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## **CIPROFLOXACIN INDUCED HORMONAL CHANGES IN THE THYROID GLAND OF RATS AND ANTI-OXIDANT VITAMIN A, C AND E AS RESCUE AGENTS**

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### **Abstract**

Ciprofloxacin is a widely prescribed antimicrobial drug belonging to a class of drugs called quinolones. The first 1 – cyclopropyl theroquinolone ciprofloxacin was synthesized in 1983 (Wentland, 1990) and this drug was remarkable among the quinolones for its high activity. Currently, the most important benefit claimed for vitamins A, C and E is their role as antioxidants, which are scavengers of particles known as oxygen free radicals (or oxidants). According to Goldberg (2005) without sufficient vitamin A, thyroid gland cannot produce thyroxine. Vitamin A is a key nutrient to produce thyroxine (Gary Null, 2005), Vitamin C is necessary for tyrosine metabolism and it is quite a delicate balance ([http://www.whatreallyworks.co.uk/start/fact\\_sheets.asp?article\\_ID=410](http://www.whatreallyworks.co.uk/start/fact_sheets.asp?article_ID=410)). Vitamin E works with iodine for proper thyroid function (Earl Mindell, 2005). Development of the thyroid gland is controlled by the thyroid stimulating hormone (TSH) secreted by the hypothalamus; TSH is also called thyrotropin (The Medicine Journal, 2000). The main function of the thyroid gland is to synthesis the iodothyronine, hormone 3, 5, 3', 5' tetraiodothyronine (T<sub>4</sub>, thyroxine) and 3, 5, 3' triiodothyronine (T<sub>3</sub>). These are peptides containing iodine. The two most important hormones are T<sub>3</sub> and T<sub>4</sub>, and these hormones are essential for life and have many effects on body metabolizing growth and development (Thyroid foundation of Canada, 1999).NSAIDs produce abnormal thyroid functions and there by alter the synthesis, transport and metabolism of thyroid hormones (Davis and Franklyn, 1991). They alter TSH response to TRH and thereby influence TSH release (Lim et al., 1995), and also compete for T<sub>3</sub> binding sites in serum and at the cell surface, which act as T<sub>3</sub> antagonists. Disturbances of the gastro intestinal tract are the most frequent side

effects observed with fluoroquinolones and occur in about 5% of treated patients. Microsomal metabolism of ciprofloxacin generates free radicals. Free radicals formation might play a role in the mechanism of producing adverse effects. Microsomal metabolism of ciprofloxacin generates free radicals. Free radicals formation might play a role in the mechanism of producing adverse effects. Ciprofloxacin is a potent inhibitor of the thyroid hormone regulated P 450 enzyme system in the liver since ciprofloxacin and enoxacin have shown the greatest inhibitory capacity (Clinical Toxicology Review, 1997).

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## **Introduction**

Taking ciprofloxacin can spur germs to mutate so that future bacterial infections become untreatable. During the last decades a dramatic increase in bacterial strains multi resistant to antibiotics, particularly CIPRO, has been reported, ciprofloxacin no longer can be used to treat gonorrhoea because the disease has become resistant to the drug. (Coronado et al., 1995; Smith et al., 1999 and CDC Special Report, USA). Currently, the most important benefit claimed for vitamins A, C and E is their role as antioxidants, which are scavengers of particles known as oxygen free radicals (or oxidants). These chemically active particles are by-products of many of the body's normal chemical processes. Their number is increased by environmental assaults, such as smoking, chemicals, toxins, and stress. The simple act of living also produces them, as we breathe in oxygen constantly. Vitamins protect us against a variety of diseases such as heart disease, Alzheimer's diseases, respiratory diseases and infectious diseases by boosting our immunity on eyes and skin function. Maintaining proper vitamin intake is quite simple with the help of a healthy diet and a high quality liquid multi vitamin taken daily. In relation to the physiology of the endocrine glands, hormones and their levels are the best indicators of glandular activity. The TSH, T<sub>3</sub> and T<sub>4</sub> level in the sera picture the drug and hormone interaction effectively. To verify if any rescue agents minimize / maximize the drug effect three excellent antioxidants vitamins, C, and E were given to animals of separate groups. To learn the recovery of the drug effect withdrawal group was maintained for each dose and durations. To observe the difference in drug effect for a short course and a long course, two different experimental periods were charted as short duration (7 days) and long duration (30 days).

