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Protective Role of Ashwagandha and Quercetin over Cypermethrin Induced Hypothyroidism: A Hematological Study in Mice

Narendra Namdev, Gayatri Rai, Payal Mahobiya*

Endocrinology Lab, Department of Zoology, School of Biological Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University) Sagar (M.P.), India
*Corresponding Author: 1607payal@gmail.com

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Abstract Cypermethrin is a synthetic pyrethroid insecticide used worldwide for domestic as well as agriculture purposes. The current research is designed to investigate the protective role of ashwagandha and quercetin on cypermethrin-induced hematological alteration via declined thyroid function. *Swiss albino* male mice weighing around 25–30 g were divided into four groups of six animals each at random. Group I served as a control with normal food and water; Group II cypermethrin treated 15 mg/kg body weight; Group III ashwagandha co-treated 200 mg/kg body weight, and Group IV quercetin co-treated 150 mg/kg body weight. Doses were applied orally for 28 days. Blood was drawn, and hematological parameters and a serum thyroid hormone calculation were performed on it. Increased body weight and decreased thyroid weight were observed in the cypermethrin exposure group. The decreased T3 and T4 and increased TSH hormone levels were observed in the cypermethrin-treated group as compared to the control signified hypothyroidism. A significant decrease in erythrocyte count, hemoglobin percentage, and platelet counts was observed in the cypermethrin exposure group. Total leukocyte counts, neutrophil%, and lymphocyte% were increased significantly in the cypermethrin exposure group as compared to the control. A significantly decreased platelet count was observed in the cypermethrin exposure group. The antioxidant co-administered animals showed significant prevention as compared to cypermethrin-exposed animals.

Keywords Cypermethrin, Ashwagandha, Quercetin, Thyroid Hormones, Hematological Indices

1. Introduction

Pesticides are used against the organism detrimental to crops, human beings, and other animals, but their poisoning causes morbidity and mortality in developing countries like India. More than 3 million cases most resulting in death have been reported due to poisoning and suicidal attempts related to pesticides [1]. Synthetic pyrethroids are the analogs of naturally occurring pyrethrin found in chrysanthemum and daisy and are chemically and synthetically modified and become more stable and toxic to target species. Increased production and use of synthetic pyrethroids were first documented in 1970 and replaced the use of organophosphates in residential and veterinary use, which increases human exposure to pyrethroids [2]. Exposure to pyrethroid and other pesticides is categorized into occupational exposure and regular exposure. Occupational exposure happens during manufacture and transportation in industries to workers and farmers during application in fields. Regular or non-occupational exposure includes ingestion of contaminated fruits, vegetables,

water, and dairy products [3].

Cypermethrin is used for plant protection by insects and is used in veterinary and households commercially. The household use of cypermethrin increases its exposure to manifolds. Mass exposure to synthetic pyrethroids leads to pathological, physiological, and behavioral changes in mammals. Numerous studies have documented the tragic effects of pyrethroid exposure on the physiology of reproduction, endocrine, neurodevelopment, and immunological system [4]. Exposure to chemical toxicants like cypermethrin cause degradation in biomacromolecules and system dysfunction *via* induced oxidative stress [5].

It is a biological phenomenon in that free radicals are produced and come into contact with antioxidants. Exposure to toxicants like pesticides, radiation, pollutants, heavy metals, etc. increases their generation manifolds leading to oxidative stress and resulting in system dysfunction. Exposure to UVB radiation imparts free radical generation, which leads to altered thyroid hormone synthesis affecting the estrous cycle and reproduction in female Wistar rats [6].

Triiodothyronine (T3) and thyroxine (T4), which are released directly into the blood, are used to control basal metabolism, body temperature, and iodine balance. Thyrotropin-releasing hormone (TRH) triggers the anterior pituitary to release thyroid-stimulating hormones (TSH) which stimulates the thyroid gland to release thyroid hormones [7]. Different kinds of synthetic pyrethroids (cypermethrin, cyfluthrin, cyhalothrin, fenvalerate, deltamethrin) have TH signaling disrupt potential and exert an antagonistic effect on TH receptors [8]. The previously reported studies show thyroid hormone regulates reproductive functioning in both males and females. The level of thyroid hormone affects fertility and infertility [9,10].

