OXIDATIVE STRESS IN EXPERIMENTAL HYPOTHYROIDISM: EFFECT OF MITOCHONDRIA TARGETED CURCUMIN SUPPLEMENTATION

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ABSTRACT

Introduction: The study was investigated to ameliorative effects of curcumin against E.coli infection induced hypothyroidism in mice. Female mice were E.coli induced intraperitoneal and mitochondrial-targeted curcumin (mt-c) give orally.

Method: The present investigation was conducted, Mitochondrial targeted curcumin synthesized by covalent linkage of curcumin with lipophilic cation. TPP (1.31 gm) reacted with HBr (350 ml) precursor to obtain lipophilicity. Reverse osmosis (RO) water 200 l in (5×10⁶) E.coli, and DMSO (2% v/v) were injected in mice by intraperitoneal, after three days 100 1 mt-c (0.12 mg/100 1), RO water and DMSO were induced in mice for seven days. Control group mice for each experimental setup were given simultaneously RO water and DMSO.

Result: The thyroid gland weight of mice increased after bacterial toxicity and ameliorate with mt-C its weight decrease, the level of T3 and T4 decerase against bacterial toxicity and ameliorate with mt-C its level increased, Lipid peroxidation and Superoxide dismutase (SOD) increased the level against bacterial toxicity and ameliorate with mt-C its value decreased, where as level of GSH after toxicity decreased and ameliorate with mt-C its value increased.

Conclusion: The data suggest that effect of mt-c positive against E.coli toxicity on thyroid gland, which could be direct involvement of mt-c on the thyroid gland of mice.

Keywords Mitochondria, mitochondria-targeted curcumin(mt-c), thyroid gland, reactive oxygen species (ROS), oxidative stress, thyroid hormones, E.coli.

INTRODUCTION

Oxidative stress may result from over production of reactive oxygen species (ROS) and the failuare of antioxidant defence system (Torun et al., 2009). Mitochondria are the vital role for the production of ROS due to incomplete reduction oxygen to water as a consequence of electron leakage in electron transport chain (Boveris and Chance,1973; Nohl et al., 2004). The free radicals, such as hydroxyl radicals, peroxide (H₂O₂) and superoxide as an important contribution to cell and tissue damage.

In the thyroid gland, thyrotropin increased the level of H₂O₂ (Corvilain et al., 1991). This H₂O₂ used as a substrate for the thyroperoxidase enzyme which catalyzes, synthesis of the thyroid hormones (T3, T4). However in hypothyroidism, hormone synthesis is decreased and H₂O₂ accumulated in the colloid of the thyroid gland and it may be destroyed thyroid follicle (Goyens et al., 1987). Abnormally high level of ROS may increase lipid peroxidation (LPO), damage nucleic acid and oxidized necessary proteins, thus leading to damage cellular.
organelles protein. One of the most crucial ways to reduce the oxidative damage is to use compounds, which has antioxidant property. Although several non-enzymatic antioxidants such as vitamin-E and vitamin-C have little or less protective value (Cocheme and Murphy, 2010). One of the potential explanations for this response, may be antioxidant therapies has non-specific nature (Murphy and Smith, 2007). Consequently, antioxidants show limited potential and they may not accumulate in sufficient amount inside the mitochondria to reduce oxidative damage. Thus, efficient mitochondrial targeted antioxidants need to develop which have higher permeability towards mitochondrial membrane (Kumar and Mahobiya, 2017, Kumar et al., 2017).

The study was investigate to effect of mitochondria-targeted-curcumin in the treatment of the hypothyroid condition. The *E.coli* produced antithyroid compound (London *et al.*, 1965; Vought *et al.*, 1967; Malamos *et al.*, 1971) and effect of antioxidants such as curcumin on control and hypothyroid mice.

**Materials and Methods**

**Chemicals and Reagents**

All chemicals were of analytical grade supplied by Merck, Hi-Media, and Sigma chemical Co. USA. nitroblue tetrazolium (NBT), Triphenylphosphonium oxide (TPP), HCl, 48 % Hydrobromic acid (HBr), thiobarburetic acid (TBA), trichloroacetic acid (TCA), ethyl acetate, ethanol, dimethosulpho-oxide (DMSO), GSH (Glutathione reduced), riboflavin, Phenazine methosulphate (PMS), NADPH, lithium lactate, H2O2 (30%), NADH.

