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Effect Of Endosulfan, An Organochlorine Insecticide On Thyroid Gland Of Rabbit

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Abstract: These studies were carried out to identify the level of the loss carried out by the application of endosulfan as an endocrine disruptor on the thyroid gland of the rabbits living in the agricultural lands and face continuous spray of the insecticides for want of good crop. About fifty animals were used in this study with the mean body weight 1400±75.5 grams. The animals were initially kept in animal house measuring 10 ′×14′ sq. ft. for acclimatization in the captive environment. All the animals were divided into four groups of 12 animals each one group was used as control and remaining three groups were kept in three different cages measuring 3x3x1.5unuts. Three doses of the insecticides were prepared as given to the each group for a period of 15 days. During the experimental period the animals exposed to higher dosage showed the clinical signs of intoxication in both males and females throughout the 15 day experiment, these signs included: Tremor, generalized convulsion, salivation, increased respiratory rate, hyperactivity and reddish nasal discharge in number of animals.

Dragging of hind limbs was also observed and these signs persisted in number of cases throughout the experiment. Histological examination of thyroid gland revealed that the damage caused in the group exposed to lowest dose was less as compared to the higher dose. Hence it is concluded that the effect of the insecticide is dose dependant.

Keywords: Thyroid gland, endosulfan, endocrine disruptor, rabbit.

1. INTRODUCTION

The rabbit has, in various times and places, been a treasured pet, a commercial farm animals, a valued subject of the hunt, a major ecological force and an economic pest. It has been hunted by the rich and eaten by the poor, despised by the farmer and praised by the landlord. It makes its home in searing deserts and freezing Alps, in suburban gardens and broad pasture lands. Its image once appeared on coins and it features in cartoons. Soil disturbance through the digging activities of rodents can have profound local effects on plant species composition and vegetation structure in the Chihuahuan Desert (Moroka *et al.* 1982). Digging activities of desert rodents intermix surface soil with subsurface soils (Abaturov 1972), and increase rainfall infiltration (Soholt 1975). Reported measures of the percentage of desert soil surface areas disturbed by rodent digging activities in desert environment range from 10% (Abaturov 1972) to 4.5% (Soholt 1975). Burrowing activities increase local soil nutrient and water status, creating favourable sites for increased plant densities, biomass production and increased species diversity (Moorehead *et al.* 1988, Mun and Whitford (1990).

The human population in Canada and in other nations throughout the world Mes *et al.,* 1990; Mes, 1992; Newsome *et a.l.,* 1995; Szymczynski and Waliszewski, 1981 a,b). While the levels of exposure for the vast majority of the population are below doses shown to cause adverse effect in laboratory studies examining the effects of single chemicals, the consequences of low level exposure to a wide variety of substances are not known and are the subject of considerable debate Pohl *et al.,* 1997)

Endocrine disrupting chemicals (EDCs) are the substances that can cause adverse effects by interfering in some way with the body's hormones or chemical messengers. These substance are therefore called hormone disruptors or endocrine disruptors. The disrupting character of the chemicals lead to suppress thyroid activity. Consequently, the metabolic pathways are directly under stress that results in the abnormal body development. Of 240 pesticides screened for carcinogenicity by the US EPA, at least 24 (10%) amongst which Organochlorine, Organophosphate, Parathroids, PCBs, herbicides.

Fungicides etc. Produced thyroid follicular cell tumors in rodents. The mechanism involved in most of the cases is through the antithyroid properties of those pesticides, which result in hormonal imbalance and hyperstimulation of the thyroid (Hurley, 1998).

Endosulfan

In Pakistan Endosulfan is commonly known as "Thiodan". It's specific uses are to control the ticks, mites and control of Rice stemborers. Endosulfan was first introduced in 1950s. It is widely used, and is important cause of pesticide poisosing in many countries. It is highly toxic, produce acute toxicity. This class of chemicals is the most important of the persistent organic pollutant or POPs. There is now a move for a worldwide ban on POPs because of their link to cancer long-term subtle effect on hormones, the immune system, and reproduction. Unlike other POPs which travel across the globe, endosulfan tends to remain in region of its use. Yet it has been found in high concentration in many areas around the world because it is so widely used. The world Health Organization (WHO) classifies endosulfan in category two (II) (moderately hazardous). The US Environmental Protection Agency (US EPA) classifies it as category Ib (highly hazardous) pesticide. It is readily absorbed through the skin, lungs or stomach, (IPCS, 1988), all routes of exposure can pose a hazard. It has many effects on the organs of body. Endosulfan has also been found to cause mutation (ATSDR, 1993), United Nation Food &Agriculture Organization has banned to its use in rice cultivation. The restriction was based on adverse effect on animals.

