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UNCOVERING THE LINK: CYPERMETHRIN-INDUCED HYPOTHYROIDISM, A GROWING CONCERN

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ABSTRACT

Objective: Cypermethrin (CYP) is a commonly used type II Pyrethroid. This pesticide tends to accumulate in the various body tissues, causing organ dysfunction. It is the major endocrine disrupter worldwide. The current study is primarily focused on investigating CYP-induced hypothyroidism in female mice, which is a growing concern for us. **Method:** The animals were divided into two groups, each comprising six mice. Group I (CN) served as a control, and Group II (CYP) was orally exposed to CYP at 15 mg/kg for 28 days. At the end, serum levels of triiodothyronine (T3), thyroxin(T4), free T3, T4, thyroid-stimulating hormone (TSH) were measured and histological study were observed. **Result:** The thyroid tissue was studied, which revealed that CYP leads to a marked elevation in TSH and a considerable decline in fT3, fT4, T3, and T4 levels. Moreover, a significant increase in the biomorphometric indices body weight and a substantial decrease in thyroid weight were seen. Marked morphometric abnormalities and hormonal dysregulation indicated the CYP-induced hypothyroidism condition. The results revealed abnormal thyroid histomorphometry in the CYP-induced hypothyroidism group. **Conclusion:** These results suggest CYP-induced hypothyroidism in female mice. This work suggests that the consumption of CYP is a growing concern because it is proven that it can cause hypothyroidism in mice.

KEYWORDS: Cypermethrin, Hypothyroidism, Environmental toxicants, Type II pyrethroid, Oxidative Stress.

INTRODUCTION

Pyrethroid pesticides are widely used as insecticides worldwide. They are grouped into two categories: type I and II. Type I has been observed to impact Na^+ channels in neural membranes, leading to a persistent neuronal discharge and a protracted negative neuronal potential.[1,2] By adding a cyano group to 4-phenoxybenzoic components, type II pyrethroids (the modified forms of type I pyrethroids) were created to increase photostability.[3] Although these are believed to be less harmful to animals, birds, and the environment[4,2], the possibility of their contamination and toxic effects can't be ruled out. In fact, Environmental samples have revealed the presence of pesticides in water, food, biota, human samples of breast milk and urine.[5,6,7] Moreover, it has been proven that pyrethroid metabolites negatively affect several physiological functions[8,9] and are linked to allergies, cancer, neurological issues, reproductive issues, and mental disorders.[10,11] Furthermore, several pyrethroids have been thought to act as possible endocrine disruptors. Thyroid function is thought to be disturbed by some Organochlorine, Organophosphate, Carbamate, and Pyrethroid pesticides[1,8] Cypermethrin often crosses the blood-brain barrier, inhibits the Na/I symporter, and causes an iodine deficiency and a rise in TSH in response to inadequate hormone production and a state of hypothyroidism.[11] The thyrotoxic potential of several pesticides is associated with decreased thyroid function, which causes hypothyroidism as inferred from pathological findings, like hyperplasia and follicular cell hypertrophy.[12] Hypothyroidism is considered the most common form of thyroid disease with deficient thyroid hormones. These symptoms include fatigue, melancholy, forgetfulness, irregular menstruation periods, and weight gain.

Chemicals

Type II Pyrethroid (Cypermethrin) of common grade brand name Superkiler (25% EC) was procured from Dhanuka Aggritech Private Limited, India. ELISA kits were procured from Calbiotech Inc., California, USA, and other biochemicals were procured from Himedia.

Experimental Design

All animals (n=12) were housed in plastic cages, provided a routine laboratory diet twice daily, and had access to adequate food and water. The experiment

comprised two groups. Each received specific doses as illustrated in (Fig: 1). The pesticide CYP was used at a concentration that was much below the acute oral toxicity threshold value (1/10th of the LD50) as used earlier.[13]

After 28 days of experimentation, the animals were vivisected via heart puncture to collect blood. The thyroid gland was taken out, thoroughly cleaned with chilled PBS, and stored at -20°C for further research. The serum was obtained by centrifuging the collected blood and stored separately for hormonal analysis after standing at room temperature (RT) for 30 minutes. For biochemical analysis, a Tris HCl buffer of 0.02 M was used to homogenise the tissues (pH 7.4). The samples were homogenised before being centrifuged at 12500 rpm for 30 minutes at 4°C. The supernatant was removed and stored at -20°C for additional biochemical examination.

