International Journal of Advanced Research and Review

www.ijarr.in

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 µl in (5×10^6) *E.coli*, and DMSO (2% v/v) were injected in mice by intraperitoneal, after three days 100 µl mt-c (0.12 mg/100 µl), 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 (H_2O_2) and superoxide as an important contribution to cell and tissue damage.

In the thyroid gland, thyrotropin increased the level of H_2O_2 (Corvilain *et al.*, 1991). This H_2O_2 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 H_2O_2 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, H_2O_2 (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 µl 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 µl mt-c (0.12 mg/100 µl), 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).

Estimatimation 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 µM NBT, 186 µM PMS, and 0.1 ml suitably diluted tissue extract. The reactions were started by addition of 780 µM NADH at 30° 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 DESCUSION

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)						
Groups	**Thyroid wt (mg)**	**Serum T3 (ng/dl)**	**Serum T4 (µg/dl)**	**MDA (nM/mg protein)**	**SOD (units/mg protein)**	**GSH level(units/mg protein)**
Control	0.123±0.011	2.32±0.09	3.6±0.2	15.53±0.07	2.01±0.002	0.29±0.022
E.coli	0.415±0.016a	1.03±0.006 b	1.8±0.26 c	34.30±0.115 a	2.56±0.01d	0.16±0.009e
mt-c+ E.coli	0.335±0.017d	1.63±0.21d	2.84±0.1 f	24.04±0.07a	2.10±0.007d	0.44±0.05e

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.

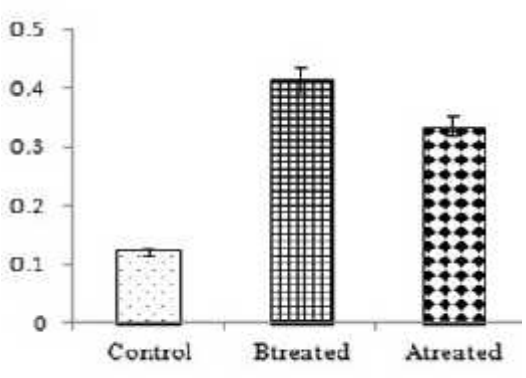

Figure: Weight of thyroid gland

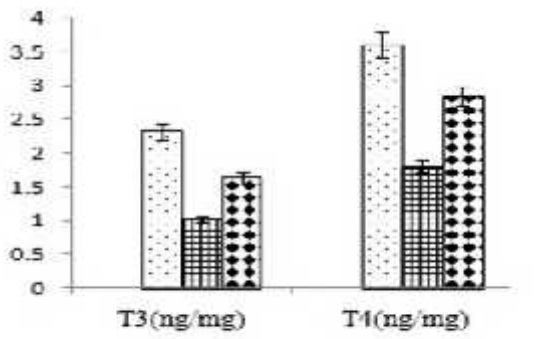

Fig. 2: Effect of *E.coli* and mt C on mice serum hormone levels in the group studied. T3 and T4.

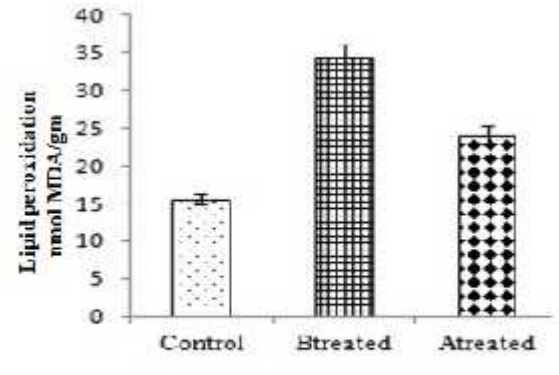

Fig. 3: Effects of *E.coli* and mt-C, LPO in the thyroid gland. Data represents mean + SD from 5 observation.

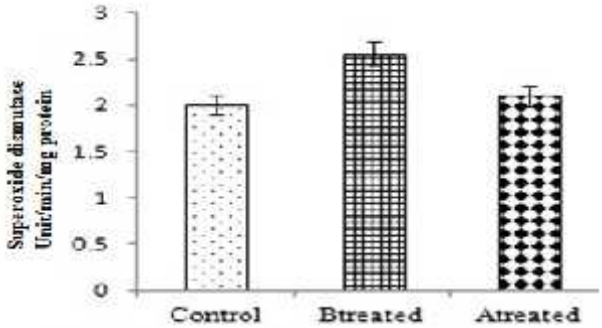

Fig. 4: Effect of *E.coli* and mt C, the specific activity of superoxide dismutase on the thyroid gland of mice. data represents mean ± SD from 5 observation.

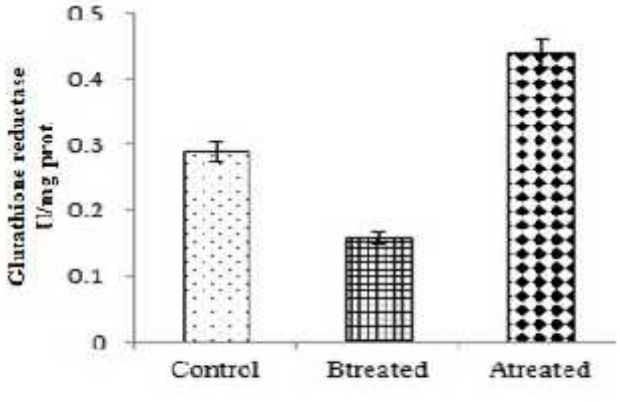

Fig. 5: Effect of *E.coli* and mt-C on specific activity of glutathione reductase on the thyroid gland of mice. data panel represents mean ± SD from 5 observation

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 first step of neutralization of O_2 is completed by synchronized activity of SOD and GPx in mice cell. As compared to control group of mice, the enzyme activity of SOD (Fig. 4) was observed to be increased significantly ($p<0.001$) when treated with *E.coli*, whereas its value decreased significantly ($p<0.01$) in the thyroid gland .

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.

ACKNOWLEDGMENT

AK thanks, Dr. Harisingh Gour Central University, Sagar for providing non-NET research fellowship. The authors are also grateful to Department of Zoology, Dr. Harisingh Gour Central University, Sagar, for providing infrastructural facilities and constant support.

Conflict of interest No conflict of interest

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