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# Antioxidants Protect Cell Damage from Free Radicals: A research study on Thyroid Hormones in Wistar Rats.

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***Abstract:*** *Antioxidant vitamins neutralize free radicals and may prevent unwanted free radical cellular damage in the body. Free radicals damage other molecules by removing electrons and destroying deoxyribonucleic acid, or DNA.The thyroid gland is an organ of "oxidative nature" as huge amount of ROS, especially of $H_2O_2$, are produced in the thyroid under physiological conditions. Aims- To evaluate the effect of antioxidants on thyroid hormones in rats, fourty wistar were used in this study and with antioxidants namely Vit. C, Vit. E and Turmeric. Methods-The present research work has been undertaken to investigate the free radical scavenging activity and antioxidant status in both hyperthyroid and hypothyroid patients.Adult male Wister rats, weighing around 150-200 gms were used in this research work and under maintained animal care facilities and veterinary supervision. All rats were fed with normal diet (20% protein) and have been administered with known amount of Vitamin C, Vitamin E and turmeric.Results-Samples has been analysed after 15 & 30 days of feeding. Results showed increased levels of thyroxin in rats after 15 days (Vit C-5.2 ± 1.2 NS,Vit E - 5.3 ± 0.5 NS and Turmeric-5.3 ± 0.87 NS).Conclusion-It was observed that the circulating levels of T3 were significantly increased in Vit. C, Vit. E and Turmeric extract treated rats (Table -2 and 3). The thyroid hormones responded to antioxydants indicating the significance of antioxydants for the prevention of occurrence of certain diseases in thyroid gland by protecting biological system against potentially harmful effects of processes or reactions that can cause excessive oxidations.*

***Keywords****: Antioxidants, Free radicals, Thyroid hormones, Turmeric, Vit. C & Vit. E*

## I. Introduction

Thyroid hormones are necessary for normal growth of our body and assumed as one of the most important involved hormonal factors in regulation of basic metabolic rate [1]. Oxygen free radicals (ROS) are produced from normal metabolic reactions after several steps. Although free radicals are potentially dangerous for organisms. Microsomal-membrane and mitochondrial electron transfer chains and also auto oxidation reactions are the main sources of free radicals [2]-[3]. Both superoxide anion and hydrogen peroxide produce highly reactive hydroxyl radicals via Haber-Weiss reaction. Hydroxyl radical can initiate lipid per oxidation, which leads to damage of membrane structure and function [4]. Lipid peroxidation and oxidative stress induced by hypothyroidism are the results of both increased production of free radicals and reduced capacity of antioxidative defense [5]–[6]. The malfunction of respiratory chain in the mitochondria, that is induced by hypothyroidism, leads to accelerated production of free radicals, which subsequently leads to oxidative stress [7]-[8]. Metabolic disorder caused by an autoimmune hypothyroidism can also increase oxidative stress [9]. The purpose of study was to see effects of the antioxidants vitamins C, E and turmeric on thyroid hormones in Wistar Rats, how these can protects cell damage from free radicals & accelerates aging. Radioimmunoassay was performed to see the results on thyroid hormones in rats.

## II. Materaials & Methods

This research study was performed at the Radiation Medicine Centre, BARC, Tata Memorial Centre, Parel, Mumbai, India. Forty adult male Wister rats, weighing around 150-200 gms has been used in this study. The rats were maintained in the animal care facilities of the Radiation Medicine Centre, Bhabha Atomic Research Centre (B.A.R.C.) Parel, Mumbai,India. Under veterinary supervision food and water were supplied ad labium to the animals. All rats were fed with normal diet (20% protein). Animal studies as performed in compliance with generally accepted guidelines governing such work. Following chemicals and nuclear medicine radioimmunoassay has been used:

1) L. Ascorbic Acid (Vitamin 'C') $C_6H_8O_6$ obtained from the LOBA chemicals private limited Mumbai.
2) D1–α-TOCOPHEROL ACETATE (Vitamin 'E') $C_{31}H_{52}O_3$. Obtained from the LOBA CHEMICALS private limited Mumbai,india.

3) Turmeric extract: fine powder of dry Thizomes of turmeric (Curcuma-Longa-L) was obtained from a local market and was subjected to cold ethanolic percolation. (Saiba Industries Pvt. Ltd. Bombay) 1% of this dry powder was mixed in normal diet (20% of proteins).
4) RIA Kits: The thyroid status of the treated animals was confirmed by serum thyroid hormone levels measured by radioimmuno-assay (RIA) KITS supplied by the Board of radiation and isotope technology (BRIT) [Vashi], Mumbai, India.
**5)** Thyroid hormones free serum: Normal rats serum was pooled and thyroid hormones free serum was prepared.