## **Methodology**

### **Animals**

Healthy, adult male albino rats of Wistar strain weighing 260-300 grams were used for the present investigation. The animals were housed in proper ventilated animal house with constant 12 ± 1 hour light and 12 ± 1 hour dark schedule. The cages were cleaned thrice daily. Experimental animals were provided with standard diet and clean drinking water *ad libitum*.

### **Experimental protocol**

The animals were weighed and divided into three groups of five animals each.

#### **Group I: Control:**

The healthy rats were selected and treated as control and they received saline orally. A separate batch of five rats was maintained for vitamin supplementation groups and received gingelly oil orally.

#### **Group II: Short duration**

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

#### **Group III: Long Duration**

Here the animals were treated with ciprofloxacin at twelve hours interval for thirty consecutive days.

Group II and Group III was further sub-divided into six groups, each group consisting of five animals. The animals received the following regimen of treatment and all the treatments were designed on the basis of adult human dosage prescribed by the physicians and interpolated to the body weights of rats.

##### **a. Low dose**

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

##### **b. High Dose**

The animals received 400mg of ciprofloxacin / 60kg body weight as an oral dose.

##### **c. High Dose + Vitamins A**

The animals received 400mg. of ciprofloxacin followed by 7.5mg of vitamin A / 60kg body weight, as an oral dose.

##### **d. High dose + Vitamin C**

The animals received 400mg of ciprofloxacin followed by 500mg of vitamin C / 60kg body weight as an oral dose.

##### **e. High dose + Vitamin E**

The animals received 400mg of ciprofloxacin followed by 600mg of vitamin E / 60kg body weight, as an oral dose.

##### **f. High dose withdrawal**

The experimental animals received 400mg of ciprofloxacin as an oral dose and were allowed a withdrawal period of the drug for further seven days for short duration and one month for long duration.

Suitable controls were maintained for each duration of treatment. However, as there was no difference in any parameter among control group, a common control was employed in the present study.

**Hormone Analysis**

Enzyme linked Immunosorbent Assay (ELISA of Hormones) of TSH, T<sub>3</sub> and T<sub>4</sub> I were carried out using kits.

**Specimen collection and preparation**

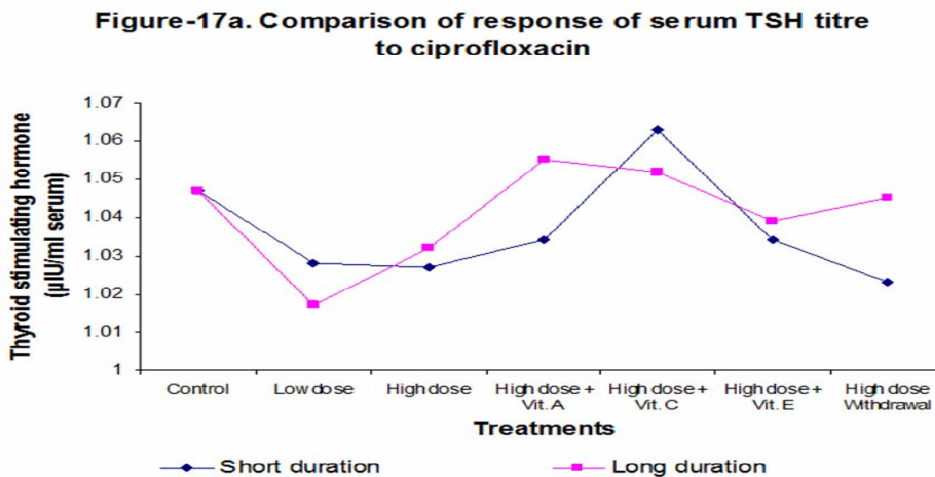
For all hormone assays fresh serum samples were acquired without additives. Serum must be stored between 2<sup>o</sup>C and 8<sup>o</sup>C for up to 48 hours prior to testing. For longer storage the serum should be stored at -20<sup>o</sup>C and the same can be preserved for up to one year. Thawed samples must be mixed prior to testing.

