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Actara 25 WG Induced Histopathological Changes in Thyroid and Intestine of Swiss Albino Mice

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Abstract **– ACTARA 25 WG is a unique systemic insecticide used as a tea garden insecticide to kill the unwanted pests that harm the tea plant. It mainly destroys bugs, beetles etc provides excellent elimination of a broad range of foliar and soil pest with a fast rate. The present study investigated the toxic effect of Actara-25 wg. On the intestine and thyroid of swiss albino mice. Four adult mice are divided into two groups of which Group-1 consists of four untreated mice maintained in laboratory condition and the Group-2 consists of four mice in same condition but given daily Actaraat the dose of 2mg/gm body weight for a period of 14 days via oral route. Histopathological study was performed following the processes of microtomyas per the method of Luna *et al.,* 1968 [1]. Results shows pathological changes as loosened muscular part, cytoplasmic degranulation, vacuolation, nucleopyknosis along with brokenvilli tips, disrupted and separated serosa in intestine. In thyroid tissues changes mainly occur as follicular enlargement, disruption of connective tissue, interstitial haemorrhage, congested blood vessels. The oral administration of Actara-25wg gives an inflammatory process in intestine and thyroid that constitute a toxicity sign in swiss albino mice.**

Keywords **– Actara 25wg, Histopathology, Insecticide, Interstitialhaemorrhage.**

I. INTRODUCTION

Nowadays more than thousand different pesticides are being used in the environment, mostly in agriculture. However the widespread use of pesticides in agriculture leads to a series of toxicological and environmental problems and this has received extensive concerns worldwide. Human exposure to pesticides is usually estimated by measuring levels in the environment such as air, food, water, etc. In some cases, information on exposure might be obtained by the analysis of concentration of specific pesticide in the human body, tissues, fluids or might analysed by designing experiments on non-target animals and to assess the risk in humans exposed to pesticides. Pesticides have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehaviour disorders, impaired immune functions and allergic sensitization reactions [2]. The individuals who are exposed to these chemicals include agricultural workers and those living near farms or consumers that exposed to pesticide residues in food. Actara is a synthetic organic insecticide included in the class of neonicotinoids, the most important new class of insecticides developed in the last three decades. Since their introduction onto the market in 1991, neonicotinoids have been the fastest growing class of insecticides, due to their expected moderate toxicity to mammals and their advantage in combating insects that are resistant to other pesticide classes [3]. Actarais used for control of aphids, whitefly, thrips, rice hoppers, rice bugs, mealy bugs and some lepidopterous species [4].

Neonicotinoids are rapidly distributed and excreted after oral exposure. Studies revealed that neonicotinoids like Actara readily permeates most tissues except for fatty tissues, the CNS and the mineral part of bone. Highest concentrations of Actarawere found in the kidney, the thyroid gland and adrenals. Neonicotinoids do not cause reproductive or developmental toxicity at low doses [5]. The present study investigates the toxic effect of Actara on the histological structure of thyroid and intestine in swiss albino mice.

II. MATERIALS AND METHODS

2.1. Test Organism

Male mice of 3-4 month old of 30-35 g body weight were selected and used for experiment. They were housed in special healthy standard cages and standard mice diets were given.

2.2. Chemicals and Experimental Setup

Actara (Thiamethoxam) was developed by Syngenta. Thiamethoxam (TMX) 25% WS (Actara®) with chemical name 3 - [(2 – Chloro - 1, 3 – thiazol – 5 - yl) methyl] – 5 – methyl – N – nitro - 1, 3, 5 – oxadiazinan - 4 - imine was obtained commercially from local market of pesticides. Thiamethoxam is a slightly cream fine crystalline powder at room temperature. Its vapour pressure is low and hence its Henry's Law Constant indicates that volatilisation is not expected to significantly contribute to the dissipation of thiamethoxam in the environment. Thiamethoxam is not considered highly flammable or explosive or oxidizing.