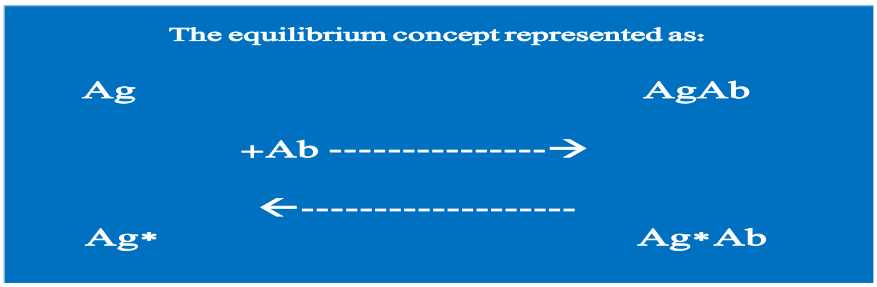
**EXPERIMENTAL DESIGN**

Total forty rats were used for this work; they were divided in to four different groups. Chemicals were mixed with their diet for 15 days.

RADIOIMMUNOASSEY OF TRIIODOTHYRONINE (T3)

Principle and Features of the Test

Unlabelled endogenous T3 competes with radiolabelled T3 for the limited binding sites on the antibody made specifically for T3. At the end of incubation, the T3 bound to antibody (Ag-Ab) and free T3 are separated by the addition of polyethylene glycol. The amount bound to the antibody in the assay tube is compared with values of know T3 standards and the T3 concentration in the patient. Sample can be calculated. The equilibrium concept is more true. As Ag increases (Ag* is constant) the equilibrium pushed to the right and Ab diminishes progressively.




Equilibrium concept(Antigen-Ag,Antibody-Ab)

RADIOIMMUNOASSEY OF THYROXINE (T4)

Principle and Features of the Test

Unlabelled endogenous T4 competes with radiolabelled T4 for the limited binding sites on the antibody made specifically for T4.At the end of incubation, the T4 bound to antibody (Ag-Ab) and free T4 are separated by the addition of polyethylene glycol (22%).The amount bound to the antibody in the assay tube is compared with values of known T4, standards and T4 concentration in the patients sample can be calculated.

**Table-1 Rats were divided into four different groups**

| S.N. | Groups | Labeling |
|---|---|---|
| 1 | Group I | -Labeled as Control |
| 2 | Group II | -Treated as Vit.C (250 mg Vit. C In Diet) |
| 3 | Group III | -Treated as Vit. E (250 mg Vit. E In Diet) |
| 4 | Group IV | -Labeled as Turmeric Extract (1 % Turmeric Extract In Diet) |

After 15 days of antioxidants doses, all rats were weighed and found healthy, under general anesthesia with Ether, blood was taken from the heart in heparinized syringes from all the animals in separate test tubes (according to group). The Blood was centrifuged for 30 minutes at $4^{o}$C. The serum was separated for the Radioimmunoassay's.

**STATISTICAL ANALYSES METHOD**

Statistical Analyses were performed by the standard method. All the results were expressed as mean ± standard deviation (S.D.). The mean of the two groups compared using the students't' test. 'P' value of less than 0.05 was considered to represent statistically significant change.

## III. Results

To evaluate the effect of antioxidants on thyroid hormones in rats, fourty wistar were used in this study and with antioxidants namely Vit. C, Vit. E and Turmeric.After 15 days of Vit. C,Vit. E and Turmeric feeding blood samples were collected and plasma was used for the radioimmunoassay of triiodothyronine (T3) and thyroxin (T4)to evaluate the effect of antioxydants on thyroid hormones.To compare the performance of KITS(T3 and T4) we have performed intra-assay (within assey system)to do the quality control of the assay system.

We observed :--

**TABLE-2 Intraassay variation of T3 & T4 Radioimmunoassay**

| No. of Observations | Triiodothyronine T3 ng/dl | No. of Observations | Thyroxine T4 ng/dl |
|---|---|---|---|
| 1. | 114 | 1. | 5.6 |
| 2. | 114 | 2. | 5.3 |
| 3. | 117 | 3. | 6.2 |
| 4. | 129 | 4. | 5.4 |
| 5. | 115 | 5. | 5.0 |
| 6. | 107 | 6. | 5.5 |
| 7. | 116 | 7. | 5.8 |
| 8. | 113 | 8. | 5.3 |
| | | 9. | 5.2 |
| | | 10. | 5.7 |
| | | 11. | 5.2 |
| | | 12. | 5.8 |
| T3 | | T4 | |
| Mean = 117<br>S.D. = 6.05<br>C.V. = 5.21% | | Mean = 5.5<br>S.D. = 0. 3<br>C.V. = 6.23% | |

**TABLE- 3** Triiodothyronine & Thyroxin Levels
After 15 Days of Administration with Vitamin C, Vitamin E, & Turmeric in Rats

| S.N. | Groups N=10 | Triiodothyronine T3 ng/dl | Thyroxine T4 ng/dl |
|---|---|---|---|
| 1. | CONTROL | 49.2 ± 10.9 | 4.6 ± 0.8 |
| 2. | VITAMIN 'C' | 76.6 ± 15* | 5.2 ± 1.2 NS |
| 3. | VITAMIN 'E' | 76.6 ± 15* | 5.3 ± 0.5 NS |
| 4. | TURMERIC | 65.3 ± 17 NS | 5.3 ± 0.87 NS |
| NS = Not significant.<br>* p < 0.01 vs. control | | | |