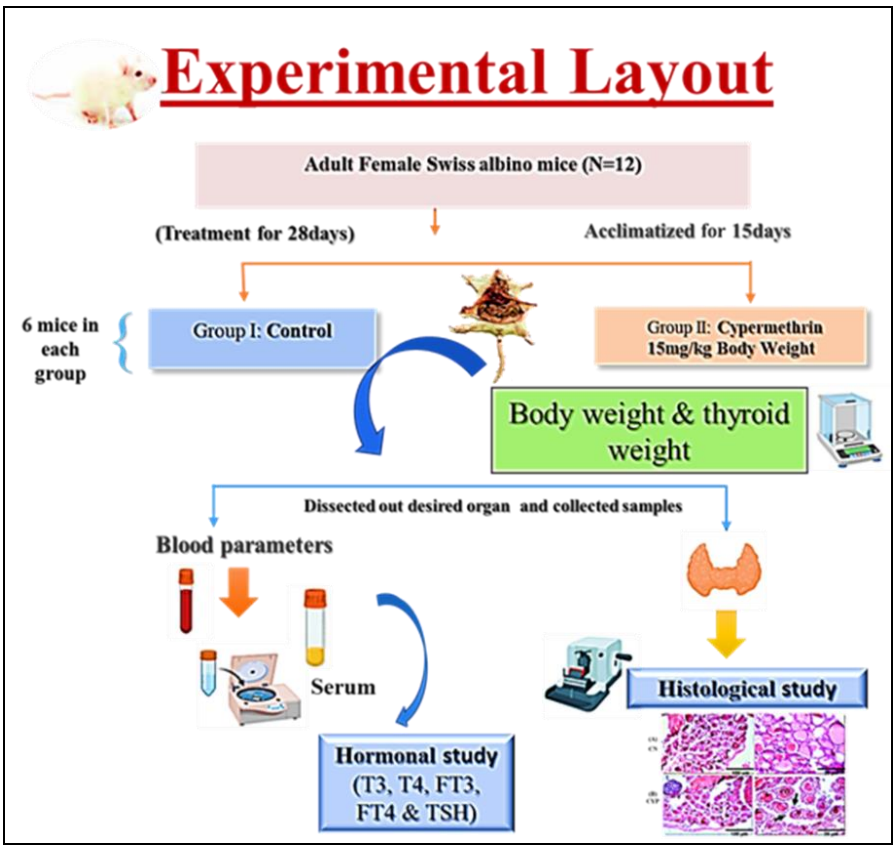

Fig. 1: Experimental design shown above.

Bio-morphometric analysis

The mice's Body weight was measured before and during the experiment. After the experiment was terminated, the weight of the thyroid gland was also taken using an electronic balance (Sartorius, BP210S).

Enzyme-linked immunosorbent assay (ELISA)

The estimation of T3, T4, fT3, fT4, and TSH levels in the serum was done using the protocol provided in the specific ELISA kit[14], and the ELISA reader measured the ELISA assay readings.

Histological Analysis

The thyroid gland was removed and preserved for histological analysis. They were then immersed in paraffin wax after being submerged for 24 hours in buffered formalin at a 10 % concentration. They underwent a dehydration series and were then embedded in paraffin wax (melting point 55–60°C). Using a rotary microtome, 5µm thick paraffin slices were obtained and stained with Hematoxylin and Eosin, and lastly, DPX mounting was performed for histological investigation.[15] The investigation included the follicle's size and structure and the characteristics of both cytoplasm and intrafollicular colloid. Using 100x and 400x magnifications, under a ZEISS Light microscope, A camera, and Quasmo software (P95-C 1/2" 0.5x; No. 415500-1812-000), histological features were observed.

Statistic evaluation

The results of the experiment were displayed as Mean±SEM. A T-test was used to analyse the data. Significance testing was performed at a $p<0.05$ confidence level using the software GraphPad Prism, version 9.0.

RESULTS

Biomorphometric results

In the CYP-treated group, body weight increased significantly, but thyroidal weight decreased significantly compared to the control group.

