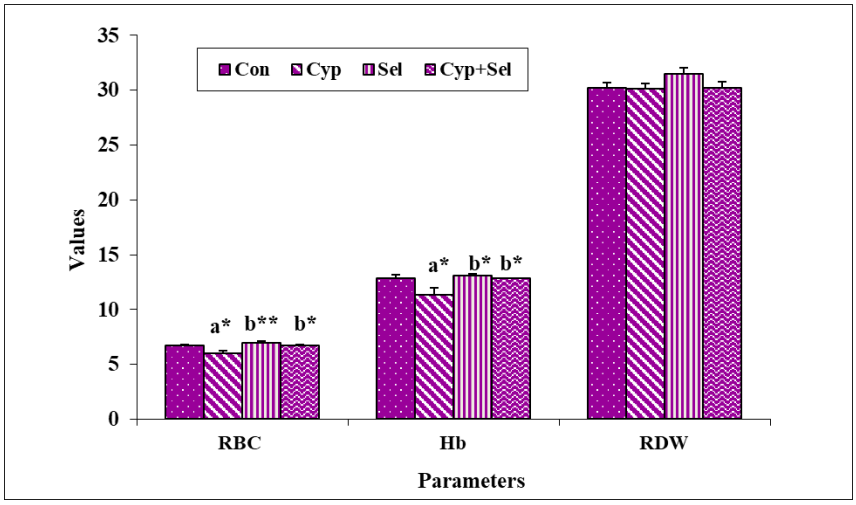
Sodium selenite resulted in increased values of red blood cell (RBC) number, haemoglobin (Hb) concentration, hematocrit (Hct), mean red blood cell volume (MCV), white blood cell (WBC) count, number of lymphocytes, mean platelet volume (MPV), platelet larger cell ratio (P-LCR), platelet count and RBC distribution width (RDW) (non-significant). While mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) decreases non-significantly (Table 1 & 2). There was a significant increase observed in red blood cells number, haemoglobin concentration, hematocrit and mean red blood cell volume when compared to cypermethrin treated group (Fig 1 & 2).

3.4. Cypermethrin + Sodium selenite treatment

Table 1 Blood parameters of the female Sprague-Dawley rats exposed to sub-chronic dose (21 days) of alpha-cypermethrin. Values are given as ± SEM (n=5), $p < 0.05$.

Blood Parameters	Control	Cypermethrin	Selenium	Cypermethrin + Selenium
RBC ($10^3/\mu l$)	6.686 ± 0.123	6.03 ±0.224 ^{a*}	6.99 ± 0.088 ^{b**}	6.712 ± 0.087 ^{b*}
Hb (g/dl)	12.88 ± 0.270	11.36 ± 0.643 ^{a*}	13.1 ± 0.173 ^{b*}	12.82 ± 0.037 ^{b*}
Hct (%)	38.26 ± 0.868	33.6 ± 1.606 ^{a*}	41.02 ± 0.795 ^{b***}	37.92 ± 0.598 ^{b*}
MCV (fl)	57.24 ± 0.463	55.58 ± 0.865	58.68 ± 0.696 ^{b*}	56.48 ± 0.606
MCH (Pg)	19.26 ± 0.278	18.78 ± 0.493	18.72 ± 0.193	19.10 ± 0.202
MCHC (g/dl)	33.66 ± 0.366	33.74 ± 0.366	31.94 ± 0.625	33.80 ± 0.484
RDW (fl)	30.22 ± 0.505	30.1 ± 0.484	31.46 ± 0.568	30.2 ± 0.600

a Significantly different from Control ; b Significantly different from Cypermethrin ; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