Female mice with body weight ranging from 30-35 g were selected and divided into 2 groups. They were housed on a 12 h light/dark cycle. Animal of control group were fed normal food with distilled water. The treated group received the test chemical at the dose of 2 mg/kg for 14 days.

2.3. Histopathological Analysis

For studying the histological and histopathology of the organs and tissues, thin paraffin sections are cut with a microtome for study under the microscope. The tissues were fixed in 4% formalin for 24 hrs and processed for

paraffin embedding. After routine processing, dehydration in several baths of ethanol in increasing degrees, paraffin sections of each tissue were cut at a thickness of 5µm and stained with haematoxylin and eosin for microscopic examination. Histopathological analysis and photograph were taken using LABOMED LX-400 Microscope.

III. RESULTS

Microscopic examination of thyroid in control mice showed the normal structure of follicles, follicular cells, parafollicular cells and a thin fibrous capsule with blood vessels, lymphatics and nerves prominent at poles. The lining of follicular epithelium ranges from flattened to cuboidal structure and the connective tissues are of regular distribution (Fig.1, 2, 3 and 4). The histopathological investigation of sections of thyroid from the treated mice showed aggregation of inflammated follicles (Fig. 6). Some follicles showed vacuolated colloid while others are without colloid (Fig. 8). Some follicles showed exfoliated cells in the lumen, lining follicular epithelium was also distorted (Fig. 5). The blood vessels become congested and connective tissues showed irregular distribution (Fig. 7 and 8). Microscopic observation of intestine from control mice showed the four layers- mucosa, sub-mucosa, muscularis and serosa. Mucosa showed glandular epithelium and lamina propria. Crypt cells, goblet cells; absorptive cells were also distinct in the glandular epithelium. Brush border showed numerous microvilli (Fig. 9, 10, 11 and 12). The sections from treated mice showed sub mucosal haemorrhage and erosion in epithelial lining (Fig.13). Distortion occurred in the four layers, villi were seen with broken ends (Fig. 14), crypts exhibited pyknotic nuclei (Fig.15). Necrotic cells (Fig. 15 and 16) and degenerated areas were also found.

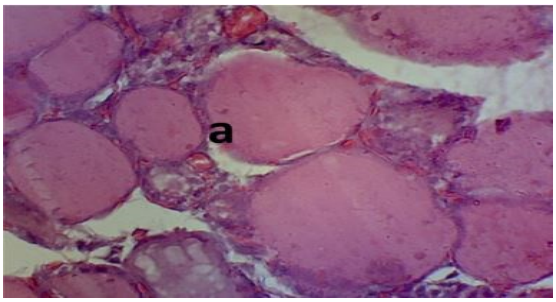

Fig. 1. Section of thyroid gland of normal mice showing thyroidfollicles with small flattened epithelial lining (40X).

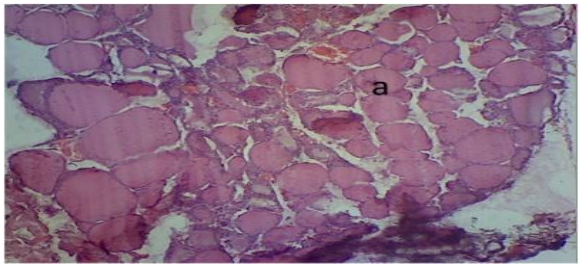

Fig. 2. Photomicrograph showing cuboidal epithelial lining in the cross sections of Haematoxylene and Eosin stained Thyroid of control Albino mice. (10X)

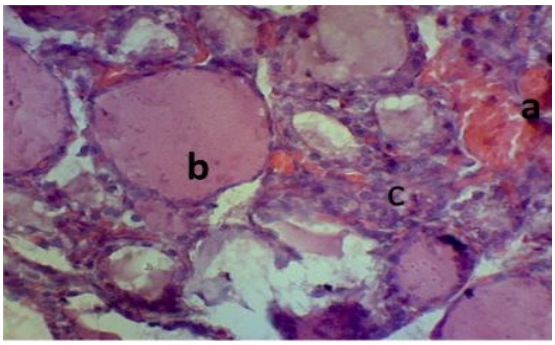

Fig. 3. Photomicrograph showing the blood vessels (a), smooth organization of follicular (b) and parafollicular cells (c) and in the cross sections of Haematoxylene and Eosin stained Thyroid of control Albino mice. (40X)