**Calculation of results**

Calculate the mean absorbance value (A<sub>450</sub>) for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance from each standard against its concentration in µIU/ml on graph paper, with absorbance values on the Y – axis and concentrations on the X – axis. Using the mean absorbance values for each sample, the concentration of TSH in µIU/ml is determined from a standard curve. The T<sub>3</sub> and T<sub>4</sub> values were expressed in ng/ml

**Result**

**Effect on thyroid stimulating hormone (TSH) (Figure - 17a)**



Each value is the mean ± SE of five animals  
 \* Control Vs Treatment, Significant at 5% level by ANOVA  
 \*\* Control Vs Treatment, Significant at 1% level by ANOVA  
 Means ± SE followed by a common letter are not significantly different at the 5% level by DMRT

**Short duration treatment**

Ciprofloxacin administration did not bring about any significant changes in the TSH levels at the doses used. Vitamins supplementations had no marked effects on this hormonal level. Similarly withdrawal of the drug also had no marked effect on the TSH titres.

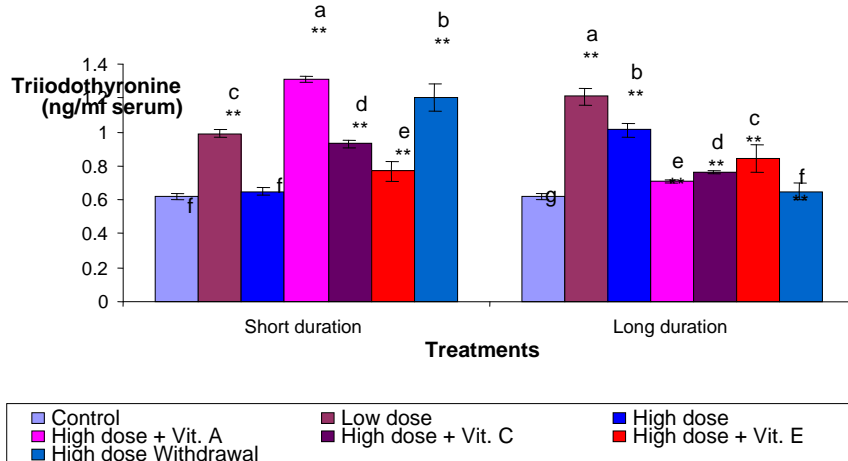
**Long duration treatment**

Similar to the short duration administration vitamins supplementations as well as withdrawal of the drug elected a similar response (i.e.) no marked effect on the TSH titres.

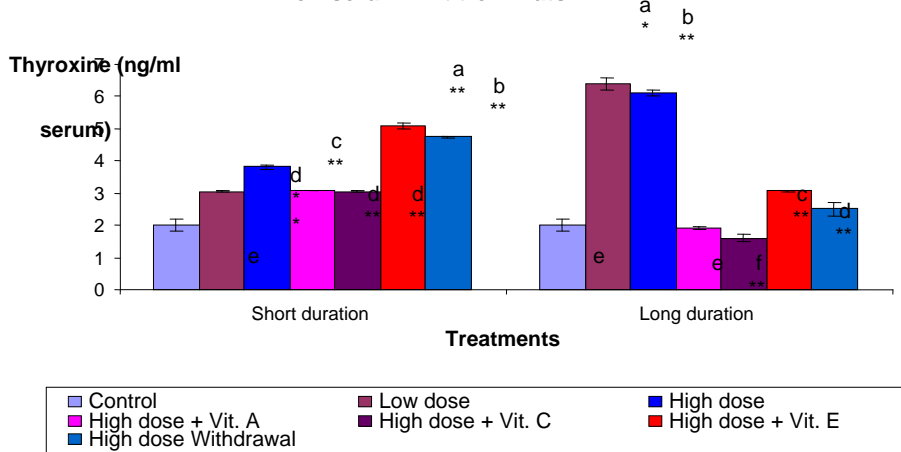
**Effect on triiodothyronine and thyroxine (T<sub>3</sub> and T<sub>4</sub>) (Figure- 2 and 3).**

**Short duration treatment**

**Figure-2. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on serum T3 titre in rats**



**Figure-3. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on serum T4 titre in rats**



Low dose of ciprofloxacin had induced a significant increase in T<sub>3</sub> (16%) and T<sub>4</sub> (53%) levels. However, high dose of the drug brought about a marked (91%) increase in T<sub>4</sub> level only. Supplementation with vitamin A to the high dose drug treated animal could not maintain the T<sub>4</sub> levels (54%) but had a marked influence on T<sub>3</sub> titres as it raised it by 112%. On the other hand, vitamin C was effective on raising the T<sub>3</sub> levels by 50% but only by 54% of the T<sub>4</sub> levels. Unlike the two-vitamin, supplementation vitamin E exerted a positive effect by raising the T<sub>4</sub> level by 155% of the control and more than 64% of the high dose groups. But this vitamin group could raise the T<sub>3</sub> levels only by 24% compared to vitamin A and C. Withdrawal of the drug was effective in raising the T<sub>3</sub> (93%) and T<sub>4</sub> (137%) when compared to the controls, and 46% and 90% compared to the high dose group.