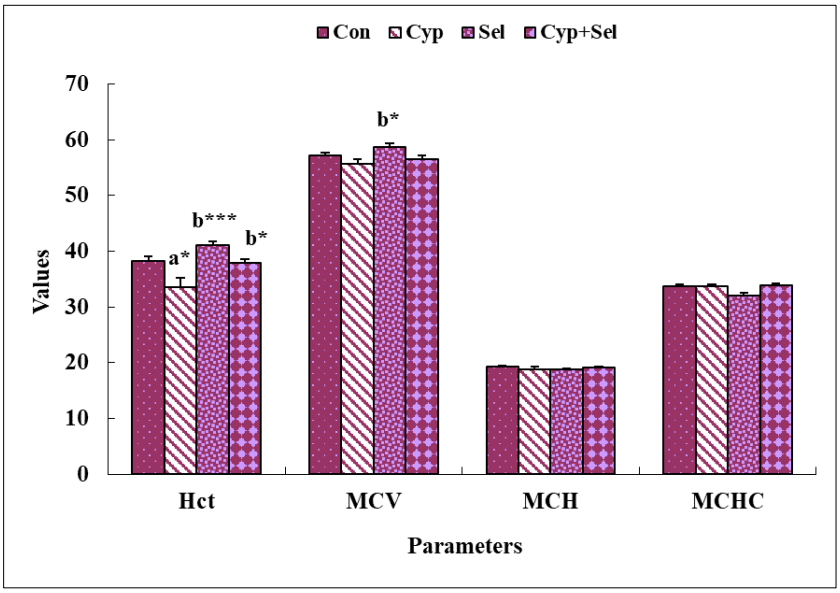


a Significantly different from Control; b Significantly different from Cypermethrin; * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$

Figure 1 Changes in red blood cell (RBC) count ($10^3/\mu l$), haemoglobin concentration (g/dl) and red blood cell ditribution width (RDW) (fl) of female rats in control and treatment groups

Selenium maintained the values of red blood cell number, haemoglobin concentration, hematocrit, mean corpuscular haemoglobin, mean red blood cell volume, mean corpuscular hemoglobin concentration and RBC distribution width close to normal (Table 1). While the values of white blood cells count, number of lymphocytes, mean platelet volume,

platelet larger cell ratio, platelet count and platelet distribution width increased non-significantly as compared to control (Fig 3-6; Table 2).



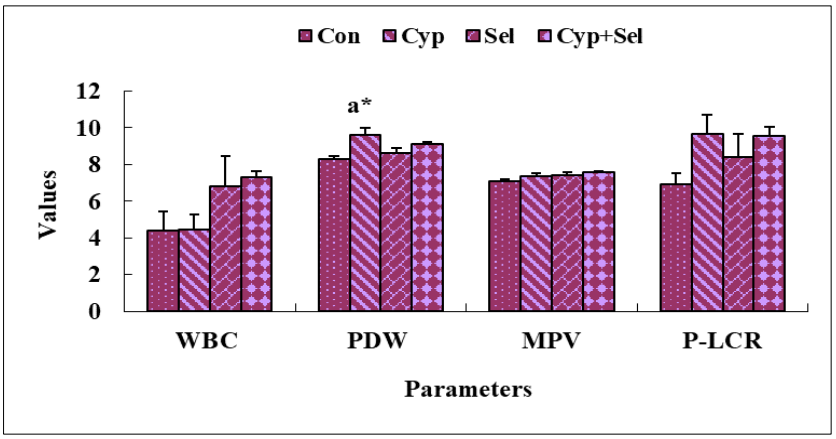
[a] Significantly different from Control ; [b] Significantly different from Cypermethrin; * p < 0.05; ** p < 0.01;*** p < 0.001

Figure 2 Changes in hematocrit concentration (%), mean red blood cell volume (MCV) (fl), mean corpuscular haemoglobin (MCH) (Pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl) of female rats in control and treatment groups

Table 2 Blood parameters of the female Sprague-Dawley rats exposed to sub-chronic dose (21 days) of alpha-cypermethrin. Values are given as ± SEM (n=5), p < 0.05

Blood Parameters	Control	Cypermethrin	Selenium	Cypermethrin + Selenium
WBC (10^3/μl)	4.42 ± 1.04	4.44 ± 0.807	6.82 ± 1.654	7.32 ± 0.293
LYM (%)	82.88 ± 7.664	83.9 ± 5.931	84.46 ± 3.211	86.7 ± 3.342
LYM (#)	2.84 ± 0.732	3.74 ± 0.666	5.88 ± 1.448	5.16 ± 0.581
PLT (10^3/μl)	924.4 ± 31.66	746.8 ± 171.8	940.4 ± 95.45	772.8 ± 31.51
P-LCR (%)	6.940 ± 0.577	9.68 ± 1.038	8.42 ± 1.256	9.560 ± 0.512
PDW (fl)	8.33 ± 0.151	9.6 ± 0.378 [a*]	8.66 ± 0.298	9.12 ± 0.128
MPV (fl)	7.08 ± 0.106	7.34 ± 0.166	7.42 ± 0.164	7.6 ± 0.044

a Significantly different from Control; b Significantly different from Cypermethrin; * p < 0.05; ** p < 0.01; *** p < 0.001



a Significantly different from Control; b Significantly different from Cypermethrin; * p < 0.05; ** p < 0.01;*** p < 0.001

