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RESEARCH ARTICLE

TOXIC INFLUENCE OF DICOFOL ON THYROID GLAND IN FEMALE PUBERTAL RATS.

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Abstract

Evidences for thyroid disruption due to organochlorine exposure are increasing. The present study investigated the toxic influence of dicofol, an organochlorine pesticide on thyroid gland in female pubertal rats. The experiments were carried out in three equal groups of animals from PND 22 to 60 day .Two groups received dicofol orally at 5.87(1/100 LD50) and 19.27(1/30 LD50) mg/kg body wt. The third group was kept as control. Daily body weight was checked during the exposure period. Upon completion of exposure blood samples were taken for thyroid hormone estimation and thyroid gland was dissected, weight determined and processed for histopathological studies. There was a dose dependent significant decrease in the body weight gain in dicofol treated groups when compared with control. A significant increase in the thyroid weight was observed in high dose treated group. Histological studies of thyroid revealed degenerative changes in a dose dependent manner. The serum T3,T4 and TSH levels also indicate such a hypoactive thyroid, similar with histological results. These data suggest that exposure to dicofol may cause a hypofunctioning of thyroid gland.

Introduction:-

Pesticides are a group of chemicals made for the purpose of killing or deterring pest. Irrational use of pesticides has led to many clinical symptoms in humans, animals and led to the deterioration of environment. Problems associated with pesticide hazard to man and environment are not confined to developing nations, but extended to developed countries as well (Nuckols JR et al.,2007). Europe is now the largest pesticide consumer in the world, followed by Asia. China is the largest pesticide producer and exporter in the world (WenJun Zhang, 2011). Most of the pesticides worldwide are used on fruit and vegetable crops.

Among the pesticides organochlorine pesticides are a major class of commonly used pesticides. They degrade relatively slowly in the environment and being lipophilic accumulates in the fatty tissues of animals. Thus they remain in the environment and food web long after application (Swackhamer D, 1988). Chronic exposure to low concentrations of organochlorine pesticides over a long period may eventually lead to the accumulation of these toxic chemicals in the body (Penelope J.E. Quintana et al., 2004). In recent years, the presences of toxic pesticides have been reported in various biological materials such as milk, blood and fatty tissues from animals and humans (John PJ et al., 2001, Minakshi Rathore et al., 2002, FujiiY etal. 2011). According to the Center for Disease Control and Prevention(CDC), presence of a low percentage of organochlorine pesticides have been detected in tissues of most humans and animals (NCEH.2005).This causes great problems in the body including the disturbances of hormones controlling the metabolism. Food is considered to be the main source of organochlorine pesticides in the human body (Morgan DP,1974). Many organochlorine pesticides are endocrine disrupting chemicals, leading to toxic effects on the body's hormonal systems (Lemaire G et al., 2004).

Dicofol is an organochlorine pesticide currently registered for use on a variety of food crops in India and many other countries. It is structurally similar to DDT. It is a non systemic miticide (a chemical that kills mites) with several

documented negative health impacts. It is sold under a number of trade names, including acarin and nomite and is approved for use on a variety of fruits and vegetables (Larry P. Pedigo, 2014).In Kerala dicofol is widely used to control a red spider mite that attack coconut. Previous studies indicate that dicofol is a potential 'endocrine disrupting compound' (Hoekstra PF et al., 2006). The evidences for thyroid disruption due to organochlorine exposure are increasing (Whitney S. Goldner et al., 2010). Many organochlorine pesticides, including DDT, endosulfan, lindane, and dieldrin are known to produce antithyroid effects (Minakshi Rathore et.al, 2002, Roman GC, 2007). There are evidences for the role of thyroid hormones in the development of several organ systems including the reproductive system (Jannini EA et.al, 1995, Metz LD et.at, 1996, Krassas GE, 2000). The normal growth and development in puberty is greatly influenced by thyroid hormones (Griffin J E, 1988). They influence estrogen and androgen metabolism, sexual maturation, ovulation and fertility in females through hormone induced transcription pathways (Krassas GE, 2000, Kaphalia BS et.al, 1985). Thus small disturbances in the thyroid function especially during pubertal age period can lead to intense and lasting effects. Recent reports indicate that the problems associated with thyroid gland are common among women than men (Whitney S. Goldner et. al, 2010) and women of lower age groups are at a greater risk of potential health hazard due to pesticides than those of higher age groups (Kaphalia BS et.al, 1985). Normal function of hypothalamus- pituitary- thyroid axis is important in human reproduction and development in both sexes (Neepa Y. Choksi et.al, 2003). The present study was carried out to investigate the toxic influence of dicofol on thyroid gland of females in pubertal age period.