The decline in circulating T3 and T4 hormones and elevated TSH levels suggest primary hypothyroidism. The oral administration of synthetic pyrethroid insecticide lambda-cyhalothrin to rats causes decreased T3 and T4 serum hormone concentration while increased TSH along with thyroid DNA damage suggesting primary hypothyroidism [11]. For many other chemical compounds like a biocide compound tributyltin chronic exposure causes declined T3 and T4 and elevated TSH concentrations pointing toward hypothyroidism along with many other thyroid disorders [12].

Through both genetic and non-genomic mechanisms, the thyroid hormone controls the maturation and synthesis of red blood cells [13]. Hypothyroidism could induce an anemic condition such as decreased RBC count and Hb percentage in humans and also in rats [14,15]. Antithyroid agents like PTU (propylthiouracil) and methimazole could reduce erythrocytes count, hematocrit value, and hemoglobin % probably due to the suppression of bone marrow. The total and differential leucocyte counts were decreased under hypothyroidism induced by PTU [16]. In rabbits, the decreased coagulating action was reported after aflatoxin intoxication. The total circulating and peripheral platelet counts were decreased in hypothyroid conditions, probably due to depressed platelet production affecting the coagulating property [17].

In the present investigation, ashwagandha and quercetin are the antioxidants that encounter cypermethrin-induced oxidative stress. Ashwagandha is traditionally being used as medicine in the ayurvedic medicine system. The steroidal lactones Withanolides and Withaferin A are two biologically active compounds of ashwagandha having antioxidant and immunomodulatory actions [18]. Ashwagandha has medicinal importance and shows corrected blood cell count, hemoglobin, and hemolytic antibody response in case of pesticide intoxication [19]. Quercetin (3,3',4',5,7- pentahydroxy flavone) is a biologically active flavonoid found in a variety of food and food products. Quercetin also has protective potential in the case of reproductive and endocrine toxicities [20]. The pharmacological importance of quercetin includes gastroprotection, immunomodulatory action, antioxidant, anticancer, antiviral, anti-inflammatory, and anti-infective properties [21].

The current work is undertaken to know the effect of 28 days of a repeated orally applied dose of cypermethrin on thyroid hormones level and their effect on hematological parameters and their correction by treatment with antioxidants ashwagandha and quercetin in male *Swiss albino* mice.

2. Material and Methods

2.1. Experimental Design

Adult male *Swiss albino* mice weighing 25-30 gm approximately were purchased from Veterinary College Mhow, Indore housed in the Animal care unit before the experiment with 12-12 hours of the dark light cycle at 25 ± 2°C temperature. Mice were given water and a conventional lab diet. The animal ethics committee granted consent for this work under approval number 379/GO/ReBi/S/01/CPCSEA. Six animals each made comprised of the four groups that the animals were divided into at random. Cypermethrin was given 15mg/kg body weight, ashwagandha 200 mg/kg body weight, and quercetin 150 mg/kg body weight orally for 28 days [22-24].

Table 1. Experimental design

Groups	Name	Dose
Group I	Control	Standard lab diet
Group II	Cypermethrin treated	15 mg/kg
Group III	Ashwagandha Co-treated	200 mg/kg
Group IV	Quercetin Co-treated	150 mg/kg

2.2. Body and Thyroid Weight

The body weight was recorded at 7- days intervals (0, 7, 14, 21, and 28) with weighing balance (Sartorius, BP210 S), and the organ weight was taken when mice were sacrificed, and individual values were noted down.

2.3. Sample Collection

The blood sample was collected by direct cardiac puncture after anesthetizing with chloroform. The whole blood was stored in two parts one K_3EDTA coated vials separately at room temperature for hematological parameters analysis and the other for the serum to measure thyroid hormone levels. Hormone levels were measured using an ELISA kit (Cal Biotech Inc., California, USA) using prescribed methods.

