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ALLEVIATING CYPERMETHRIN-INDUCED HYPOTHYROIDISM AND HEMATOLOGICAL CHANGES IN FEMALE *SWISS ALBINO* MICE USING *BACOPA MONNIERI*

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ABSTRACT

Hypothyroidism is a common outcome resulting from the interaction of various drugs, environmental pollutants (insecticides), and industrial chemicals with the endocrine glands. The thyroid gland is an important endocrine gland in the human body. Thyroid hormones play a crucial role in proper development, metabolic balance, differentiation, and physiological function of nearly all tissues. The thyroid gland produces hormones such as triiodothyronine (T3) and tetraiodothyronine or thyroxine (T4). Under physiological condition, thyroid hormone influences hematological parameters. Cypermethrin is a type II synthetic pyrethroid insecticide used worldwide to reduce crop loss and pest population. Although *Bacopa monnieri* has been used for various diseases, it is best recognized as an antioxidant and also for brain-boosting properties. This study was done to evaluate the effect of cypermethrin-induced Hypothyroidism on different hematological parameters and the ameliorative effect of *Bacopa monnieri.* Female *Swiss albino* mice were used in the investigation and were divided randomly into four groups viz. Group I control, Group II cypermethrin (15mg/kg body weight) treated, Group III cypermethrin and *Bacopa monnieri* treated (15mg/kg body weight, and 200mg/kg body weight simultaneously), and Group IV *Bacopa monnieri* administered (200mg/kg body weight) via oral gavage for 28 days. Results indicate that the Cypermethrin induced-hypothyroidism group II showed significant alterations in hematological parameters suggesting that significantly increased WBC and Platelets count, likewise a significantly decreased RBC, and Hb% lead to anemia, are all associated with hypothyroidism, whereas thyroid hormonal parameters also showed significantly decreased serum T3 and T4 (***$p<0.001$) and increased TSH (***$p<0.001$) levels are shown in induced- hypothyroidism group. The *Bacopa monnieri* showed therapeutic efficacy in the BM co-administered group when compared to control and treated groups. The investigation showed the elucidating potential of *bacopa monnieri* on hematological alterations in Female *Swiss albino* mice associated with CYP-induced hypothyroidism. The study revealed that thyroid diseases impacted females more frequently than males. Emphasizing a strong link between hematological parameters and thyroid dysfunction in mice. The medicinal application of this plant and its numerous possibilities for further investigation remain in relatively new areas of study. Analyzing the chemical composition of this plant will enable further exploration of its remediation and hematological effects in mice.

KEYWORDS: Hemoglobin, Hypothyroidism, Hematological Parameter, Cypermethrin, *Bacopa monnieri.*

INTRODUCTION

Pesticides are intended for killing pests that harm humans, crops, and other animals, their poisoning results in illness and even death in impoverished nations like India. Pesticide poisoning and attempted suicides have been linked to more than 3 million instances, most of which have ended in death.[1] Commercial applications for cypermethrin include home and veterinary use for pest control of plants. Cypermethrin usage in the home exposes them to more factors. Mammals that are exposed to synthetic pyrethroids in large quantities develop pathological, physiological, and behavioral abnormalities. The devastating consequences of pyrethroid exposure on the immune system, endocrine system, neurodevelopment, and reproductive physiology have been reported in several studies.[2] Chemical toxicants such as cypermethrin can degrade biological macromolecules and lead to systemic dysfunction by oxidative via induced oxidative stress.[3] The thyroid gland is situated just below the larynx on the anterior side of the neck. It is the biggest and most essential endocrine gland in the human body. With a type of epithelial tissue origin, it is formed of many thin follicular cells and has two lobes. Thyroid hormones in the form of thyroglobulin molecules are stored in these follicles until the body needs them. The two main