**Experimental design**

**Synthesis of targeted antioxidant**

Mitochondrial targeted curcumin synthesized by covalent linkage of curcumin with lipophilic cation. TPP (1.31 gm) reacted with HBr (350 ml) precursor to obtain lipophilicity (Hercout A *et al.*, 1998). To synthesize the targeted derivative of curcumin, a solution of lipophilic cation refluxed with curcumin and evaporated to obtained mitochondrial targeted curcumin (mt-c) (Smith *et al.*, 2003, Kumar and Mahobiya, 2017).

**In vivo study**

Reverse osmosis (RO) water 200 l in (5×10^6) *E.coli*, and DMSO (2% v/v) (Kunwar *et al.*,2006) were injected in mice by intraperitoneal, after three days 100 l mt-c (0.12 mg/100 l), RO water and DMSO were induced in mice for seven days. Control group mice for each experimental setup were given simultaneously RO water and DMSO. The animals were euthanizing by decapitation for ten days from treatment. Thyroid gland with trachea was dissected out, washed in ice-cold saline (0.9% NaCl), and stored frozen at -80°C for further studies.

**Preparation of tissue extract**

Thyroid gland extract was prepared in 0.02 M tris-Cl (pH 7.4) and homogenate (10% w/v). Homogenate was centrifuged at 1000 rpm for 10 min at 4°C. After the first centrifugation, the pellet was discarded and the supernatant fluid was recentrifuged at 12500 rpm for 20 min. So obtained was stored at -20°C for the study of biochemical assay.

**Biochemical estimation**

**Protein estimation**

Protein content was measured by the method of Lowery *et al.*, (1951).

**Estimation of T3, T4, and TSH**
Detection of T3, T4 measured using ELISA provided by The Calbiotech Inc. (California, USA). All the assay were performed in triplicate.

Assay of lipid peroxidation

Lipid peroxidation was determined by the measuring of thiobarbituric acid reactive substance (TBARS) in terms of malonaldehyde (MDA) following as described the method (placer ZA et al., 1966) with some modification. Briefly, 1ml of the tris-maleate buffer of pH 5.9, and 10 μl of the tissue extract was incubated at 37º C for 30 minutes. After, 1.5 ml of TBA reagent was added and the mixture was incubated at boiling water (100-120º C) for 10 min. After cooling at room temperature pyridine: n butanol (3:1, v/v) mixture and 1N NaOH is added. The contents were thoroughly shaken and allowed to stand for 10 minutes. The photometric measurement was carried out at 548 nm and the level of lipid peroxidation was expressed as nmol MDA/g.

Assay of Superoxide dismutase (SOD)

The activity of superoxide dismutase (EC: 1.15.1.1) was determined method (Kakkar et al., 1984). The reaction mixture consisted of 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M), 300 M NBT, 186 M PMS, and 0.1 ml suitably diluted tissue extract. The reactions were started by addition of 780 M NADH at 30 º C and stopped after 90 s by the addition of 1 ml of TCA. The reaction mixture was stirred with 4 ml of n-butanol and allowed to stand for 10 min. The control set without tissue extract run simultaneously. The unit of the enzyme was defined as 50% inhibition of NBT/min and the activity was expressed as units/mg protein.

Glutathione reductase assay

The activity of glutathione reductase (EC: 1.6.4.2) was measured following the method of Carlberg and Mannervik (1975) with some modification. The reaction mixture (1 ml) containing 0.2 M potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 2 mM oxidized glutathione (GSSG) and 0.30 mM NADPH. The reaction was initiated by addition of 10 μl tissue extract and NADPH was recorded as a decrease in absorbance at 340 nm for 5 min. Nonspecific oxidation of NADPH was calibrated by the absorbance measured in the absence of GSSG. Unit of the enzyme was defined as μmol NADPH/min and the activity of the enzyme was expressed as units/mg protein.

Statistical Analysis

Results expressed as mean ± SD and student t-test was applied for determining the level of significance between controls and treated groups Fisher (Fisher 1953).