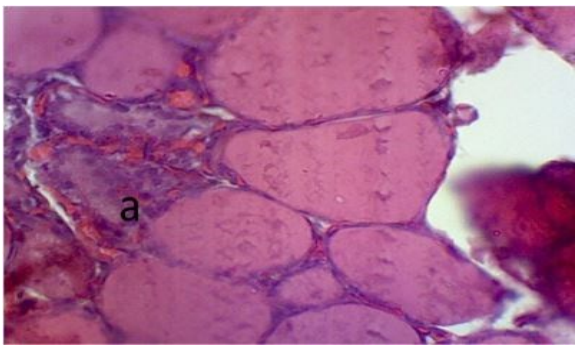

Fig. 4. Photomicrograph showing regular distribution of connective tissue in the cross sections of Haematoxylene and Eosin stained Thyroid of control Albino mice. (40X)

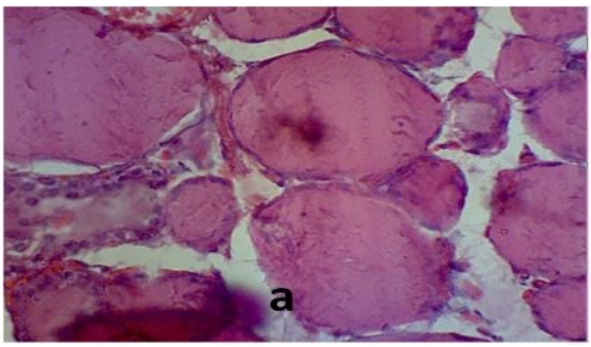

Fig. 5. Photomicrograph showing distortion of follicular lining in the cross sections of Haematoxylene and Eosin stained Thyroid of treated Albino mice. (40X)

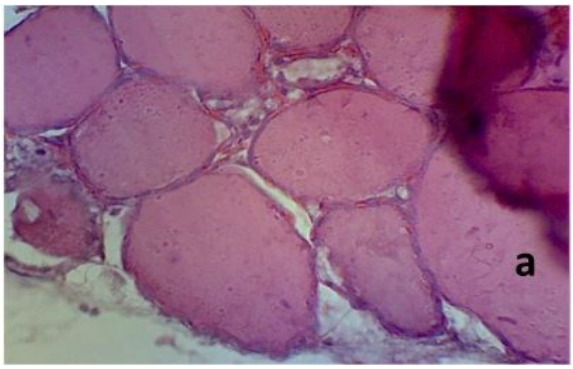

Fig. 6. Photomicrograph showing inflammation of follicle in the cross sections of Haematoxylene and Eosin stained Thyroid of treated Albino mice. (40X)

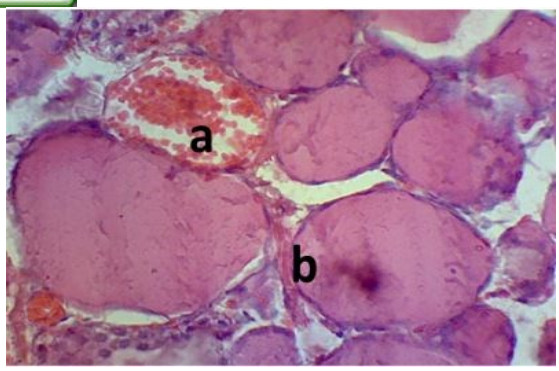

Fig. **7.** Photomicrograph showing congested blood vessels (a) and irregular distribution of connective tissues (b) in the cross sections of Haematoxylene and Eosin stained Thyroid of treated Albino mice. (40X)