hormones produced by the thyroid gland are thyroxin, sometimes referred to as 3, 5, 3', 5'-tetraiodothyronine (T4), and 3, 5, 3'-triiodothyronine (T3). The hormones in concern are essential for regulating the metabolic rate, or the rapidity at which the body burns and stores energy. These metabolites are also essential for the maturation of bones, somatic growth, protein synthesis, early brain development, and red blood cell regulation. The binding of the thyroid hormone T3's active form to certain nuclear receptor family members (TR□ and TR□) controls all of these processes. Thyrotropin, or thyroid stimulating hormone, is a hormone that the anterior pituitary secretes and regulates the thyroid's hormonal production. Thyrotropin-releasing hormone (TRH), which is released by the hypothalamus, mediates the production of thyrotropin itself. Thyroid hormones also have an impact on the development of hemoglobin in the fetus and the adult.[4-6] Thyroid abnormalities may also have low iron levels, which affect hemoglobin levels. They may also have reduced levels of folate and B12, which have been detected in up to 25% of patients. Both of these factors eventually affect blood parameters, including hemoglobin and RBCs. The causes of anemia may include bone marrow suppression and other related comorbid diseases.[7] A prevalent clinical issue, anemia mostly affects females who are of childbearing age and the elderly. Its occurrence in the general population can reach up to 10% in various regions of the world. Anemia is characterized by a decrease in hemoglobin (Hb) or red blood cells (RBC), which impairs the blood's capacity for carrying oxygen to bodily tissues. Thyroid hormones stimulate erythrocyte precursors, which directly affect blood parameters, and they also indirectly affect erythropoietin synthesis.[8] The small creeper *Bacopa monnieri*, sometimes known as Indian pennywort, is a member of the Scrophulariaceae family that grows naturally in moist areas and marshlands. The herb is used to treat dermatitis, epilepsy, insanity, neurological breakdown, and memory improvement. It has been linked to several medical benefits.[9] There have also been reports of its anticancer and antioxidant properties.[10] The purpose of this study is to assess the possible therapeutic benefits of *Bacopa monnieri* as well as the impact of anomalies related to CYP-induced hypothyroidism on several blood parameters. And also provides potential associations between various blood parameter levels and abnormal thyroid function for future studies.

MATERIALS AND METHODOLOGY

Chemicals

Cypermethrin was procured from Dhanuka Agrotech Private Ltd, New Delhi.

Experimental design and treatment

Female Wistar mice weighing 25-30 gm were purchased from the College of Veterinary Sciences and Animal Husbandry Mhow, India. Animals were acclimatized to the animal housing with an ambient temperature of ~20-25°C, relative humidity of 50±5%, and a 12 h light:12 h dark cycle. Ethical approval was obtained from the Department of Pharmaceutical Sciences Dr. Harisingh Gour Vishwavidyalaya (Registration - 379/CPCSEA/IAEC-2021/005), Sagar (M.P.). The care and use of laboratory animals followed international guidelines. All animals (n=24) were housed in plastic cages and given standard food and water ad libitum. The experimental animals were divided into four groups, Group I to Group IV, and administered different doses as illustrated in Figure 1.