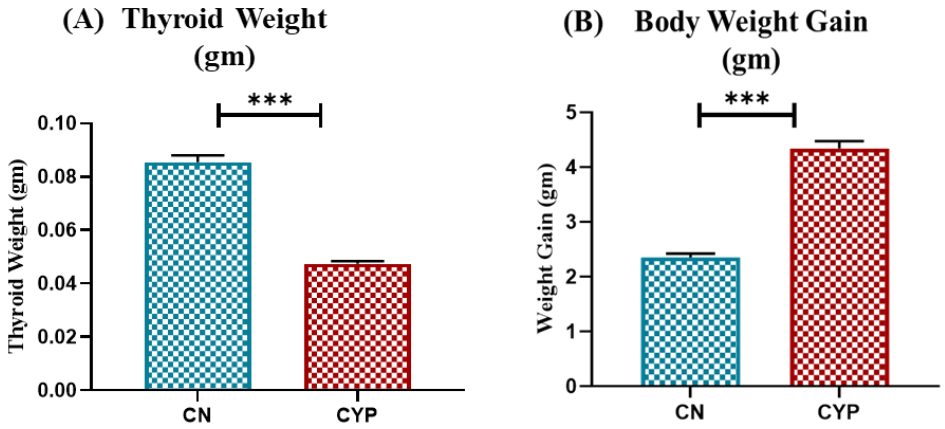

Fig. 2: Effect of Cypermethrin (CYP) exposure on (A) Thyroid weight and (B) Body weight gain. The results have been represented as mean ± S.E. The values are expressed in grams (gm), respectively. Data were analyzed using t-test (*p < 0.05, **p < 0.01 and ***p < 0.001; statistically significant with respect to the control group).

Hormonal results

Our results showed that in the CYP-exposed group, there were significant alterations in the levels of hormones (T3, T4, fT3, fT4, and TSH) compared to those of the control group. The CYP-treated group showed a significant increase in the serum TSH level compared to the control group, whereas there was a substantial decrease in the serum T3, T4, fT3, and fT4 compared to the control group.

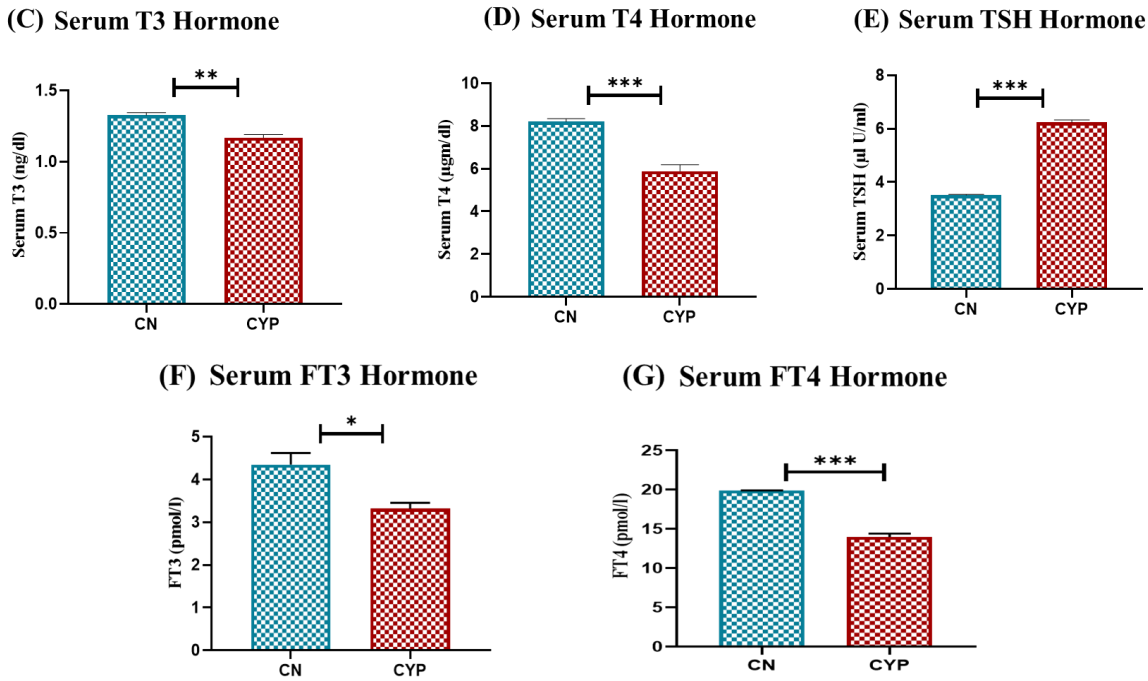

Fig. 3: Serum T3 Hormone (C), T4 Hormone (D), TSH Hormone (E), FT3 Hormone, and FT4 Hormone level in control (CN), and CYP treated groups. The results have been represented as mean ± S.E. Data were analyzed using a t-test (*p < 0.05, **p < 0.01, and ***p < 0.001; statistically significant with respect to the control group).

Histological results

An organized histological structure was found in the thyroid tissues of control mice. With a typical arrangement of different diameters, the thyroid follicles looked healthy. They were primarily surrounded by cubical follicular cells with rounded vesicular nuclei and a few parafollicular cells, with an acidophilic homogeneous colloid in the middle. Follicular cells and

blood capillaries interacted with one another in healthy ways between follicles. Additionally, the interfollicular gaps showed connective tissue and blood vessels (Fig: 4.A). However, the thyroids of mice treated with CYP displayed a distinct histological structure. Reduced follicle diameters and cell exfoliation within some follicle's lumens were observed. The shapes of thyroid follicles appeared to vary, with some resembling atrophied and shrunken. Other follicular cells had exfoliated cells in the follicular lumen or vacuolation in their cytoplasm. Comparatively to the control group, the lumen of a substantial number of follicles had vacuolation, while the lumen of other follicles was devoid of colloids (Fig: 4.B).