Endosulfan, amongst many insecticides/ pesticides having been used indiscriminately and have caused much more damage to the animals throughout the world that is reported in the literature. Now it has been totally banned in the world but it is still being used in Pakistan indiscriminately. After searching worldwide literature, no any effort has been made on the histopathological effects caused by endosulfan on the Thyroid gland. This study was made to understand such type of study at the preliminary level on the rabbits that live in the agricultural crops and always remain under stress due to the indiscriminate use of the pesticides/insecticides like Endosulfan.

2. MATERIAL AND METHODS

About fifty animals, the Rabbits *Oryctolagus cuniculus* mean body weight 1400±75.5 grams were purchased from the market of Hyderabad and brought to the laboratory. They were kept in the animal house measuring 10 ′×14′ sq. ft. for acclimatization in the new environment for 15 days. During this period the animals were fed three times a day with the grass Alfalfa (*Medicago sativa*) which is commonly known as Lucerne. After the expiry of the acclimatization period the animals were transferred to the experimental cage which was especially designed as per natural requirements of the animals. Six animals (Three males and three females) were kept for each, control and three experimental groups. The animals in this condition were again left for three days to acclimatize them in the new environment.

Endosulfan EC-35

The insecticide Endosulfan EC-35 was purchased from the local market of Hyderabad. The stock solution was prepared by diluting it in the deionized water. The dosage given to the animals kept for experimental purpose was prepared as per procedure given below.

Body weight of animal x Dose to be administered.
Percentage of product / liter.

According to the formula the dose of insecticide was fixed for single animal which is as under:

Group I	15.9µl. / kg. body weight / day.
Group II	38.3µl. / kg. body weight / day.
Group III	66.0µl. / kg. body weight / day.

The dosage fixed for the experimental schedule for each day was taken from stock solution and again diluted in 10ml deionized water. The application of the experimental doses was continued for 15 days.

Experimental Procedure:

All the control and experimental animals were weighed and kept individually by tagging the control and experimental males and females in separate cages. The diluted dose of the insecticide was administered daily after the feed was consumed by the animals. The control group was also maintained by giving same quantity of feed (Lucerne) and water as per requirement. At the termination of experiment the animals were anesthetized by using chloroform. All the animals (Control and Experimental) were dissected out and required tissue the thyroid gland was removed carefully from all the control and experimental animals.

Removal Of Thyroid Gland:

By using magnifying glass, the thyroid gland was first traced and identified. It is situated below the skin and muscle layer in the anterior region of the neck. It is butterfly shaped with the two wings being represented by the left and right thyroid lobes that wraps around the trachea. Each gland was isolated and initially kept in the patry dish containing physiological solution (0.9% NaCl solution) before fixing in the Bouins fluid for further process.

Histological Procedure

Dehydration

The Thyroid gland, fixed in Bouin's fluid, dehydrated for embedding in the paraffin wax. The sections of the tissue was cut by using rotary microtome at a thickness of 6µ. The sections were stretched on the albumenized glass slides by placing on the warm plate maintained at 60ºC. The stretched sections were left in the oven at 45ºC in order to fix the sections on the glass slides firmly.

Staining procedure

The sections of thyroid of control as well as experimental rabbits were stained in haemotoxlyne and Eocene. The stained slides were mounted and covered by cover slip.

The thyroid follicles were measured by ocular micron meter to calculate indices of the thyroid function. Follicle dimensions were measured automatically from colloid cross section based on a manual tracing around the basement membrane.

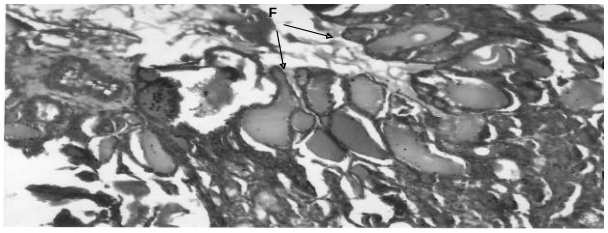
The method used for measuring epithelial height was calculated by the

formula given below:

$$\frac{(Lf - Lc) + (Wf - Wc)}{4}$$

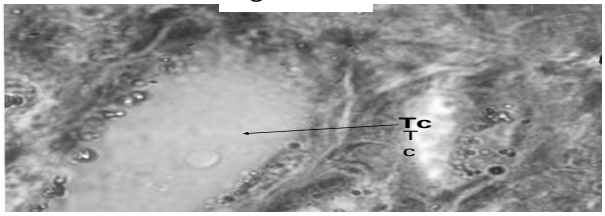
Lf= length of follicle, Lc= lenghth of colloid, Wf= width of follicle, Wc= width of colloid. In order to capture the relative changes in epithelial height and volume of colloid in a single parameter, the ratio of area of epithelium (Total area colloid) to colloid area was calculated for the five target follicles