Materials and methods:-

Technical grade dicofol ,formulation 18.5 % Emulsifiable Concentrate (EC), supplied by Fil Industries Ltd.,Jammu-181133b (J& K) ,India was used for this investigation (Commercial name; Nomite). Its acute oral LD_{50} value is 587mg/kg body weight in rats (Dr. B Vasantharaj David, 2003).All other chemicals used for this experiments were of analytical grade obtained from Sigma – Aldrich (St. Louis-USA). Female Sprague – Dawley rats, body weight 34± 2g, weaned on day 21 were used for the study. The experimental protocol was met by the guidelines of CPCSEA (Ministry Of Environment And Forests, New Delhi, India) and was duly approved by the Animal Ethics Committee of the Institute (1415/GO/a/11/CPCSEA). The animals were housed in plastic cages under controlled conditions of temperature (24 ±2 ^{o}C) and humidity (60 ± 5 %) in a photoperiod controlled room (light: dark; 12h : 12h) with free access to complete and balanced laboratory diet and tap water ad libitum. Doses given were below the acute oral LD_{50} level of intoxication.

Experimental design:-

Rats were randomly divided into 3 equal groups- 6 each- and treated as follows.

Group-1 (Control):- Received distilled water at an equivalent volume which is used for preparing dicofol doses.

Group-2(Low Dose):- Dicofol at a dose of 5.87mg/ kg body weight/ day which equals 1/100 of LD_{50} of dicofol

Group -3 (High Dose):- Dicofol at a dose of 19.27mg/ kg body weight/day which equals1/30 of LD_{50} of dicofol

Dicofol was emulsified in drinking water to obtain the desired concentrations. The samples were given orally once daily with the help of a metallic gavage starting from PND22 to PND 60. From the initial day onwards the rats were inspected daily, body weights recorded and doses adjusted accordingly.

Collection of blood & Thyroid gland:-

At one day after the last day of treatment, all the animals were ansesthetized using ketamine – xylazine combination and blood sample was collected from each animal from the jugular vein. They were then decapitated; thyroid gland was dissected out quickly from the posterior aspect of trachea and the wet weight taken in an electrical monopan balance. The thyroid was then fixed in 10% buffered formalin for histological evaluation. Blood samples were allowed to clot at room temperature, centrifuged to obtain serum, which was individually kept at -20^{0}C until the hormonal assay.

Estimation of T3, T4 & TSH:-

The levels of T3 **(Tri-** iodo thyronine), T4 (Tetra-iodo thyronine) and TSH (Thyroid Stimulating Hormone) in serum were estimated by sandwich ELISA technique. The ready to use kits were procured from the Siemens Diagnostics. The protocols of the kit were strictly followed.

Histological Study:-
The fixed thyroid were embedded in paraffin, sectioned at 5µm thickness with the help of a microtome, stained with hematoxylin and eosin and examined by light microscopy. Morphometrical study was done with the help of a grided eyepiece lens.

Statistical analysis:-
The data are expressed as Mean ± standard deviation (SD). All statistical analysis were performed using analysis of variance (ANOVA), followed by Post Hoc Tukey HSD test for multiple comparison of the two treated groups with controls. Differences were considered as statistically significant at P value ≤0.05.