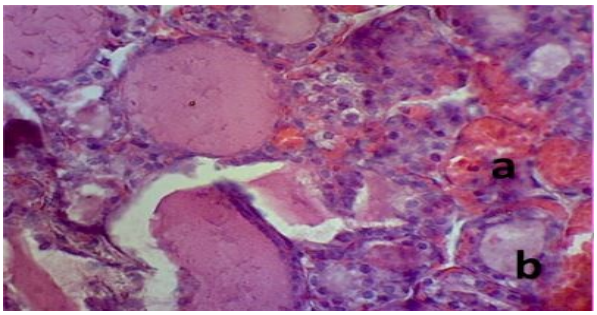

Fig. 8. Photomicrograph showing congested blood vessels (a) with vacuolated colloid (b) in the cross sections of Haematoxylene and Eosin stained Thyroid of treated Albino mice. (40X)

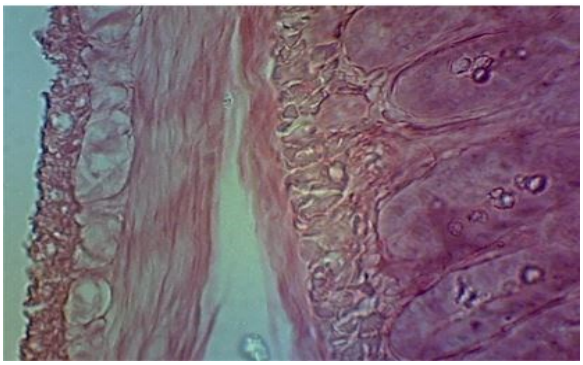

Fig. 9. Photomicrograph showing normal organization of intestinal layers–mucosa, sub-mucosa, muscularis and serosa in cross section of intestine in normal albino mice (40X)

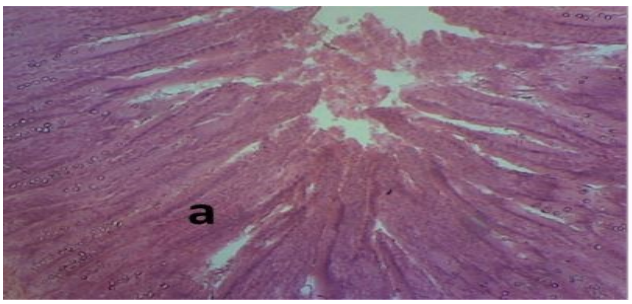

Fig. 10. Photomicrograph showing normal organization of brushborder showing numerous goblet cells (a) in cross section of intestine in normal albino mice. (40X)

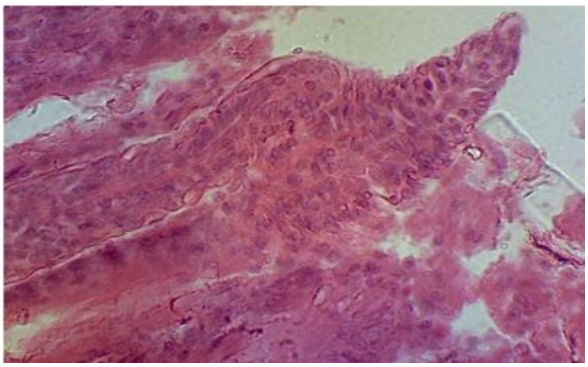

Fig.11. Photomicrograph showing glandular epithelia, crypts, laminapropria in cross section of intestine in normal albino mice. (40X)

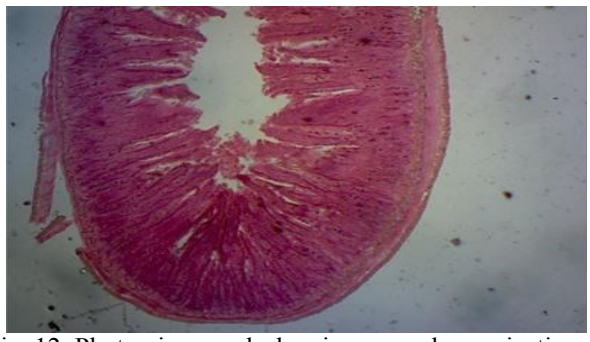

Fig. 12. Photomicrograph showing normal organization of intestinal layers –mucosa, sub-mucosa, muscularis and serosa, villi in cross section of intestine in normal albino mice. (4X)