2.4. Statistical Analysis

The results were presented as Mean±SE for all statistical data analyses, which were conducted using a one-way Analysis of Variance. Significant was defined as *$p<0.05$ level of significance.

3. Results

Blood, a fluid connective tissue, accounts for the largest percentage of fluid and is responsible for the route of transportation for food, drug, and foreign substances. Tracking the toxicity of foreign substances in the physiological system requires careful consideration of hematological markers. Clinic-pathological situations are caused by the buildup of foreign substances in excess or at low concentrations. The toxicity of compounds is usually associated with excess or deficit of markers, enzymes, and hormones. An increase or decrease in blood variables indicates imbalanced cell production and cell destruction.

3.1. Body Weight and Thyroid Weight

The body weight of cypermethrin-exposed animals increases from the day before the experiment till the end of the experiment and ashwagandha and quercetin help in preventing the exaggerated increase in body weight (Figure 1). The thyroid gland weight significantly declined (***$p<0.001$) in the cypermethrin exposure group and thyroid gland weight decreased significantly in ashwagandha and quercetin (**$p<0.01$) co-administrative groups along with cypermethrin exposure (Figure 2).

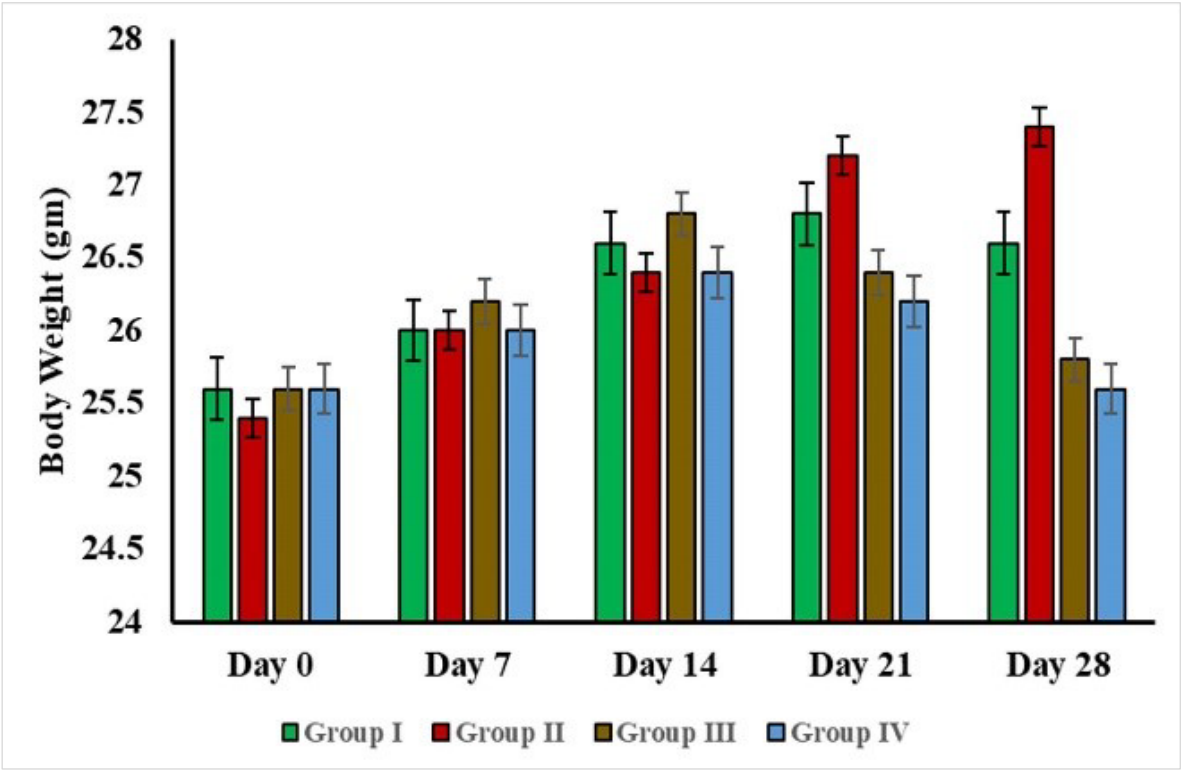

Figure 1. Efficacy of ashwagandha and quercetin on alterations in body and thyroid weight after cypermethrin exposure