Results:-
1. Effect on body weight and thyroid weight:-
The animals treated with dicofol showed a dose related toxicity in terms of body weight. Both groups of dicofol treated rats showed a significant decrease ($p \leq 0.05$) in body weight gain during the experimental period with noticeable change in high dose group, when compared with the control. Dicofol increased the thyroid weight, and these increases were significant in high dose group when compared with the control (Table.1)**.**

Table.1: Effect of exposure to Dicofol on body weight and thyroid weight in pubertal rats.

Experimental Group	Initial Body Weight(g) Mean ±SD	Final Body Weight(g) Mean ±SD	Thyroid Weight (g)
Control	33.00 ±2.4	144.33±3.39	0.0076±0.00067
Low Dose	34.66±2.06	117.66±3.14*	0.0084 ±0.00047
High Dose	34.33 ±2.25	114.00± 2.25*	0.0091±0.00069*

*= significant p value < 0.05 compared to control.

2. Effect on T3, T4 and TSH:-
Dicofol treated rats at both doses showed a significant decrease in serum tri- iodo thyronine (T3) and tetra-iodo thyronine (T4) levels. A significant increase in serum thyroid stimulating hormone (TSH) levels observed in a dose dependent manner with a marked effect in high dose group when compared with the control (Table .2).

Table.2: Effect of exposure to Dicofol on T3, T4 & TSH in pubertal rats.

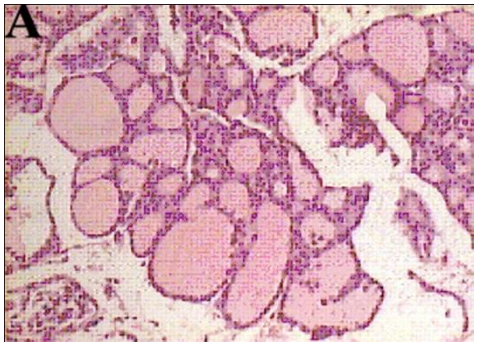
Experimental Group	T3 (ng / dl) Mean± SD	T 4(µg/ dl) Mean ± SD	TSHµIU/ml) Mean ±SD
Control	77.3333 ± 2.25093	3.8333 ± 0.13663	1.2033 ±0 .17896
Low Dose	68.0000 ± 1.78885*	2.8667 ± 0.13663*	2.0933 ±0 .08066*
High Dose	55.0000 ± 2.36643*	2.5000 ±0 .08944*	2.5267 ± 0.23347*

*= significant p value < 0.05 compared to control

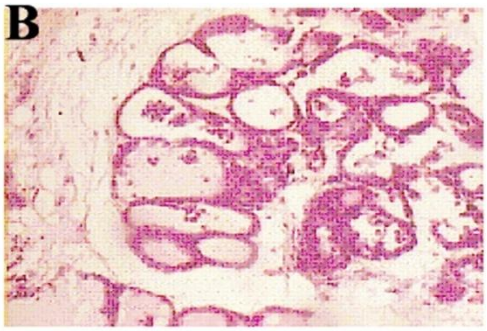
3. Effect on thyroid histology:-

Light microscopic examination of thyroid sections from control rats revealed variable sized thyroid follicles lined mainly by cuboidal cells which were intact on the basement membrane. The luminal spaces of the follicles were filled almost with colloid with an average colloid area of 800µm$^{2.}$ Sections from dicofol treated rats revealed exfoliated follicles with a decrease in follicular cell height. Amount of colloid had also become so reduced in a dose dependent manner. Colloid reduction was associated with an increased number of follicles in which the lumens had a decreased diameter. The average colloid area was found to be 765 µm^2 in low dose group and 625µm^2 in high dose group (Figure .1).