RESULTS AND DISCUSSION

Production of free radical, a natural event in cells, because highly dangerous if overproduction in cells. However, so many antioxidant defense systems are present with the cell to help to protect them and neutralized it. Vitamin D is a most important chain breaking antioxidant inhibitor of lipid peroxidation and membrane stabilizer (Burton G W & Ingold K U. 1989).

The level of T3, T4, weights of the thyroid gland, MDA/mg, SOD and GSH level of thyroid protein in control mice. E.coli induced hypothyroid mice and mt-c treated mice along with E.coli for 14 days were shown in tables A.
Table - A

Effect of mt-con E.coli induced hypothyroid mice for 14 days 
(Mean ± SD of 5 animals)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thyroid wt (mg)</th>
<th>Serum T3 (ng/dl)</th>
<th>Serum T4 (μg/dl)</th>
<th>MDA (nM/mg protein)</th>
<th>SOD (units/mg protein)</th>
<th>GSH level(units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.123±0.0</td>
<td>2.32±0.09</td>
<td>3.6±0.2</td>
<td>15.53±0.07</td>
<td>2.01±0.002</td>
<td>0.29±0.022</td>
</tr>
<tr>
<td>E.coli</td>
<td>0.415±0.0</td>
<td>1.03±0.006</td>
<td>1.8±0.26</td>
<td>34.30±0.115</td>
<td>2.56±0.01d</td>
<td>0.16±0.009e</td>
</tr>
<tr>
<td>mt-c+ E.coli</td>
<td>0.335±0.0</td>
<td>1.63±0.21d</td>
<td>2.84±0.1</td>
<td>24.04±0.07a</td>
<td>2.10±0.007d</td>
<td>0.44±0.05e</td>
</tr>
</tbody>
</table>

P value -a <0.001, b<0.002, c<0.01, d>0.05 as compared with control.
f<0.02, d>0.05, f<0.05, a<0.001, d>0.05 as compared E.coli & mt-c treated.

Fig. 1: Weight of thyroid gland

Fig. 2: Effect of E.coli and mt Con mice serum hormone levels in the group stained T3 and T4.

Fig. 3: Effects of E.coli and mt LPC in the thyroid gland. Data represents mean ± SD from 3 observation.

Fig. 4: Effect of E.coli and mt C, the specific activity of superoxide dismutase on the thyroid gland of rats. Data represents mean ± SD from 3 observation.
Thyroid weight of *E. coli* treated mice were observed (Fig. 1) as significantly (p<0.001) increased as compared to an average weight of control mice, mt-c along with *E. coli* treated mice also showed an increased (p<0.002) in thyroid weight. However, these increased weight of thyroid glands were less in mt-c treated group as compared to *E. coli* treated group (p<0.02).

A drastic decrease in both T3 and T4 values in mice serum were *E. coli* treated 14 days. T4 value was found to be decreased in *E. coli* treated mice (64-70%) of controls in 14 days (Fig. 2). These value also decrease in the mt-c along with *E. coli* treated mice and were not statistically significance as compared to *E. coli* alone.

*E. coli* treated significantly reduced of T3 concentration of mice serum in all groups compared to controls (p>0.001) 42-68% in 14 days (Fig. 2). However less suppress of T3 value in mt-c treated group as compared to *E. coli* group (*E. coli*-65% and mt-c - 42% in 14 days). The significant difference in T3 value was observed in mt-c treated mice for 14 days.

The level of MDA of the thyroid gland is increased in *E. coli* treated mice as compared to controls, were not statistically significant in the table (Fig. 3). However mt-c along with *E. coli* treated mice showed significantly (p<0.001) decrease MDA level in 14 days as compared to *E. coli* treated group.

The level of GSH of the thyroid gland is reduced in *E. coli* treated mice as compared to control (Fig. 5). However mt-c along with *E. coli* treated mice showed significantly (p>0.05) increase GSH level in 14 days as compared to *E. coli* treated group.

**CONCLUSION**

In view of the above results, it may be concluded that treatment of mt-c with hypothyroidism, increased the antioxidant defense of the cells and attenuate infectious effects of reactive oxygen metabolism.

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**Conflict of interest** No conflict of interest
REFERENCES