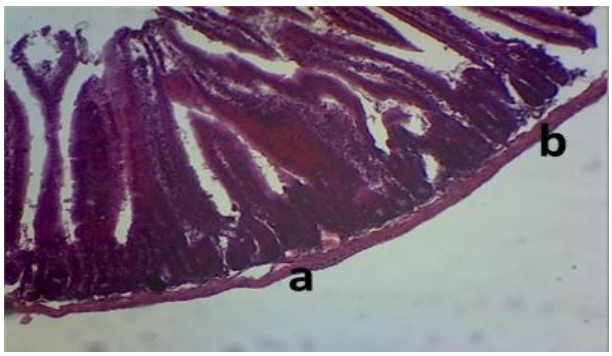

Fig.13. Photomicrograph showing erosion of layers (a) and sub-mucosal haemorrhage (b) in cross section of intestine in treated albino mice. (10X)

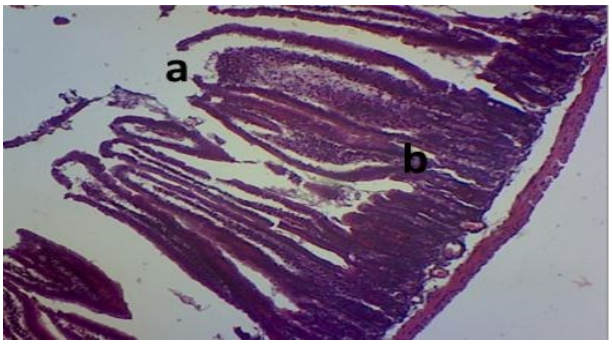

Fig. 14. Photomicrograph showing villi with broken tips (a) and necrotic cells (b) in cross section of intestine in treated albino mice. (10X)

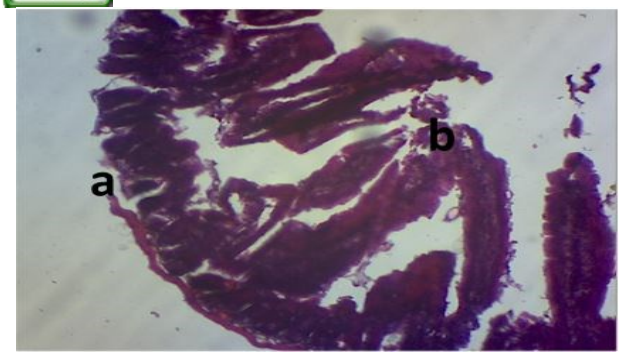

Fig. 15. Photomicrograph showing distorted epithelial lining (a) crypt with pyknotic nuclei (b) in cross section of intestine in treated albino mice. (40X)

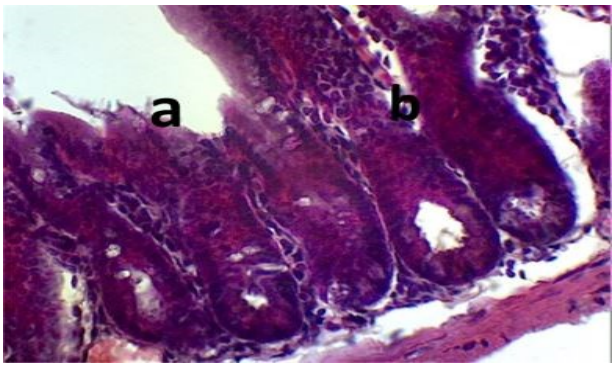

Fig. 16 Photomicrograph showing distorted villi (a) and goblet cells (b) in cross section of intestine in treated albino mice. (40X)