### **Long duration treatment**

Though ciprofloxacin administration for the longer duration, stimulated the response elicited by the short durational drug administration the magnitude of response was higher. The T<sub>4</sub> levels were raised by 220% and T<sub>3</sub> by 95%, whereas, high dose could raise the T<sub>4</sub> levels by 205% and T<sub>3</sub> by 63% only. Vitamins supplementations appeared to lower the raised T<sub>3</sub> and T<sub>4</sub> levels like the drug withdrawal group. While vitamin A brought down the T<sub>3</sub> levels by 48% and the T<sub>4</sub> levels were drastically reduced to normal. Vitamin C supplementation however could bring only 23% decrease in T<sub>3</sub> titres but the T<sub>4</sub> titres were considerably reduced nearly 20% compared to controls. On the other hand vitamin E restored the control values and caused a 152% decrease in the T<sub>4</sub> titres. Drug withdrawal induced a 180% decrease of T<sub>4</sub> compared to high dose group and restored the T<sub>3</sub> levels to near normalcy.

### **Comparison of T<sub>3</sub> and T<sub>4</sub> response to ciprofloxacin.**

T<sub>3</sub> and T<sub>4</sub> levels were raised by the ciprofloxacin treatment and especially more effectively in the long durational groups. Longer durational vitamin supplementations could restore the T<sub>3</sub> and T<sub>4</sub> levels more effectively than the short durational treatments. Similarly, drug withdrawal of longer durational treatment could restore the T<sub>3</sub> levels to normalcy and T<sub>4</sub> levels to near normalcy.

## **Discussion**

### **Effect on serum thyrotropin and thyroid hormones (TSH, T<sub>3</sub> and T<sub>4</sub>)**

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid glands to disrupt one of several steps in the biosynthesis and secretion of thyroid hormones (Capen, 1994). The mammalian thyroid gland is composed of two distinct endocrine cell populations concerned with the synthesis of two different classes of hormones. One is the follicular cells, which secrete the metabolically active iodothyronines, and C- (parafollicular) cells, which produce calcitonin. The synthesis of metabolic thyroid hormones is different than in other endocrine glands because the final assembly of hormone occurs within the follicular lumen. This extracellular synthesis of thyroid hormones is made possible by thyroglobulin, a glycoprotein synthesized by follicular cells. The secretion of thyroid hormones, under the influence of pituitary thyrotropin (TSH) from stores in the luminal colloid is initiated by elongation of microvilli and formation of pseudopods. D and C Red No.3 are tetraiodinated derivatives of fluorescein which in lifetime studies increases the incidence of thyroid follicular cell adenomas in male rats (Capen and Martin, 1989)).

In a normal thyroid gland the follicles secrete  $T_3$  and  $T_4$  after an elaborate synthesis that involves iodide trapping, oxidation of iodide to iodine and its condensation with tyrosine molecules attached to thyroglobulin. This results in the formation of monoiodothyrosine (MIT) and an iodination at the 5<sup>th</sup> position forms diiodothyrosine (DIT). Two DIT molecules then undergo on oxidative condensation to form  $T_4$  or DIT and MIT couple to form  $T_3$ . The hormone remains unbound to thyroglobulin until secretion. When they are decreased, colloid is ingested by the thyroid cells, the peptide bonds are hydrolysed and free  $T_3$  and  $T_4$  are discharged into the capillaries (Ganong, 1995).

The secretion of TSH by the adenohypophysis is partially determined by the level of circulating thyroid hormones and partially by neural influences transmitted from the hypothalamus by a thyrotropin releasing factor which reaches the pituitary through the hypothalamic-pituitary portal system of veins (Volpe,1979). TSH exerts profound effects on many aspects of thyroid gland function such as increase in gland size and vascularity. It also increases iodine uptake, iodide clearance from the plasma, formation of iodotyrosine and iodothyronine, thyroglobulin proteolysis, and  $T_4$  and  $T_3$  release from the thyroid gland (Ragevendra Puri, 2003).