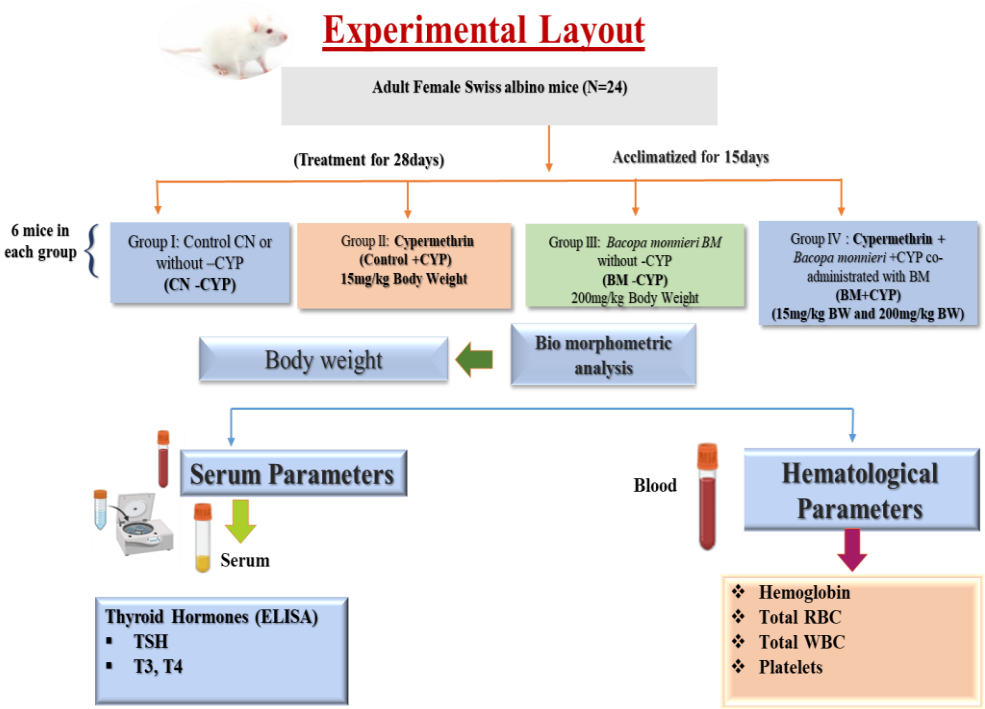

Figure 1: Experimental design is shown above.

Body and Thyroid Weight

The body weight was recorded at 7-day 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.

Collection of blood samples

Each animal group was sensitized to chloroform after the exposure period, and following heart puncture, blood samples were obtained for use in various blood parameter determinations. Then, according to previous findings, the hematological parameters were completed, and the blood was collected into the proper EDTA tubes (anticlotting tubes).[11]

ELISA Assay (Enzyme-Linked Immunosorbent assay)

Blood is kept at room temperature; then, serum is collected and stored at -20 ºC for hormone estimation and measurement of T3, T4, and TSH using an ELISA kit provided by Calbiotech Inc. (California, USA).[28]

Determination of Hematological Parameters

The hematological study was followed by Dacie and Lewis., 1991 and Osman et al., 2010;.[12-13]

Hemoglobin (Hb)

The acid hematin technique developed by Osman's 2010 was used to determine Hb (g/dl). A glass tube filled with HCl was filled with twenty microliters of blood. The acid hematin solution was then vigorously stirred with a glass rod and left to stand for 10 min. After 10 min, distilled water was added to the acid hematin solution while stirring constantly. Water was added gradually until the solution's hue precisely matched the standard. Reading expressed in grams.[12]

Red blood cells (RBC)

Blood was precisely filled to the 0.5 mark on the red cell pipette; then, fluid was added to reach the 101 mark and mixed solution. Some drips were added to the chamber and left there for a little while after mixing. Neubauer's chamber's center double-ruled squares are used to count RBCs (million/cmm) under a microscope.[13]

White Blood Cells (WBC)

The WBC pipette was loaded with blood to 0.5 marks, then diluted with fluid to mark 11. After it had been well mixed, a few drops of the mixture were added to the Neubauer chamber. For a few minutes, undisturbed the room. The Neubauer chamber's single-ruled four corner squares were used to conduct the WBC (million/cmm) count.[13]

Estimation of platelet count on smears using the multiplication factor

Platelet count was estimated by using the previously published method by Sahu Nageshwar 2022. In samples, the multiplication factor 9.42 x 10^3 was multiplied by the average number of platelets in the smear per OIF (PS2) to estimate the TPC (PE).[27]

Statistic evaluation

The results of the experiment were displayed as Mean±SEM. A Student T-test was used to analyze the data, identifying the bio-morphometric, hormonal, and hematological alterations with treatment +CYP or CYP co-administration with BM, CN, and BM or without -CYP followed by the T-test. Significance testing was performed at a $p<0.05$ confidence level using the software GraphPad Prism version 9.0 for graphing.