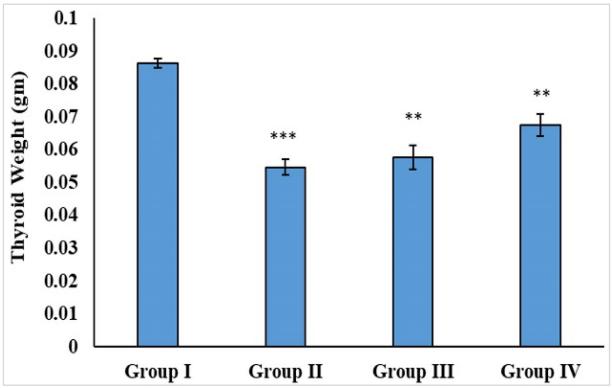

Figure 2. Cypermethrin exposure (Group II) caused decreased (***p<0.001) thyroid gland weight and increased thyroid gland weight after co-administration of ashwagandha and quercetin (**p<0.01) (Group III & IV) as compared with group II

3.2. Thyroid Hormones

All of the experimental groups' serum T3, T4, and TSH levels were examined in our investigation.

3.2.1. Triiodothyronine (T3)

The serum level of T3 decreased significantly (**p<0.01) after exposure to cypermethrin as compared to control and the co-administration of ashwagandha and quercetin (**p<0.01) helped in significant elevation of T3 hormone as compared to cypermethrin exposed group (Figure 3).

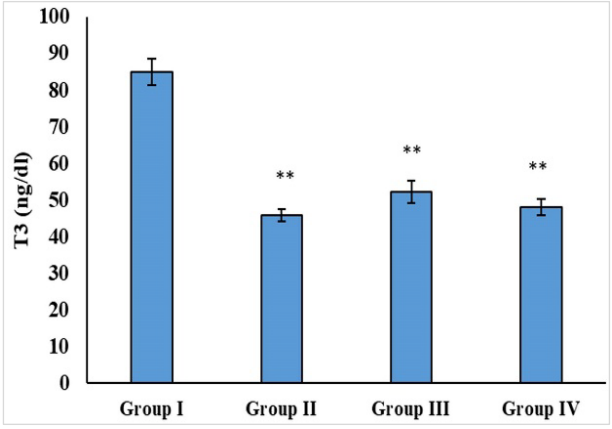

Figure 3. Serum T3 concentration decreased significantly in the cypermethrin (**p<0.01) exposed group as compared to the control and increased significantly in ashwagandha and quercetin (**p<0.01) co-administrative groups

3.2.2. Tetraiodothyronine (T4)

The serum concentration of the T4 hormone was decreased significantly after exposure to cypermethrin (**p<0.01) as compared to control and the ashwagandha (*p<0.05) and quercetin (**p<0.01) co-administrative groups (Group III & IV) shows significantly elevation of T4 hormone as compared to cypermethrin exposed group (Figure 4).