In the present investigation with ciprofloxacin, the TSH,  $T_3$  and  $T_4$  were altered by the drug. The drug had increased the  $T_3$  and  $T_4$  levels in long duration of its treatment with no change in TSH titres. Tricyclic anti depressants (TCAs) interface with the hypothalamic – pituitary – thyroid (HPT) axis via the serotonergic and nor adenergetic systems, this lead to a decrease in TSH (Beckett *et al.*, 1993).. The same study also observed that, by long term administration of clomipramine decrease  $T_4$ , f $T_4$  (free  $T_4$ ) and r $T_3$  (reverse  $T_3$ ).

Many drugs affect tests of thyroid function through alterations in the synthesis, transport and metabolism of thyroid hormones as well as via influences on thyrotropin (TSH) synthesis and secretion (Davis and Franklyn, 1991). Lower dose of proadrenomedullin N-terminal peptide had significantly lowered blood TSH concentration, without affecting the total, free  $T_3$  and  $T_4$  concentrations in rat (Ginda *et al.*, 2000). Morris and Gasca (1985) found no significant effect on mean basal plasma concentration of thyroxine or  $T_3$  among horses given phenyl butazone. In a study with neurotensin, the thyroid gland weight was observed to be increased, but the serum  $T_4$  level was decreased with no changes in serum TSH and  $T_3$  levels (Malendowicz and Miskowiak, 1990). These workers also observed that neuropeptide – Y had no effect on thyroid gland function. Nishrimura *et al* (2002) observed a low dose of 2, 3, 7, 8- tetrachlorodibenzo-p-dioxin, to affect thyroid hormone regulation especially that of TSH in the pituitary and of  $T_4$  in the thyroid gland of rat. A long-term administration of salicylate depressed serum levels of both  $T_4$  and  $T_3$  and  $rT_3$  to about 75% as well serum as TSH concentrations (McConnel, 1999).

NSAID's are reported to produce adverse effect on endocrine systems, especially cause abnormality on thyroid function. In the absence of clinical features thyroid dysfunction is noticed by alterations in the synthesis, transport and metabolism of thyroid hormones (Davies and Franklyn, 1991). NSAID attenuate TSH response to TRH and thereby influence TSH release (Lim *et. al.*, 1995). Several drugs and supplements can affect thyroid hormone absorption. An interaction of potentially much greater clinical significance recently reported is “ the effect of calcium carbonate on thyroxine absorption”. (Paauw, 2000). NSAIDs compete for  $T_3$  binding sites in serum. In cytosol NSAIDs were stronger competitors than in the nuclear extract (Barlow *et.al.*, 1994). Diclofenac sodium inhibits binding of  $T_3$  and  $T_4$  in the protons (Limet.*al.*,1988; Topliss *et.al.*, 1988 and Aoyama *et.al.*, 1990). NSAID administration increase the risk of hypoaldosteronism ( $E_1$  – Deiry *et.al.*, 1997) and also alter the hypothalamic – pituitary – adrenal axis(HPA) response (Hall *et.al.*, 1994).

The increased excretion of thyroid hormones causes decrease in serum  $T_4$  and promote TSH release from the pituitary gland thereby decreasing  $T_3$  and  $T_4$  in the serum and increasing TSH, by the administration of diniconazole (Hosokawa, 1993). The raised and lowered levels of these hormones are controlled by iodine, which controls the thyroid hormone synthesis and metabolism. The low dose of ciprofloxacin might have triggered the hydrolysis of thyroglobulin, so the  $T_3$  level of low dose drug treated group increased significantly in the present study of both long and short duration. A same effect with  $T_4$  secretion in long duration of ciprofloxacin treatment was also seen.

The thyroid gland secretes mostly  $T_4$  and very little  $T_3$ . Under certain conditions the conversion of  $T_4$  to  $T_3$  decreases and more reverse  $T_3$  is produced from  $T_4$  (Metabolic Health.com 1999). TSH and thyroid



hormone levels vary during the day and from day to day during the week, and the levels of TSH and T<sub>4</sub> were within the normal range at some time and the levels were abnormal at other times (Metabolic Health.com 2000). Diclofenac competes for T<sub>3</sub> and T<sub>4</sub> binding sites in serum at the cell surfaces (Bishnoi *et al.*, 1994). But in the present study, ciprofloxacin did not compete for T<sub>3</sub> and T<sub>4</sub> but it induces the gland to secrete both the hormones to maximum and was dosage dependent. The TSH is known to stimulate both differentiation and proliferation of thyroid follicle cells and its decreased level affects the above said processes of thyroid follicle cells (Pietrzik *et al.*, 1998). The decrease in metabolism of T<sub>4</sub> and T<sub>3</sub> appears to be responsible for the decrease in the level of TSH.