RESULTS AND DISCUSSION

Our results suggest that the THs participate in the maintenance of body weight in different ways. In this study, the body weight increased significantly (***$p<0.001$) in the cypermethrin-induced hypothyroid group as compared to the control group and decreased significantly (***$p<0.001$) in the *Bacopa monnieri* co-administrative group. (Figure-2A.) The thyroid gland weight decreased significantly (***$p<0.001$) in the cypermethrin-induced hypothyroid group as compared to the control group and increased significantly (***$p<0.001$) in the *Bacopa monnieri* co-administrative groups. *Bacopa monnieri* administration showed nonsignificant changes in gain in body weight and thyroid weight (Figure 2B). The level of thyroid hormones (T3 and T4) decreased significantly (***$p<0.001$) in the cypermethrin-exposed group as compared to the control and BM co-administrative groups increased significantly (*$p<0.05$) as compared to the cypermethrin-exposed group. The serum level of TSH increased significantly (***$p<0.001$) in the cypermethrin-exposed group as compared to the control and BM co-administrative groups decreased significantly (*$p<0.05$ respectively) as compared to the cypermethrin-exposed group (Figure- 3A-C). To examine the effect of hypothyroidism on hematological parameters, the result suggests as a consequence, insufficiency of thyroid hormones during development leads to involvement in hemoglobin synthesis in adults and maturation of hemoglobin in the fetus[14,15] and by affecting the hematopoietic process, hypothyroidism results in anemia through slowing the oxygen process.[16] In line with these studies, our data shows a significant decrease in the Hb% (**$p>0.01$), RBC (**$p>0.01$), and a significant increase in WBC (***$p>0.001$) and Platelets counts(***$p>0.001$). whereas the BM co-administration restores the level of all the parameters towards normal (Table- 1). Kawa MP et al. in 2010 reported that RBC, and Hb, were decreased in patients with hypothyroidism.[17] Mechanistically, the THRA (NR1A1) and THRB (NR1A2) genes encode the highly conserved nuclear receptors known as thyroid hormone receptor (TR)-α and TR-β (18, 20). Early research revealed that changing thyroid function in vitro[19] and in vivo[21] may have an impact on the hematopoietic system. To supplement these observations, new research on the molecular processes underlying the relationship between alterations in hematopoiesis and

thyroid hormones has shown that altering the expression of TR genes has an impact on hematopoietic progenitors in vivo.[17] Based on this information, it is widely accepted that THs can alter bone marrow cell production.[22] Dönmez and Keskin[23] explained in different research that a rise in platelet counts could be a restorative reaction to a bleeding tendency. Platelet numbers are correlated with platelet aggregation.[25] Furthermore, there is an inverse relationship between thyroid hormone levels and platelet aggregation.[24] According to Nejar Bruno et al.[24] animals with hypothyroidism had higher platelet 5'-nucleotidase activity, an enzyme that breaks down adenosine monophosphate into adenosine, while rats with hyperthyroidism showed lower platelet activity. The findings suggest that variations in platelet 5'-nucleotidase activity during hypo- and hyperthyroidism may have affected adenosine levels, which in turn may have affected platelet aggregation.[26] Despite the available research, we recommended that CYP-induced hypothyroidism be routinely assessed for hematological alterations that may result in anemia-like symptoms, and decreased RBC counts, as well as increased WBC and PLT counts in female mice, whereas BM co-administration could potentially alter all of the symptoms related to CYP-induced hypothyroidism in mice.

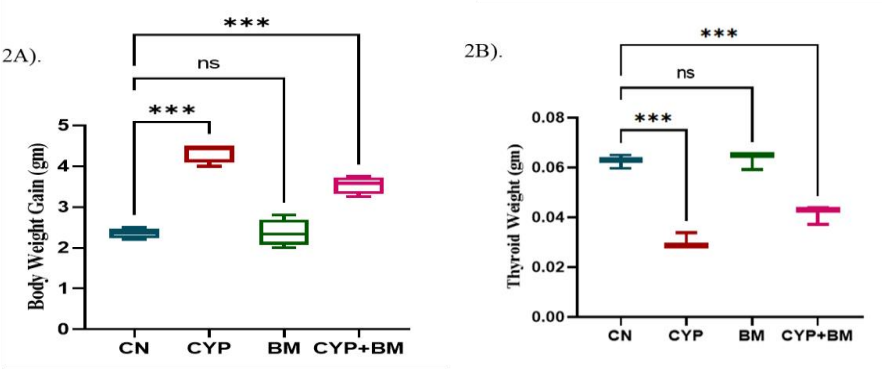

Figure 2: Effect of BM on Morphometric (Body Weight and Liver weight) in all animal groups; Data are presented as mean ± SEM and calculated by T-test with comparison to control. Statistical significances were noted as ($p < 0.05$*, $p < 0.01$** and $p < 0.001$***). CN: Control; BM: *Bacopa monnieri*; CYP: Cypermethrin; CYP+BM: Cypermethrin and *Bacopa monnieri* administered