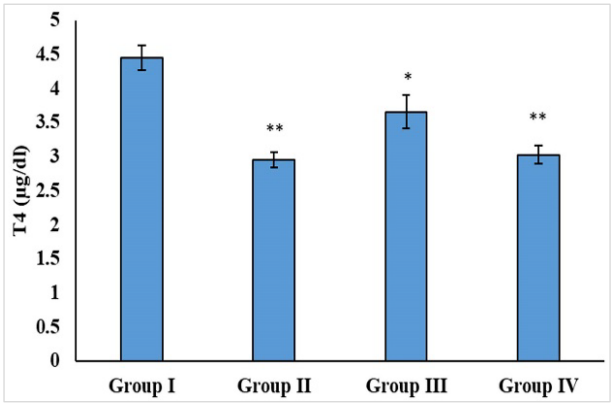

Figure 4. Serum T4 concentration decreased significantly in the cypermethrin (**p<0.01) exposed group as compared to the control and increased significantly in ashwagandha (*p<0.05) and quercetin (**p<0.01) co-administrative groups (Group III & IV)

3.2.3. Thyroid Stimulating Hormone (TSH)

The serum concentration of thyroid-stimulating hormone increased significantly in the cypermethrin exposed group (**p<0.01) as compared to control and the ashwagandha and quercetin (**p<0.01) co-administration (Group III & IV) caused a significant elevation in TSH level as compared to cypermethrin exposed group (Group II) (Figure 5).

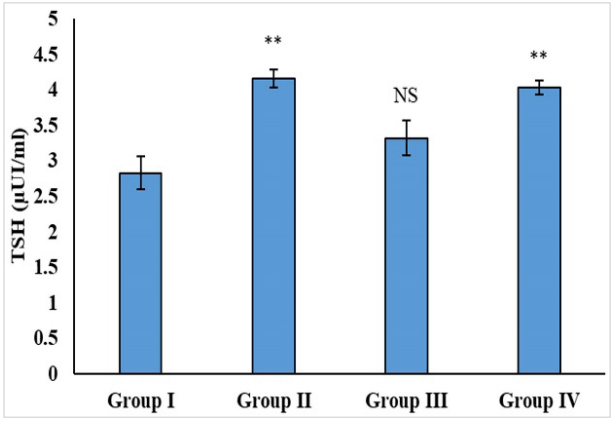

Figure 5. The level of TSH increased after cypermethrin exposure (**p<0.01) as compared to control and increased after co-administration of ashwagandha and quercetin (**p<0.01) (Group III & IV)

3.3. Hematological Profile

Hemoglobin, total erythrocyte count, total leukocyte count, neutrophil, lymphocyte, and platelet count were examined and measured in the current study from each experimental group.

3.3.1. Hemoglobin Percentage and Erythrocyte Count

The exposure to cypermethrin caused a significant reduction (***p<0.001) in hemoglobin percentage as compared to control while ashwagandha (***p<0.001) and quercetin (***p<0.001) co-administration (Group III & IV)

prevents the further decrease in hemoglobin (Figure 6).

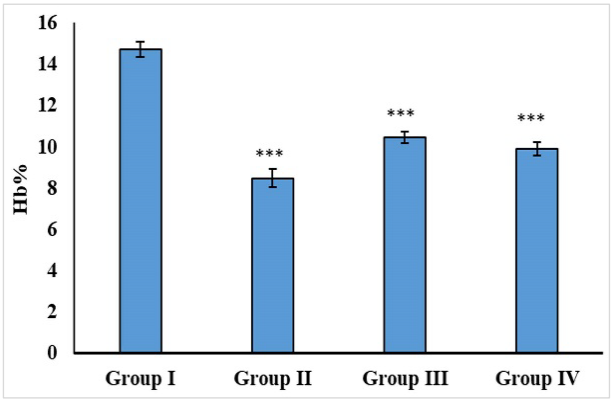

Figure 6. The hemoglobin% decreased significantly after exposure to cypermethrin (***p<0.001) and significantly increased after co-administration of ashwagandha (***p<0.001) and quercetin (***p<0.001) (Group III & IV) along with cypermethrin exposure

The current study's findings revealed that cypermethrin intoxication caused a significantly decreased total erythrocytes count (***p<0.001), and that co-treatment with ashwagandha and quercetin considerably increased erythrocytes count in mice (**p<0.01) (Figure 7).