### **Effect of Antioxidant vitamins**

An adequate host defense activity critically depends upon the micronutrient status of an individual among which the cellular oxidant – antioxidant balance is an important determinant. Oxidative burst is part of the physiological function of phagocytes connected to a massive production and release of reactive oxygen intermediates. At the same time maintenance of the functional capacity of the host defense system in fighting against foreign antigens is significantly affected by the various reactive oxygen species. To compensate this critical condition there must be a high intracellular concentrations of antioxidants (Peake,2003).

Vitamin E is considered the most important radical scavenging vitamin of the lipid soluble compartment and vitamin A comprises all retinoids with properties like trans-retinol, and the individual surfactant proteins are selectively regulated by retinoic acid (Bauernfeind, 1998). The antioxidative vitamins, ascorbic acid and the tocopherols, are important not only for limiting tissue damage but also in preventing increased cytokine production, and also exerting anti-inflammatory effects in studies in man and animals (Bland, 1998). In the present study also the three antioxidants have prevented the tissue damage caused by ciprofloxacin especially when administered in high dose during the long duration study. Stimulation of gap junctional communication and intercellular communication are some of the main activity of carotenoids, which increase the oxidative property of vitamin A (Helmut Sies and Wilhelm Stahl, 1997).

In the present study also vitamins helped to bring back the normal status of T<sub>3</sub> and T<sub>4</sub> production which was raised by ciprofloxacin administration to maximum level. Vitamin C, vitamin E and the B complex vitamins may help in the treatment of hyperthyroidism and hypothyroidism because they play key roles in improving the overall healthy function of the thyroid gland and the immune system ([http://www.readers.digest.co.uk/health/thyroid\\_disease.htm](http://www.readers.digest.co.uk/health/thyroid_disease.htm)). Vitamin E and coenzyme Q play some role as scavengers in thyroid follicular cell hyper function or dysfunction (Mano *et al.*, 1998). In the present study also vitamin E showed its stimulant effect by restoring T<sub>3</sub> and T<sub>4</sub> levels from the elevated levels caused by the drug.

The drug withdrawal effectively restored the hormone status by 85%-99%, in a duration dependent manner. This may be due to the readopting of the thyroid tissue to its normal condition in due course of time. Deivendran (2004) observed a similar reversal of male reproductive hormones with ciprofloxacin on drug withdrawal.

## Conclusion

Ciprofloxacin administration did not bring any significant changes in the TSH levels in the doses used at both the duration of treatments. Neither vitamins nor drug withdrawal could bring any marked changes on the TSH titres. On the other hand T<sub>3</sub> and T<sub>4</sub> levels were raised especially in the long durational groups. Vitamins supplemented along with the drug were found to restore the T<sub>3</sub> and T<sub>4</sub> levels to control values more effectively in long duration treatment groups than in the short duration groups. By drug withdrawal the T<sub>3</sub> level was restored to normalcy and T<sub>4</sub> level to near normalcy.