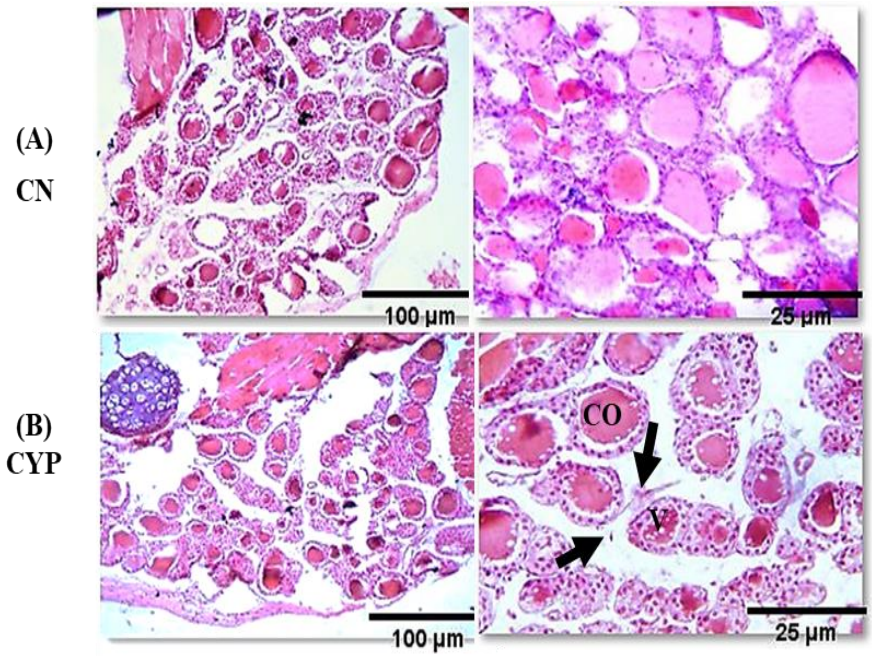

Fig. 4: Photomicrograph showing hematoxylin and eosin (H & E) staining of the vertical sectional view of the mice thyroid of all experimental groups precisely; A) (CN) normal control group showing typical thyroid architecture with variable follicles lined with cuboidal follicular epithelium**, B) (CYP)** CYP- treated thyroid displaying a decrease in colloidal material (CO), vacuolation in the cytoplasm of follicular cells (V), irregularities between follicles and cytoplasm (are denoted with Black arrow). The images were taken at 10X and 40X magnifications.

DISCUSSION

Results of this investigation clearly indicated the adverse effects of Cypermethrin on the thyroid functions of mice as evidenced by a decrease in serum thyroid hormones, fT3, fT4, T4 and T3, and an increase in TSH serum level. Although an earlier study had indicated the possible interference of CYP with TH signaling[16], no systematic evaluation was made on this aspect in any animal model before we attempted this investigation. In fact, only a few reports are there on the effects of pesticides in general on thyroid functions[8,17,18,19,20] However, our present report clearly indicates the thyroid-inhibitory effects of CYP. The histological findings of this experiment showed that the CYP-induced mice indicated the changes seen in the mouse thyroid are distorted, the thyroid follicles and cytoplasm were vacuolated. The outcomes supported the notion that CYP makes mice heavier. The current study shows a significant increase in body weight gain and a decline in thyroid weight ($p<0.001$***) in the CYP-treated group, similar to that observed by others.[21] The study indicated that CYP - induced the hypothyroidism in female mice. Further, we can suggest that environmental toxicant pesticides like insecticides are the major endocrine disrupters, or they can be the reason for hypothyroidism in today's scenario, so we can say the indoor & outdoor exposure of CYP will be one of the causes of hypothyroidism.

CONCLUSION

Our findings indicate that CYP adversely affects the structure and hormonal synthesis of the thyroid gland, resulting in reduced thyroid hormone production and the development of hypothyroidism. This concludes that exposure to CYP type II pyrethroid will be the cause of hypothyroidism.

Ethical Approval

The Animal Husbandry Mhow and College of Veterinary Sciences in India (22.55º N, 75.75º E, M.P.) provided female adult mice weighing 25–30 grams. Our university has an IAEC with ethical permission registered under the Ministry of Environment (Registration-379/CPCSEA/IAEC-2021/005), which provides guidelines for laboratory animal use.

Conflict of interest: There is no conflict of interest**.**

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