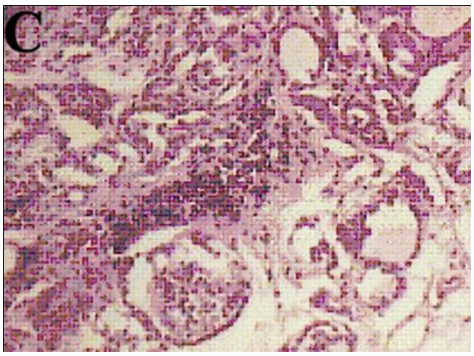
Figure. 1(A,B &C)



(A) Thyroid gland at magnification (40 xs) from female rats of control group showing variable sized follicles. The epithelial cells are intact on the basement membrane and filled almost with colloid. Average colloid area 800µm^2



(B): The thyroid gland of rats exposed to a low dose of dicofol showing epithelial cells that have been shifted to the colloid region. Amount of colloid has been reduced compared to the control. Average colloid area 765µm^2.



(C): The thyroid gland of rats exposed to a high dose of dicofol showing greater toxicity on the epithelium. Almost all the epithelial cells have been entered the colloid. Average colloid area 625µm^2.

Discussion:-

The results of the present study indicate that body weight (loss/gain) measurement is a good qualitative parameter to evaluate the toxicity of a pesticide and that dicofol can cause dose related toxicity in terms of body weight. There was a marked decrease in the body weight gain after high dose of dicofol treatment. This agrees with the effects of some other organochlorine pesticides on body weight (Camon L et al, 1988). Decreased body weight gain could be referred to metabolic derangements in the body by organochlorine pesticides (Somayyeh Karami- Mohajeri and Mohammad Abdollahi, 2011) which might be due to decreased level of thyroid hormones which plays a critical role in the regulation of carbohydrate and protein metabolism in all cells (Choksi et al., 2003).Thus the toxicity related decrease in body weight may be due to the poor tissue development and differentiation in the developing animal.

The present study results indicate an enlarged and hypoactive thyroid gland in a dose dependent manner with dicofol which was evidenced by significantly decreased level of thyroid hormones, T3 and T4 and from the elevated levels of TSH. Moreover there are histological evidences of decreased amount of colloid in dicofol exposed rats when compared with control group. Decreased colloid area has also been reported following exposure to polychlorinated biphenyls (Ness DK et.al, 1993). Hypothyroidsm due to dicofol exposure is already reported in adult male rats in which dicofol induced a significant decrease in serum thyroxine (T4) and tri- iodo thyronine (T3) level in a dose dependent manner. A previous study evaluating the risk of thyroid disease in pesticide exposed women in agricultural areas have shown that there was a higher incidence of hypothyroidism with the use of organochlorine pesticides (Whitney S. Goldner et al). The present study results also indicate hypothyroidism due to dicofol which is an organochlorine pesticide. This hypoactivity of thyroid gland may be due to the degeneration of thyroid follicular cells which was noticed in dicofol treated rats as the histological evidences of exfoliated follicles and areas of atropy and fibrosis. This degeneration might be due to free radical induced oxidative damage of cells mediated by dicofol toxicity. There are evidences for elevated lipid peroxidation due to several organochlorine pesticides like dicofol (Afaf A.EI-Kashoury et al, 2009). Data from previous studies have also shown that some of the organochlorine pesticides reduce the thyroid hormone levels by their interaction with transthyretin, a hormone carrier protein (Van den Berg KJ et.al,1991).Transthyretin is the major protein responsible for the transport of T_3 in animals. Thus there might be some inhibitory effects on transthyretin due to dicofol and thereby reducing the available binding sites for T_3 and T_4 on transthyretin to cause a decrease level of circulating thyroid hormones. The decreased level of thyroid homones may also be due to an inhibitory effect of dicofol on their biosynthesis by inhibiting the key enzymes like thyroperoxidase (Song M et al., 2012).

Conclusion:-

In the present study we investigated the toxicity of dicofol an organochlorine pesticide on thyroid gland in female pubertal rats. The results showed that chronic exposure to dicofol during the pubertal age period may cause a hypo functioning of thyroid gland. Several other OCPs are identified to be significantly associated with thyroid abnormalities.

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