## References

1. Ayoma, A., Natori, Y., Yamaguti, N., Koike, S., Kusakabe, K., Demura, R. and Demura, H. (1990). The effect of diclofenac sodium on thyroid function test *invitro* and *invivo* Rinsho Byori (Japan), 38 (6). P. 688-92.
2. Barlow, J.W., Curtis, A.J., Raggatt, L.E., Loidl, N.M., Topliss, D.J. and Stockigt J.R. (1994). Drug competition for intracellular tri-iodothyronine binding sites. *Eur. J. Endocrinol.*, (Norway), 130 (4) P. 417 – 21.
3. Bauernfeind, J.C.(1988). Vitamin A deficiency, a staggering problem of health and sight. *Nutrition Today*,. pp. 34.
4. Beckett, G.J., Nicol, F., Rae, P.W., Beech, S., Guo, Y. and Arthur, J.R.(1993). Effect of combined iodine and selenium deficiency on thyroid hormone metabolism of proadrenomedullin N-terminal peptide. *Horm. Metab. Res.*, 32 (1):10-4
5. Bishnoi, A., Carlson, HE., Gruber, BL., Kaufman, L.D., Bock, J.L. and Lindonni, M. (1994). Effect of commonly prescribed NSAIDs on thyroid hormone measurements. *Am. J. Med.*, Mar 96(3): 235 – 8.
6. Bland, G.W. (1998). Vitamins: molecular and biological function. *Proc Nutr. Soc.* 53: 251-262.
7. Capen, C.C and Martin, S.L.(1989).The effects of xenobiotics on the structure and function of thyroid follicular and C- cells. *Toxicol.Pathol.*, 17(2): 266-93.
8. Capen, C.C.(1994). Mechanisms of chemical injury of thyroid gland. *Prog. Clin. Biol. Res.*, 387:173-91.
9. Centres for Disease Control (CDC) Special Report (2001). Emerging Mechanisms of Fluroquinolone Resistance. David C. Hoop Massachusetts general Hospital, Harvard Medical School, Boston, Massachusetts, USA.
10. Clinical Toxicology Review. (1997). What are Fluroquinolones? CTR, Massachusetts Poison Control system, Vol. 20, No. 3.
11. Coronado, V.G., Edwards, J.R., Culver, D.H. and Gaynes, R.P.(1995). Ciprofloxacin resistance among nosocomial *pseudomonas aeruginosa* and *staphylococcus aureus* in the United States. National Nosocomial Infections Surveillance (NNIS) System. *Infect. Control. Hosp. Epidemcol.*, 16(2): 715.
12. Davies, P.H and Franklyn, J.A.(1991). The effects of drugs on tests of thyroid function. *Eur. J. Clin. Pharmacol.*, 40(5): 439-51.
13. Deivendran,R.(2004). Beneficial role of anti-oxidant vitamins C, E and A on ciprofloxacin induced oxidative stress in epididymis and seminal vesicle of rats. Ph.D. Thesis, Bharathiar University, Coimbatore, India.