Fig. No. 1



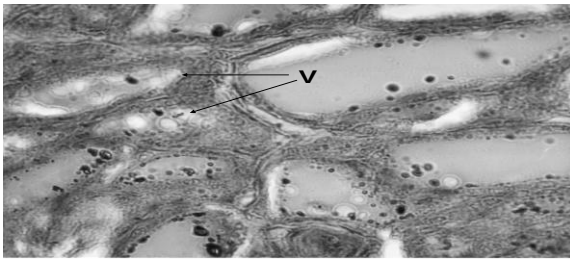
Photomicrograph of the section of the thyroid gland of control rabbits showing variable sized Follicles (F). X10

Fig.No.2



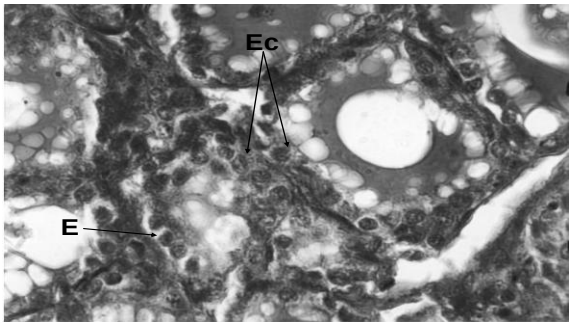
Photomicrograph of the section of the thyroid gland of control rabbits showing, the colloid was filled thickly (Tc), and scattered throughout the space under epithelium. X 100.

Fig.No. 3



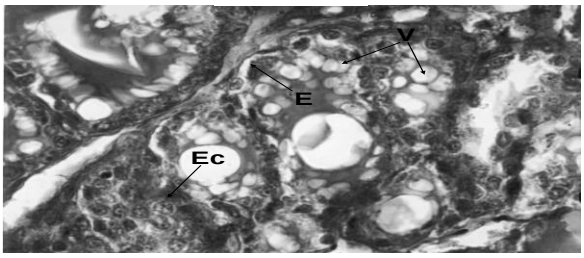
Photomicrograph of the section of the thyroid gland of control rabbits showing, that in some follicles, the vacuolization (V) was also noted. X 40.

Fig.No. 4



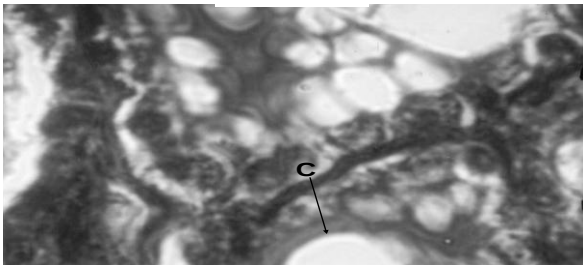
Photomicrograph of the section of the thyroid gland of Group1 rabbits showing, the mean epithelial lining (E) was reduced to 15.0 mm. The epithelial cells (Ec) increased in size (hyperplasia) and also become unseen. X 40.

Fig.No. 5



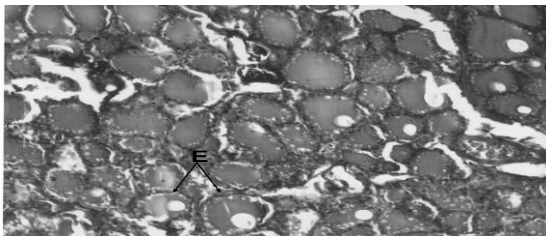
Photomicrograph of the section of the thyroid gland of Group 1 rabbits showing, the epithelial lining (E) that was intact in controls has been disintegrated. Epithelial cells (Ec) have been shifted to the colloid region. Vacuolization (V) increased as compared to controls. X 40.

Fig.o.6



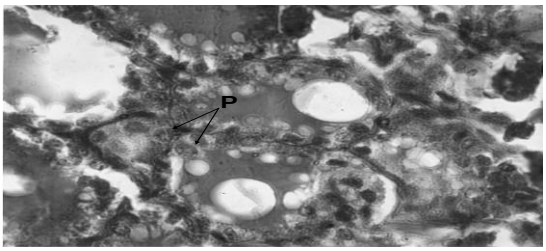
Photomicrograph of the section of the thyroid gland of Group 1 rabbits showing, amount of colloid (C) has also been affected drastically as it has been reduced enough as compared to the controls. X 100.