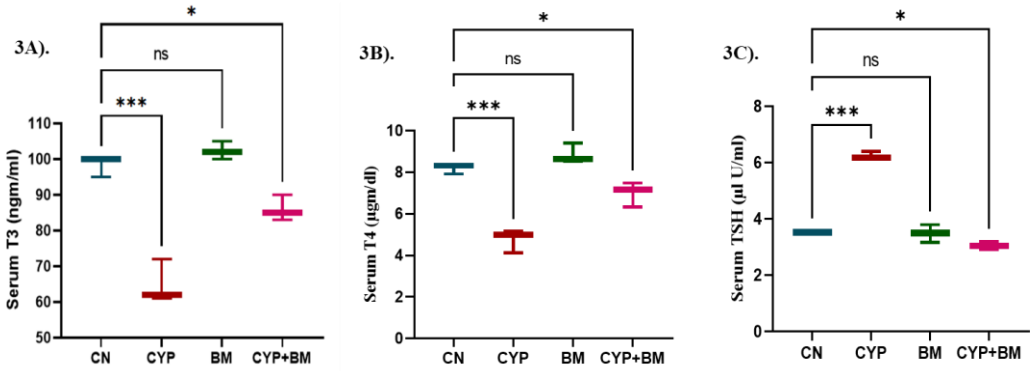

Figure 3: Effect of BM on Hormonal Parameters (TSH, T3, and T4) in all animal groups; Data are presented as mean ± SEM and calculated by T-test with comparison to control. Statistical significances were noted as ($p < 0.05$*, $p < 0.01$** and $p < 0.001$***). CN: Control; BM: *Bacopa monnieri*; CYP: Cypermethrin; CYP+BM: Cypermethrin and *Bacopa monnieri* administered.

Table 1: Demonstrates the Effect of BM on Hematological Parameters (Hb%, RBC, WBC, and Platelets Count) in all animal groups

Group	CN (-CYP)	CN (+CYP)	CN BM (-CYP)	CYP+BM(+CYP)
Hemoglobin (Hb)%	14.8±0.30	11.73±0.31**	15.1±0.24	13.7±0.30*
Total RBC (Erythrocytes count) (mil/cmm)	7.43±0.05	6.08±0.056***	7.54±0.035	7.15±0.04*
Total WBC (Leucocytes Count) (/cmm)	14333.3±248.3	16233.3±177.9**	14066.6±267.7	13466.6±285.7*
Platelets (Thrombocytes Count) (lac/cmm)	**5.37±0.0.05**	**8.51±0.300*** **	**5.706±0.46**	**7.54±0.45***

Data are presented as mean ± SEM and calculated by T-test with comparison to control. Statistical significances were noted as ($p < 0.05$*, $p < 0.01$** and $p < 0.001$***). CN: Control; BM: *Bacopa monnieri*; CYP: Cypermethrin; CYP+BM: Cypermethrin and *Bacopa monnieri* administered

CONCLUSION

The majority of these individuals see improvement following ameliorative effects on hypothyroidism and thyroid hormonal normalization. Anemia and other blood abnormalities are prevalent in thyroid function abnormalities, primarily hypothyroidism. The study found that females are more likely than males to suffer from thyroid diseases. All blood parameters are impacted by thyroid dysfunction; however, platelets and WBC are less affected than other parameters, suggesting that thyroid hormones play a critical role in blood formation. On the other hand, BM reverses the transformation and also lessens the hematological alterations, which may be beneficial to the medical field.

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Conflicts of interest

The authors hereby declare that there is no conflict of interest in this study.

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