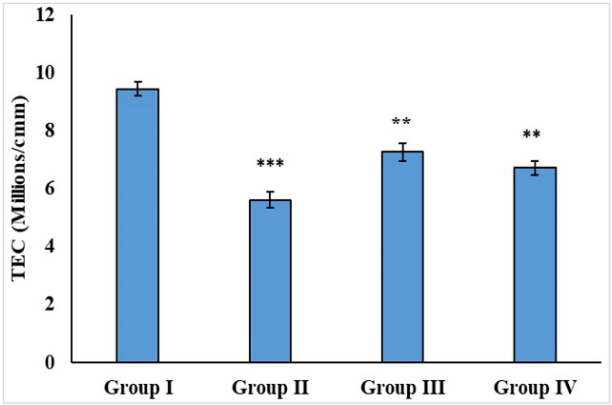

Figure 7. The total erythrocyte count decreased significantly after cypermethrin (***p<0.001) intoxication and increased after ashwagandha and quercetin (**p<0.01) co-administration (Group III & IV)

3.3.2. Leukocyte Count, Neutrophil% and Basophil%

The intoxication to cypermethrin (*p<0.05) causes increased total leukocyte counts as compared to control and decreased significantly after ashwagandha (*p<0.05) and non-significantly after quercetin co-administration in mice along with cypermethrin intoxication and suppressing the immune system of the body (Figure 8).

According to the investigation's findings, exposure to cypermethrin dramatically boosted the neutrophil percentage. (*p<0.05) as compared to control, and the neutrophil% decreased after co-administration of ashwagandha and quercetin (**p<0.01) as compared to cypermethrin intoxicated group (Figure 9).

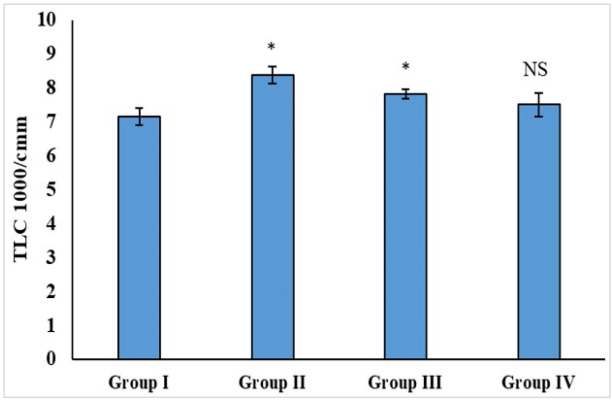

Figure 8. The leukocyte counts increased after cypermethrin (*p<0.05) exposure and decreased after ashwagandha (*p<0.05) and quercetin co-administration

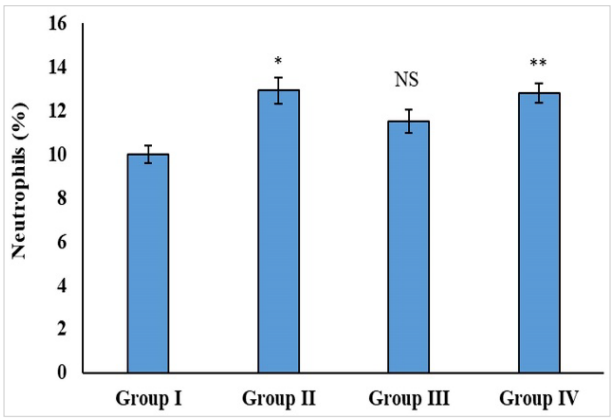

Figure 9. The neutrophil% increased significantly after cypermethrin (*p<0.05) intoxication and decreased significantly after ashwagandha and quercetin (**p<0.01) co-treatment (Group III & IV)