Fig. No.7



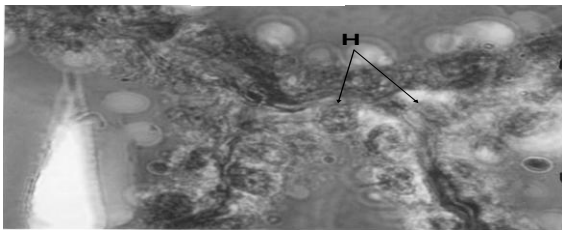
Photomicrograph of the section of the thyroid gland of Group 2 rabbits showing, epithelial lining (E) has not remained intact. X 10.

Fig. No. 8



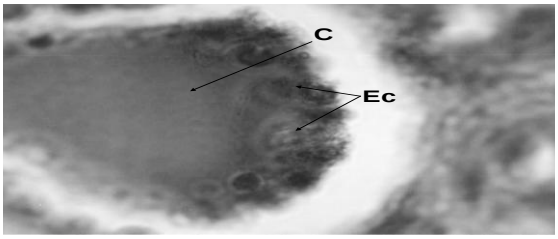
Photomicrograph of the section of the thyroid gland of Group2 rabbits showing Pyknosis (P) was noted in few of the epithelial cells. X 40.

Fig.No. 9



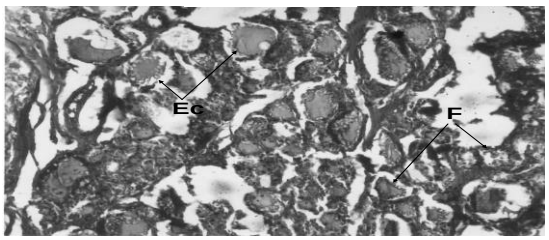
Photomicrograph of the section of the thyroid gland of Group 2 rabbits showing, the acute hyperplasia (H) in the epithelial cells. X 100.

Fig. No. 10



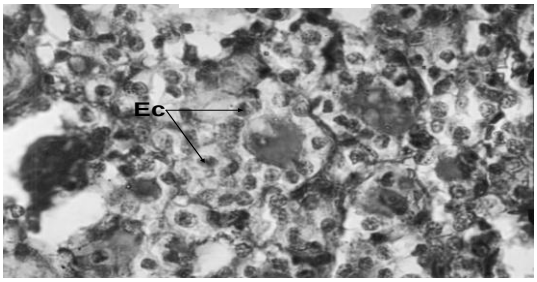
Photomicrograph of the section of the thyroid gland of Group 2 rabbits showing, the colloid (C) has become thin and completely isolated from disintegrated inner epithelial lining. All the epithelial cells (Ec) have invaded/entered the colloid that indicates the severe action of the toxicity on the epithelium. X 100.

Fig. No. 11



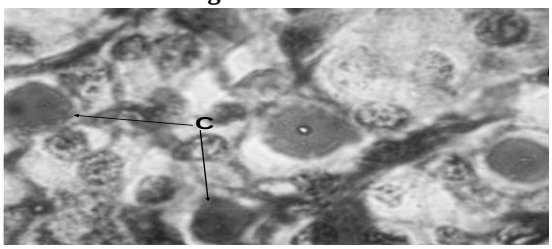
Photomicrograph of the section of the thyroid gland of Group-3 rabbits showing, the overall diameter of the follicle (F) has been reduced further as compared to the two experimental and control groups. The epithelial lining (E) is almost disintegrated or has become thin (17.75 mm).

Fig. No. 12-a



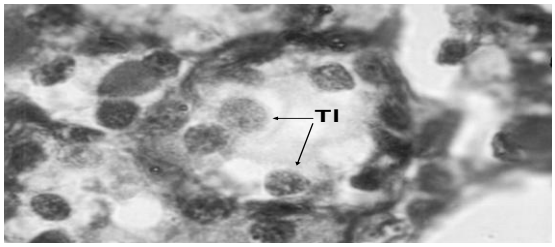
Photomicrograph of the section of the thyroid gland of Group 3 rabbits showing, that epithelial cells (Ec) are found scattered throughout the follicle. X 40.

Fig. No. 12-b



Photomicrograph of the section of the thyroid gland of Group 3 rabbits showing, that colloid (C) is totally eliminated from most of the follicles or it has become so reduced that can be equalized with the hyper plastic epithelial cells. X 100.

Fig. No. 12-c



Photomicrograph of the section of the thyroid gland of Group 3 rabbits showing, the epithelial cells have become tumor like (Tl).