Figure 3 Changes in white blood cell (WBC) count ($10^3/\mu l$), platelet distribution width (PDW) (fl), mean platelet volume (MPV) (fl), platelet larger cell ratio (P-LCR) (%) of female rats in control and treatment groups

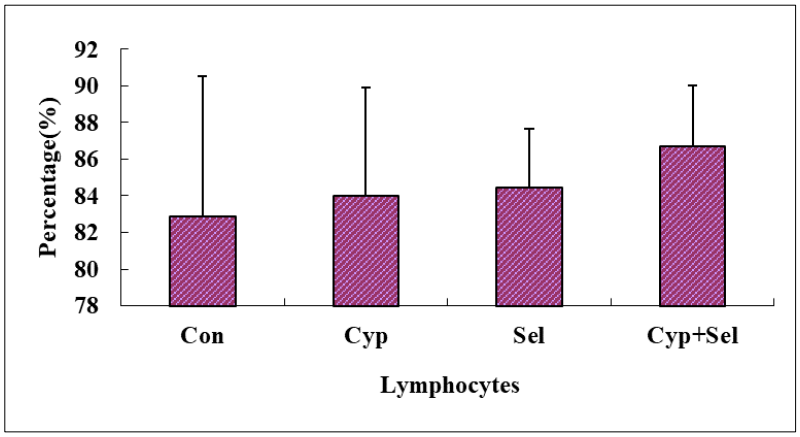


Figure 4 Changes in lymphocytes percentage (%) of female rats in control and treatment groups

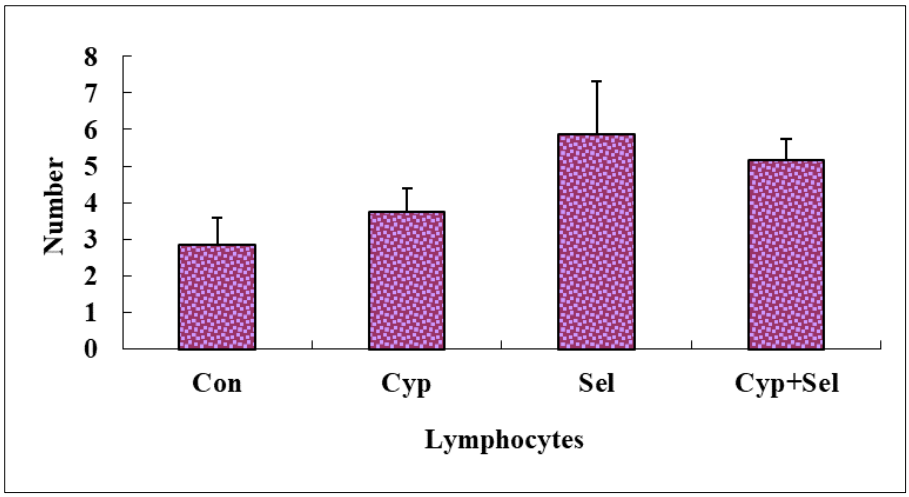


Figure 5 Changes in lymphocytes number of female rats in control and treatment groups

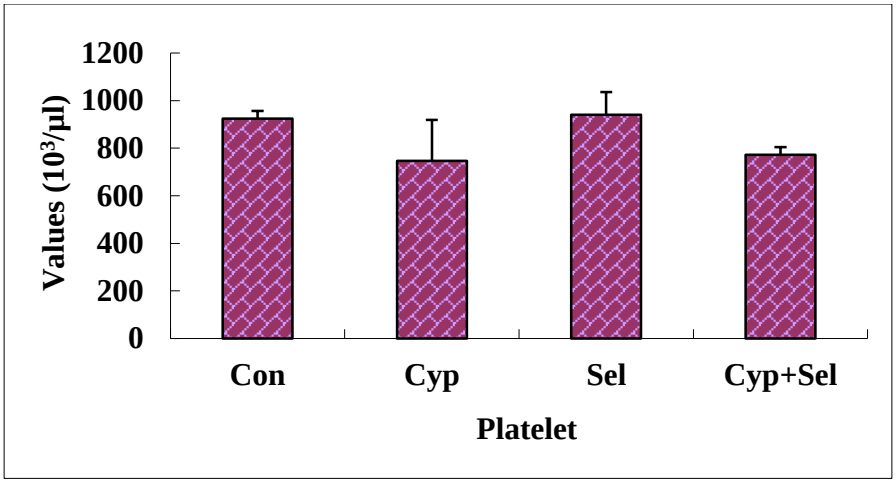


Figure 6 Changes in platelet count ($10^3/\mu l$) of female rats in control and treatment groups