IV. Discussion and Conclusion

The findings of the present study clearly established that Actara possess adverse physiological effects on 14 days of exposure to actara in swiss albino mice resulted in inflammation of follicles, vacuolated colloid, exfoliated cells in lumen, distortion of epithelium, and congestion of blood vessels in thyroid. There is increasing evidence that environmental exposures, specifically to pesticides, should also be considered potential risk factors for thyroid disease. Certain insecticides, herbicides, and fungicides have been previously reported to be endocrine disruptors and, more specifically, thyroid disruptors acting through diverse mechanisms such as inhibition of thyroidal iodine uptake, interference at the thyroid hormone receptor, binding to transport proteins, interference with iodothyronine deiodinases, increased clearance of thyroid hormones, interference with cellular uptake of thyroid hormones, and interference with thyroid hormone gene expression (6, 7, 8). The thyroid gland is associated with many physiological functions. The functional components of the thyroid glands are individual thyroid follicles. C cells present within the follicular wall and extracellular space between follicles are source of calcitonin required for calcium homeostasis. The follicular cells synthesize thyroglobulin which is substrate for subsequent synthesis of thyroid hormone. The thyroid is richly supplied with sympathetic postganglionic neurons that along with performing the vasomotor function, in some species also innervate the individual follicular cells. In mice, sympathetic stimulation induces secretion of thyroid hormones from those stimulated areas. Thus the Actara-25wg induced pathological changes in the structure of follicles, in the blood vessels and neurons can affect the normal functioning of the gland. As the thyroid hormone is required for bone growth and maturation, nervous system differentiation in early development, pituitary prolactin and other growth hormone synthesis, increased absorption of intestinal glucose etc., under these pathological conditions the abnormalities in thyroid function can lead to gross alterations in the normal physiology of an individual [9]. Since thyroid gland is an important endocrine gland in our body that plays an essential and pervasive role in regulation of metabolic processes such as maintenance of homeostasis, regulation of growth, reactions to exterior stimulations (stress, infection etc) and regulation of reproduction [10] disfuctioning of the gland leads to many physiological abnormalities. Pesticides like chlorophenols, chlorophenoxy acids, organochlorines, and quinones have been shown to alter thyroid gland function and to reduce circulating thyroid hormone levels [11, 12]. Reduction in thyroid hormone levels can compromise the catalytic activity of hepatic cytochrome P450 monooxygenases, resulting in an altered hepatic androgen metabolism [13].

Similarly intestine was also adversely effected by oral administration of actara-25wg and the effects were hypertrophy of lymphocytes, sub-mucosal haemorrhage, erosion in epithelial lining, the villi showed broken ends, crypts exhibited pyknotic nuclei and necrotic areas were also found. Intestine is a major functional organ of digestive tract. Final digestion of most oligomers and dimers, peptides and dipeptides, fatty acids occur at small intestinal brush border. Absorptive surface of small intestine includes the folds or crypts, villi, and the brush border epithelium. Absorptive cells, goblet cells that are present at the base of crypt are important for normal functioning of small intestine [14]. Thus Actara-25wg induced breakage of villi, distortion of epithelial surface affect the absorption by reducing the absorptive area. Again thyroid hormone is also required for active absorption of glucose in small intestine. Thus it can be concluded that pathological changes in intestine together with thyroid on exposure to Actara-25wg alter the normal absorptive function of small intestine. Carcinogenic effects have also been reported on exposure to Actara [15, 16]. The thymic tissues toxicated with imidacloprid showed lymphocytic depletion, invasion by lymphocytes, and pyknotic nuclei [17]. The infiltration of lymphocytes and cell lining proliferation in the intestine have been reported by many investigators exposed to pesticide Dimethoate 40EC [18, 19], noticed accumulation of lymphocytes in the intestine of Nubian goats previously treated with Sevin as an indication of inflammation.

The findings of the present study have shown a noticeable resemblance with the results of previously performed work. Hence oral administration of actara established an adverse effect on thyroid and intestine and constituted a toxicity sign in Swiss albino mice. The use of all insecticides especially actara should be limited and be under many precautions.

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