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Research Article

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Therapeutic role of mitochondria-targeted curcumin and gallic acid against *Escherichia coli* induced oxidative stress in thyroid gland of female *Swiss albino* mice

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Abstract

The thyroid problem is affecting the public health worldwide. Thus the present investigation is aimed to find out the toxic effects of *Escherichia coli* on thyroid gland and preventive role of mitochondria targeted curcumin and mitochondria targeted gallic acid in bacterial induced thyroid toxicity in female *Swiss albino* mice. The animals are separated in different groups. The result shows that mice exposed to bacteria have decreased T3 and T4 levels as compared to control and co-treated mice have elevated level of T3 and T4 hormones as compared to treated mice. In the biochemical study it is found that the increased lactate dehydrogenate, catalase activity, superoxide dismutase and lipid peroxidation and decreased glutathione reductase points towards the induced oxidative stress in thyroid gland. It is concluded that the exposure to *Escherichia coli* causes thyroid problem and treatment with mt-C and mt-G prevents the induced toxicity.

Keywords: Thyroid gland; mt-Gallic acid; mt-Curcumin; *Escherichia coli*

1. Introduction

Thyroid dysfunction, considered as the most common problem across worldwide. The effective form of thyroid hormone is T3, because T3 has higher affinity towards thyroid hormone receptor as compared to T4. Many factors such as pollution, stress, pesticide, radiations, infection and imbalanced gut microflora affect thyroid hormone synthesis. We focus on the imbalanced population of gut microflora. The balanced microbiota population helps in iodide uptake from intestine and helps in thyroid hormone synthesis while imbalanced population does not. *Escherichia coli* are gram-negative, facultative anaerobic, rod shaped bacteria and found in the lower intestine of warm-blooded organisms.

Gallic acid provides effective defense against oxidative damage by reactive oxygen species such as hydroxyl (HO**.**), superoxide (O_2-) and peroxyl (ROO**.**) radicals, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) (1). Due to kaleidoscopic properties, turmeric is used traditionally as medicine as a general tonic, stimulant, and cosmetic and also used in the treatment of coughs, colds, asthma, arthritis, sore throats, wound healing, and dyspepsia including peptic ulcers and as an antibiotic. Thus, it is needed to develop mitochondrial targeted antioxidants having higher permeability towards mitochondrial membrane (2). The present study has been undertaken to investigate the effect of mitochondrial targeted

antioxidants on attenuation of free radical induced thyroid mitochondrial damage. Further, *in vivo* experiments were carried out to observe the efficacy of targeted compounds.

2. Materials and Methods

2.1 Sampling of bacteria

The *Escherichia coli* (MTCC-68) used in this experiment were purchased from IMTECH Chandigarh, India. Bacteria were cultured in antifungal media up to 72 hrs on shaker.

2.2 Experimental animal

16-18 weeks old female mice about 28-32 gm weighted, housed in animal facility of ADINA institute of pharmaceutical sciences, Sagar M.P, India with 12h:12h light-dark cycle at room temperature were used for experiments. This work was approved by Ethics review committee of ADINA institute of pharmaceutical sciences, Sagar, India (Ethics approval no.-AIPS/2015/2459/IAEC-03).

2.3 Experimental design

The mice were randomly divided into four groups having 5 mice in each group.

Group i - Control with DMSO (0.02%).
Group ii - *E.coli* treated, *E. coli* (5×10^6/kg bw/day, ip) For 14 days.
Group iii - *E.coli* (5×10^6/kg bw/day, ip) and mt-C (0.12 mg/kg bw/day) For 14 days.
Group iv - *E. coli* (5×10^6/kg bw/day, ip) and mt-G (0.12 mg/kg bw/day) For 14 days.

2.4 Chemical Synthesis

Synthesis of mitochondrial targeted curcumin (mt-C) and gallic acid (mt-G) was performed by described method (3).