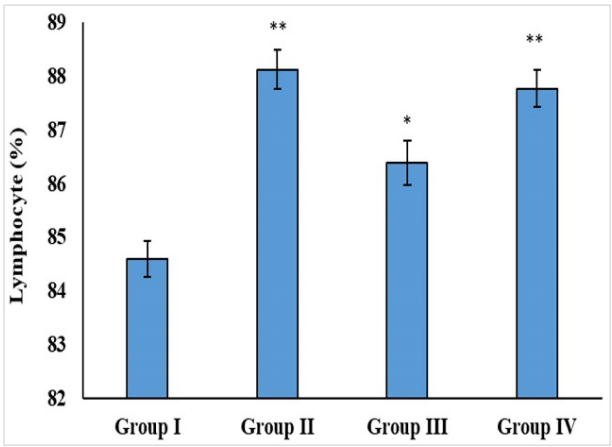

Figure 10. The lymphocyte% increased significantly after cypermethrin (**p<0.01) toxicity and decreased significantly after co-treatment with ashwagandha (*p<0.05) and quercetin (**p<0.01) (Group III & IV)

The current investigation showed a significantly increased lymphocyte% after cypermethrin (**p<0.01) intoxication in mice as compared to control, and the lymphocyte% decreased significantly after

co-supplementation with ashwagandha (*p<0.05) and quercetin (**p<0.01) as compared to cypermethrin intoxicated group (Figure 10).

3.3.3. Platelet Count

In the present investigation it is observed that the platelet counts in the cypermethrin (**p<0.01) exposed group significantly decreased as compared to the control and increased significantly after co-supplementation with ashwagandha (*p<0.05) and quercetin (**p<0.01) as compared to cypermethrin intoxicated group (Figure 11).

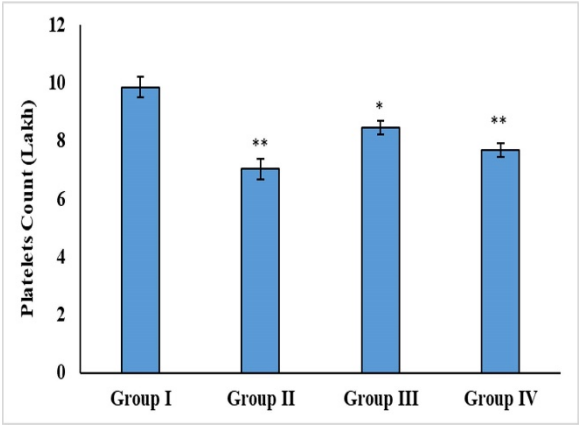

Figure 11. Cypermethrin (**p<0.01) caused a significant reduction in platelet count and a significantly increased platelet count after co-administration of ashwagandha (*p<0.05) and quercetin (**p<0.01) (Group III & IV)

4. Discussion

The alteration in physiology after exposure to a toxicant is the result due to free radical-induced oxidative stress. Loss and gain in body weight are remarkable symptoms of toxicity. Toxicants after entering the body cause an alteration in metabolic activities resulting in a change in body weight. In this investigation, cypermethrin caused a gain in body weight which was similar to the pattern of body weight gain observed when different doses of cypermethrin (60, 150, and 300 mg/kg) were given orally to rats [25].

The results showed, declined T3 and T4 and high TSH after cypermethrin exposure as compared with control, leading to hypothyroidism. The levels of T3 and T4 were increased, and TSH levels decreased significantly in ashwagandha and quercetin co-treated rats administered cypermethrin 20 mg/kg for successive 14 days significantly caused a reduction in serum T3 and T4 concentration and elevated TSH levels as compared to control and damaged the thyroid follicular cells which cause imbalanced thyroid hormone synthesis and transportation which can be examined with the help of histological investigations [26]. Synthetic pyrethroids like cyhalothrin, deltamethrin, permethrin including cypermethrin, and their active compound 3-PBA were potential thyroid disruptors. This is also previously demonstrated that synthetic pyrethroids exert an antagonistic effect on TH receptors. The mixed pesticide exposure leads to a change in the serum thyroid concentrations. The TSH elevates, T3 and T4 were decreased marginally in pesticide workers [27]. In previously reported works, ashwagandha and quercetin show ameliorative effects against hypothyroidism [23,28]. Ashwagandha prevents hypothyroidism *via* acting through the hypothalamus-pituitary-adrenal (HPA) axis and lowers the cortisol level which in turn stimulates the hypothalamus-pituitary-thyroid (HPT) axis and stimulates the thyroid hormone synthesis [29]. Quercetin prevents thyroid follicular cells, it decreases the level of thyroid-restricted sodium/iodide symporter gene *via* TSH, which caused decreased TSH and increased thyroid hormone synthesis [30].