3. RESULTS

As per studies of the sections of the thyroid gland of the control and experimental rabbits, the results are as under:

Histological examination:

Control rabbits

The thyroid gland of the control animals when studied under light microscope reveal that the thyroid gland consists of variable sized, oval shaped follicles.

Each follicle consists of colloid in the centre and epithelium in the periphery. The colloid containing a jelly like substance iodinated glycoprotein, thyroglobulin and the epithelium consists of the cuboidal cells arranged in the circular lining.The histological sections of the thyroid of the control group.

It consists of variable sized, oval-shaped follicles (F) in **(Fig.No.1)**. Epithelial size was 14.00mm.Epithelial cells were simple cuboidal arranged in thin epithelium.

The colloid was filled thickly (TC), and scattered throughout the space under epithelium in **(Fig. No. 2)**

In some follicles, the vacuolization (V) was also noted as in **(Fig. No. 3).**

EXPERIMENTAL GROUP ─ 1:

The thyroid gland of the rabbits exposed to the toxicity i.e 15.9 µl/kg. body weight/day revealed that:
Overall size of the thyroid follicle was reduced.

The mean epithelial lining (E) size was reduced to 15.0 mm. in **(Fig. No.4).**

The epithelial cells (Ec) increased in size (hyperplasia) and also become unseen cell boundary. The epithelial lining (E) that was intact in controls has been disintegrated. Epithelial cells have been shifted to the colloid (Ec) region. Vacuolization increased as compared to controls in **(Fig. No.5).**

Amount of colloid (C) has also been affected drastically as it has been reduced enough as compared to the controls in **(Fig. No.6).**

EXPERIMENTAL GROUP ─ 2

Histological studies of the thyroid sections of the animals exposed to the toxicity i.e. 38.3 µl/kg. body weight/day reveals that although, there was no significant change noted with regard to the epithelial size of group 1, however:

The mean epithelial size was16.75 mm. Epithelial lining (E) has not remained intact in **(Fig. No.7).**

Pyknosis (P) was noted in few of the epithelial Cells in **(Fig. No. 8).**

Acute hyperplasia (H) the epithelial cells in **(Fig. No.9).**

The colloid has become thin and completely isolated from disintegrated inner epithelial lining. All the epithelial cells have been invaded/entered the colloid that indicates the severe action of the toxicity on the epithelium in **(Fig. No.10).**

EXPERIMENTAL GROUP ─ 3

The Rabbits exposed to highest toxicity i.e 66.0 µl/kg. body weight/day reveals that:

Overall diameter of the follicle (F) has been reduced as compared to the two experimental and control groups. The epithelial (E) lining which is almost disintegrated or have become thin 17.75 mm. in **(Fig. No.11).**

The epithelial cells found scattered throughout the follicle in **(Fig. No.12 (a))**

Colloid is totally eliminated from most of the follicles or it has become so reduced that can be equalized with the hyper plastic epithelial cells in **(Fig. No. 12 (b))**

The epithelial cells have become tumor-like. (TI) in **(Fig. No. 12(c))**.

4. DISCUSSION

Increased follicular cell vacuolization and height, increased nuclear vesiculation and decreased colloid area have also been reported following polychlorinated biphenyls (PCBs) by few workers (Ness, *et al.,* 1993; Khan, 1999; Saeed and Hansen, 1997 and Waritz, *et al.,* 1996). Many other studies (Pamela, *et al.,* 1998) on the pesticides have been reported to affect because synthesis and /or metabolism. Of 240 pesticides screened for carcinogenicity by the U.S. Environmental Protection Agency (EPA) office of pesticide programme at least 24, Acetochlor, Amitrole, Bromacil, Clofentezine, DCPA, Ethiozin, Ethofenprox, Ethylene thiourea, Fenbuconazole, Fipronil, Mancozeb, Pendimethalin, Pentachloronitrobenzene, Piperonyl butoxide, Prodiamine, Pronamide, Pyrethrins, Pyrimethanil, Thiazopyr etc. produced thyroid follicular cell tumor in rodents. Some mutagenic data are available on all 24 pesticides producing thyroid tumors. Suspected tumor like changes pointed out in the present study agree with already reported data in rodents and other animals exposed to different kind of insecticide/pesticides (Mann, 1997; Pamela, et al., 1998; Wynford-Thomas D, *et al.,* 1989; Hill RN, *et al.,* 1998; U.S. EPA; 1997; Parker SL, *et al.,* 1997; McConnell EE, 1992). Twenty seven from all the pesticides tested with studies carried out and reviewed by cancer peer reviewer committee (CRPC) also supposted.

These have been determined to induce thyroid follicular cell tumor in rodents. The neoplastic changes like tumor structures in the thyroid follicles can also be compared with earlier findings.

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