14. Earl Mindell.(2005). In: Vitamin Bible for the 21<sup>st</sup> century. p. 90  
<http://www.newstarget.com/008902.html>.
15. El-Deiry, S.S., Naidu, S., Blevins, L.S.and Ladenson, P.W.(1997).Assessmeent of adrenal function in women heterozygotes for adrenoleukodystrophy. *Journal of Clinical Endocrinology and Metabolism*, 82(3) pp.856-60.
16. Ganong, W.F. (1995). The thyroid gland. In: Review of medical physiology, 17<sup>th</sup> edn., Appleton and Lange, Philadelphia. pp.290-305.
17. Gary Null. (2005). Complete guide health nutrition. In: The experts speak on iodine and thyroid. P.410.  
<http://www.newstarget.com/008902.html>.
18. Ginda, W.J., Nowak, K.W and Malendowicz, L.K. (2000). Decrease of TSH level and epithelium/colloid ratio in rat thyroid glands following administration of proadrenomedullin N –terminal peptide (12-20). *Horm. Metab .Res.*, 32(1): 10-4.
19. Goldberg.B. (2005). How iodine accelerates weight loss by supporting the thyroid gland.
20. Hall, J., Morand, E.F., Medbak, S., Zaman, M., Perry, L., Goulding, N.J., Maddison, P.J and O’Hare, J.P. (1994). Abnormal hypothalamic-pituitary-adrenal axis function in rheumatoid arthritis. Effects of nonsteroidal anti-inflammatory drugs and water immersion. *Arthritis Rheum.*, (United States of America), 37(8). Pp1132-7.
21. Helmut Sies. and Wilhelm Stahl.(1997).Carotenoids and Intercellular communication via gap junctions. *International Journal for Vitamin and Nutrition Research.*, Band 67, Heft 5© Verlag Hans Huber, Bern.
22. Hosokawa, S., Nakamura, J., Ito, S., Murakami, M., Ineyama, M., Yoshioka, K., Yamada, T., Seki, T., Matsu, M and Yamada, H. (1993). Hormonal disregulation mechanism in the rat thyroid tumor induced by diniconazole. *J. Toxicol. Sci.*, 18(1): 57-67.
23. Lim, C.F., Bai, Y., Topliss, D.J., Barlow, J.W, and Stockigt (1998). Drug and fatty acid effects on serum thyroid hormone binding. *J. Clin – Endocrinol Metab* (United States), 76 (4): 682 – 8.
24. Lim, C.F., Lordl, N.N., Kennedy, J.A., Topliss, D.J. and Stockight, J.R. (1995). Effects of loop diversities and NSAIDs on thyrotropin release by rat anterior pituitary cells. *Invitro. Metabolism* (United States), 44 (8): p. 1008-12.
25. Mano, T., Iwase, K., Hayashi, R.,Hayakawa, N., Uchimura, K., Makino, M., Nagata,M. and Ioth, M.(1998). Vitamin E and Coenzyme Q concentration in the thyroid tissue of patients with various thyroid disorders. *Am. J. Med Sci.*, 315 (4):230-2.
26. Malendowicz, L.K. and Miskowiak, B. (1990). Effects of prolonged administration of neurotensin, arginine-vasopressin, NPY, and bombesin on blood TSH, T3 and T4 levels in the rat. *In Vivo.*, 4(4): 259-61.
27. McConnell, R.J.(1999). Changes in thyroid function tests during short-term salsalate use. *Metabolism*, 48(4): 501-3.
28. Morris, D.D. and Garcia, M.C. (1985). Effects of phenylbutazone and anabolic steroids on adrenal and thyroid gland function tests in healthy horses. *Am. J. Vet. Res.*, 46(2): 359-64.
29. Nishimura, N., Miyabara, Y., Sato, M., Yonemoto, J. and Tohyama, C.(2002). Immunohistochemical localization of thyroid stimulating hormone induced by a low oral dose of 2, 3, 7, 8 –tetrachlorodibenzo-p-dioxin in female Sprague-Dawley rats. *Toxicology*, 171(2-3): 73-82.
30. Paauw, D.S. (2000). Drug Absorption Problems J A M A. 283. 2822.
31. Peake, J. M. (2003). Vitamin C: effects of exercise and requirements with training. *Int. J Sport. Nutr. Exerc. Metab;* 13(2): 125-51.
32. Pietrzik, C.U., Hoffmann, J., Stober, K., Chan-Yan-Chun., Christophbauer, Debooh, A,O,C., Roch, J. and Herzog, V.(1998). From differentiation to proliferation: The secretory amiloid precursor as alocal mediator of growth in thyroid epithelial cells. *Proc. Natl. Acad. Sci.*, (USA), 1770-1775.

33. Raghvendra Puri. (2003). Thyroid gland. In: Mammalian Endocrinology. 1<sup>st</sup> edn., Dominant Publishers and Distributors, Moujpur, Delhi. pp.157-254.
34. Smith, K.E., Besser, J.M., Hedberg, C.W., Leano, F.T., Bender, J.B., Wicklund, J.H., Johnson, B.P., Moore, K.A. and Osterholm, M.T.(1999). Quinolone resistant *Campylobacter jejuni* infections in Minnesota, 1992, 1998. *N Engl J Med* 340 (20): 1525- 32.
35. Thyroid Foundation of Canada. (1999). The Thyroid Gland. A General Introduction. In: Health Guides on Thyroid Disease. Copyright © 1999 Thyroid Foundation of Canada / La Fondation Condiennne de la Thyroide. Copyright©2004 Abbott Lab Abbott park Illinois U.S.A.
36. Topliss, O.J., Hambling, P.S., Kolliniatis, C., Lim, F. and Stockigt, J.R.(1998). Furosemide fenclofenac, diclofenac, mefenamic acid and meclofenamic acid inhibit specific T<sub>3</sub> binding in isolated rat hepatic nuclei. *J.Endocrinol.Invest.* (Italy), II (5), P.355-60.
37. Volpe.R (1979). The Thyroid In: Systematic endocrinology 2<sup>nd</sup> edn.,Harper and Row, Publishers, Inc., 2350 Virginia Avenue, Hagerstown, Maryland, U. S.A.pp.84-136.
38. Wentland, M.P. (1990), Structure, activity, relationship of fluoroquinolones. In: Siporin, C., Heifetz, C.L., Domagala, J.M., (eds). The new generation of quinolones, Marcel Dekker., New York, pp 1 – 43.
  - <http://www.whatreallyworks.co.uk/start/factsheets.asp> article ID = 410
  - <http://www.readersdigest.co.uk/health/thyroiddisease.htm>.