Blood stem cell development and proliferation are significantly influenced by thyroid hormones. Similar to previous findings, which indicated that recurring oral administration of alpha-cypermethrin at a dose of 14.5 mg/kg for 30 days to rats decreased both erythrocyte count and hemoglobin percentage, cypermethrin reduces hemoglobin percentage and erythrocyte counts in the current study. [31]. Similarly, 60, 150, and 300 mg/kg doses of cypermethrin to rats for repeated 28 days also caused the same as in previous research works [32]. The dose-dependent cypermethrin toxicity for 14 successive days in rats also showed decreased erythrocyte count and hemoglobin percentage [33]. Similar results were shown in our study, which indicated that the declined erythrocyte count and hemoglobin concentration after cypermethrin intoxication is due to hemolysis. Previous studies showed that pyrethroid exposure causes hemorrhage and reduced erythropoiesis [34,35]. In this investigation, hemoglobin concentration decreased after cypermethrin intoxication. The reduction in hemoglobin concentration is probably due to impaired heme synthesis of in the bone marrow [36].

Leukocyte counts significantly increased in this investigation's total and differential groups exposed to cypermethrin compared with the control group, while leukocytes count significantly decreased in those treated with ashwagandha and quercetin. It was also noted in earlier research studies that Swiss mice exposed to dose-dependent oral alpha-cypermethrin for 28 days experienced a considerable increase in their leukocyte count, both overall and differentially [37,38]. Similarly, it was reported earlier that rats exposed to cypermethrin have increased total and differential leukocyte count [39]. It was found that cypermethrin has an immunostimulatory effect on rats and mice, which can be examined with more parameters [40,41]. The increased leukocyte counts were also reported in earlier published research work. Leukocyte production may have grown as a result of the body's immune system and overactive defensive mechanisms.

Leukocytes aid in the body's defense against foreign substances, which are produced through leukocytosis and the generation of antibodies. Exposure to chemicals, as well as acute bleeding and hemolysis, can cause leukocytosis [39]. Altered thyroid hormones inhibit the maturation of WBC and cause immunosuppression by increasing immature WBC counts [42]. The decreased Hb%, RBC, and platelets count under hypothyroidism reflect hypoplasia myeloid cell lineage [43].

In the present study, the total circulating platelet counts decreased in the cypermethrin-exposed group as compared to the control, whereas increased platelet count was observed in the ashwagandha and quercetin administration groups. Similarly, the decreased platelet count was reported previously when the different doses of cypermethrin were applied orally. The pyrethroids imiprothrin, phenothrin, and trans allethrin exposure for three weeks also cause reduced platelet counts suggesting a suppressive effect on thrombopoietin [32]. Ashwagandha and quercetin showed protective efficacy against hematological toxicity.

5. Conclusion

The present investigation was conducted to find the protective effectiveness of ashwagandha and quercetin against cypermethrin, a synthetic pyrethroid insecticide-induced alteration in plasma thyroid hormone concentrations, and their deleterious effects on hematological parameters. Based on the current investigation and available research works in the database, it is concluded that cypermethrin and other synthetic pyrethroids affect health adversely. This investigation concluded that long-term cypermethrin treatment causes decreased thyroid hormone concentrations resulting in hypothyroidism. The alterations in hemoglobin percentage, total erythrocyte count, and total and differential leukocyte count were also observed under hypothyroidism. The co-administration of ashwagandha and quercetin helps in recovery from cypermethrin-induced thyroid hormone alterations.

Conflict of Interest

None.

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