4. Discussion

Pesticides are extremely toxic compounds that are introduced intentionally in huge quantities to the environment unlike other pollutants. There is usually a requirement to apply pesticides periodically in conventional forms like concentrated emulsions, granules or powders, which are directly sprayed or dusted on the required area. Such types of applications results in many environmental problems including soil, water and atmospheric contaminations, thereby affects the aquatic and terrestrial ecosystems. There can be a direct contact with the insecticide during involuntary exposure or its application in treated zones (buildings etc.) and indirectly by consuming contaminated water, vegetables and meat [28].

In Pakistan, about 70% of the population resides in villages and most of them are directly or indirectly involved in agriculture. The farmers apply synthetic pesticides on crops to protect them from pest attacks. The use of synthetic pesticides by the farmers is alarmingly increasing. There are also many malpractices such as spraymen do not follow the necessary precautions. It causes hazardous accidents and many problems including resistance in pests, pesticidal pollution, accumulation of pesticide residues in the body of human beings and animals [29]. Moreover, most of the pesticides commonly employed in agriculture, have the ability to cause DNA damage as shown by different studies [30-32].

Ripcord is a widely used commercial product which contains pyrethroid compound. In general, people are not exposed to pure chemical compounds but to the commercial products. Hence, the commercial product with known concentrations of cypermethrin in a suitable solvent was selected for the current study. Another reason to choose commercial product is the low solubility of pyrethroids in water, which would make the use of solvents necessary in any case [33].

A significant decline was observed in the hematocrit values, haemoglobin and red blood cell number in the rats treated with cypermethrin. The decrease in red blood cell count could be due to haemolysis caused by pyrethroids (type II) which leads to reduced erythropoiesis and haemorrhages [34]. Lymphatic vessels absorb some erythrocytes (autotransfusion) in internal haemorrhages specifically in body cavities. Rest of the red blood cells are phagocytosed or lysed [35]. Similar results have been reported by various authors with the cypermethrin treatment in rats [36], rabbits [37, 38], goats [39] and sheep [40].

The reduction in rate of red blood cell formation or increased rate of destruction and impaired biosynthesis of haem in bone marrow can cause reduction in Hb contents [41]. The disruptive action of the pesticides on the erythropoietic tissue might affect the viability of the cells which could also be a contributing factor in the decline of haemoglobin contents and red blood cells. The changes in the haematological parameters by cypermethrin resulted in anemia due to decreased synthesis of red blood cells [42]. Moreover, the hyperactivity of bone marrow may attribute to reduction in RBC and Hb [43] by producing red blood cells with impaired integrity which were easily destroyed by reticulo-endothelial system in circulation. Shakoori *et al.,* [44] suggested that the decline in red blood cell count in rabbits is either an indicative of inhibition of erythrocyte formation or excessive damage to erythrocytes.

Mean corpuscular volume (MCV) decreases non-significantly, while the values of MCHC and MCH revealed no pronounced effect in cypermethrin treated group. These results agree with the work of El-Zayat *et al*., [45] on deltamethrin.

There was no significant change observed in these parameters in the rats treated with selenium alone as compared to control, and improved the toxic effects of cypermethrin. Selenium increases the values of RBC, Hb, Ht, MCV and RDW, while MCH and MCHC decreases non significantly. In cypermethrin and selenium combined group these parameters remained close to the control values.

Percentage of lymphocytes, P-LCR, MPV, WBC and PDW increases in cypermethrin treated group as compared to control (non-significantly). The rise in white blood cells might indicates activation of immune and defense system of the body [37]. It may causes an increase in release of WBC into the blood from bone marrow storage pool. The excitement, apprehension, fear and pain encountered by the rats during the treatment may have resulted in lymphocytosis [46].

All the above parameters were increased in selenium alone and combined group as compared to control except platelet number which were less than those of control in cypermethrin and selenium combined group. Bednarek *et al*., [47] observed that injection of selenium to calves resulted in higher WBC count. We observed similar results in this study.

5. Conclusion

The overall results of this study shows that cypermethrin induces hematological alterations in rats. Co-administration of selenium provided protection against cypermethrin toxicity. Further studies are required to assess the dose and route of administration of selenium to enhance its protection against cypermethrin.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All animal handling and subsequent killings were done according to the guidelines provided by the ethics committee of the Department of Zoology, Quaid-i-Azam University, Islamabad.

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