**TABLE- 4** Triiodothyroxine T3 & Thyroxin T4 Levels
After 30 Days Of Treatment With Vitamin 'C', Vitamin 'E' And Turmeric in Rats

| S.N. | Groups N=10 | Triiodothyronine T3 ng/dl | Thyroxine T4 ng/dl |
|---|---|---|---|
| 1. | CONTROL | 60.4 ± 16.3 | 3.8 ± 0.54 |
| 2. | VITAMIN 'C' | 89.7 ± 8.0* | 4.3 ± 0.84 NS |
| 3. | VITAMIN 'E' | 93.7 ± 19* | 4.7 ± 0.58 NS |
| 4. | TURMERIC | 84.8 ± 11* | 4.8 ± 0.76 ** |

NS = Not significant.

*p < 0.01 vs. control
**p < 0.02 vs. control

The present study was undertaken to evaluate the effects of antioxydants on thyroid hormones.It was observed that the circulating levels of T3 were significantly increased in Vit. C, Vit. E and Turmeric extract treated rats (Table -3 and 4)**.** However, T4 levels were significantly increased after 30 days of Vit. E and Turmeric extract treatment (Table-4).Hence; our results show positive effects of antioxydants (Vit. C,Vit. E and Turmeric)on thyroid hormones levels, which could be due to direct involvement of antioxydants on thyroid gland or on deiodinase enzyme activity. This needs further detailed study to prove the exact pathway of the mechanism of action of antioxydants.

## IV. Discussions

Free radical production, a natural event in cells, becomes highly dangerous if overproduction occurs. However, a number of antioxidants defense systems are present within the cell, which helps to protect it from the deleterious effects of oxidative stress. The most important chain breaking antioxidant inhibitor of lipid peroxidation is oc -tocopherol, a physiological antioxidant and membrane stabilizer [10]. The blood levels of T3, T4. Total cholesterol as well as the eights of thyroid gland. and *MDAlmg* of liver protein in control rats, MMI-induced hypothyroid rats and rats treated with vit. C, vit. E or TE, along with MMI for 15 days and 30 days are shown in Tables 1 and 2 respectively [11].Thyroid function is known to be altered by many environmental factors, such as energy intake and dietary composition in addition to ambient temperature. However, it is not clear yet whether thyroid hormone induced by ambient temperature, nutrient deficiency etc., influences growth in Animals **[**12**].** The data suggests that the positive effect of antioxidants on thyroid gland could be due to direct involvement of antioxidants on thyroid gland [13]. The clinical significance of dietary antioxidants it is necessary to understand what cellular oxidants are and why they are potentially harmful to the body. During the normal course of metabolic events, which occur within all cells, there results the production of free radicals and what are termed reactive oxygen species, ROS and reactive nitrogen species, RNS**.**Most discussion of oxidants and antioxidants focuses on the production, uses, and harms associated with ROS. ROS and RNS are formed either as necessary components of vital biological processes such as in the transmission of nerve impulses and in the normal course of inflammatory reactions. The most common ROS are the non-radicals hydrogen peroxide (H2O2) and ozone (O3) and the radicals superoxide ion (O2.–) and hydroxyl radical (.OH). Two physiologically important RNS include nitric oxide (NO, but most correctly written with the radical designation: NO.) and peroxynitrite (ONOO–). Hydrogen peroxide, superoxide, and nitric oxide react with only a selective group of biological molecules in the body, whereas the hydroxyl radical will instantaneously react with virtually any molecule with which it comes into contact [14].As a major water soluble antioxidant, vitamin C is capable of maintaining sulphydryl compounds in a reduced state, particularly in several redox reactions [15].The possible future enhancements in this study; vitamin C increases the resistant power of the body and protect cellular damage from free radicals and alpha-tocopherol, the biologically and chemically most active form of vitamin E, is by far the most abundant lipid-soluble antioxidant in humans accelerates aging . Our study shows antioxidants may possibly reduce the risks of cancer. Antioxidants clearly slow the progression of age-related macular degeneration.

## V. Conclusions

Triiodothyronine and thyroxine designated as designated T3 and T4 are two physiologically important thyroid hormones. The clinical syndrome produced by excess hormone is usually referred to as thyrotoxicosis or as hyperthyroidism. In this research study it is clear that the administration of antioxidants namely Vit. C,Vit. E and turmeric significantly increased in the circulating levels of T3 and T4.The thyroid hormones responded to antioxydants indicating the significance of antioxydants for the prevention of occurrence of certain diseases in thyroid gland by protecting biological system against potentially harmful effects of processes or reactions that can cause excessive oxidations.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing

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