2.4.1 Synthesis of triphenyl phosphonium bromide

To 48 % aqueous HBr (350 ml) is mixed with Ph_3P (1.31 gm, 0.5mol). After stirring at 70° C for 20 min, the solution is cooled and extracted with $CHCl_3$ (3 X 150 ml). The compound organic phase is dried over Na_2SO_4 and the organic phase was removed and dried in rotator vacuum. The residue is washed with ethyl acetate (300 ml) to remove traces of Ph_3P, yield 1.67 gm (97 %) and its melting point is 185-195° C.

$$(C_6H_5)_3 + HBr \xrightarrow{CHCl_3 + Na_2SO_4} (C_6H_5)_3PHBr$$

2.4.2 Synthesis of mitochondrial targeted curcumin (mt-C) and gallic acid (mt-G)

Synthesis of mt- C and mt-G, curcumin and gallic acid bind with the lipophilic cation TPP (triphenyl phosphonium oxide) results the formation of mt-C and mt-G. Curcumin/Gallic acid of 0.678 gm; 2mMol mixed with 0.524 gm; 2mMol TPP in the presence of $Ph_3P.HBr$ and N_2, mixed in a round bottom flask which was sealed and heated at 70°C with magnetic string.

2.5 Body Weight

Body weight of mice noted in every two days starting from the day of treatment with the help of weighing balance and the individual and mean values were noted in gain weight/animal.

2.6 Preparation of samples for biochemical studies

For biochemical studies, five mice from each group were sacrificed, thyroid gland with trachea dissected out and wash in ice cold saline solution (9%) and stored at -80° C for the biochemical study. For the study of a thyroid hormone of blood sample collected by cardiac puncture into sterilized tubes and allow clotting at room temperature (25° C). After centrifuged at 1500 rpm for 15 min and collect serum for measurement of T3, T4 and TSH using ELISA kit provided by The Calbiotech Inc. (California, USA). The entire assay was performed in triplicate.

2.7 Assay of Lactate dehydrogenase (LDH)

The activity of LDH was measured by the Kornberg (1955) method. The reaction mixture (1ml) was composed of 20mM Tris-Cl, 3 mM NADH, diluted tissue extract and 1 mM sodium pyruvate. Decrease optical density (OD) was noted at 340 nm for 10 minutes. One unit of the enzyme, conversion of 1 µmole of pyruvate to lactate is equal to 1 µmole NADH to NAD/minutes at 30° C and units were presented as unit/mg protein.

2.8 Lipid peroxidation (LPO)

Lipid peroxidation was performed by the method of Placer *et al.*, (1966). In which 1 mol of malondialdehyde (MDA) reacts with 2mol of TBA in an acid medium to form tri-methionine, appeared as pink colour and its maximum absorbance at 548 nm, and incubated in water bath at 37º C for 30 minutes. After incubation 1.5 ml of TBA reagent (2 volume: 1 volume PCA) was added, after cooling in mixture added 3.0 ml pyridine: n- butanol (3:1, v/v) and 1 ml 1 N NaOH was added.

2.9 Superoxide dismutase (SOD)

SOD activity was determined according to the Beauchamp and Fridovich (1971). In reaction mixture oxidised riboflavin in the presence of EDTA to reduce flavin. It oxidised and simultaneously reducing oxygen to O_2^-, the formation of formazan blue to reduces NBT. Hence the amount of SOD is directly proportional to the reduction of formazan amount.

2.10 Catalase activity

The Catalase activity (EC:1.11.1.6) was measured as with some modification. Briefly, the reaction mixture1mlconsisted of 0.067M phosphate buffer (pH 7.0) and 0.003% H_2O_2. By the addition of diluted tissue extract, the reaction was started and a decrease in absorbance at 240 nm was recorded for 10 min. The activity of catalase was expressed as µmol of H_2O_2 consumed/min/mg protein.

2.11 Gram staining of bacteria

A thin smear of the bacterial culture was prepared on a slide and heat fixed. The smear was stained (Gram's, 1984) with crystal violet for 60 sec and followed by washing with slowly running tap water. Violet or purple color indicated for gram positive bacteria whereas pink color indicated gram negative bacteria.

2.12 Statistical Analysis

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

3. Results

3.1 Synthesis of targeted antioxidant mt-C and mt-G

The synthesis of mitochondria-targeted curcumin (mt-C); triphenylphosphonium cation covalently binds to alkyl chain of curcumin. So, these compounds are still hydrophobic in nature.

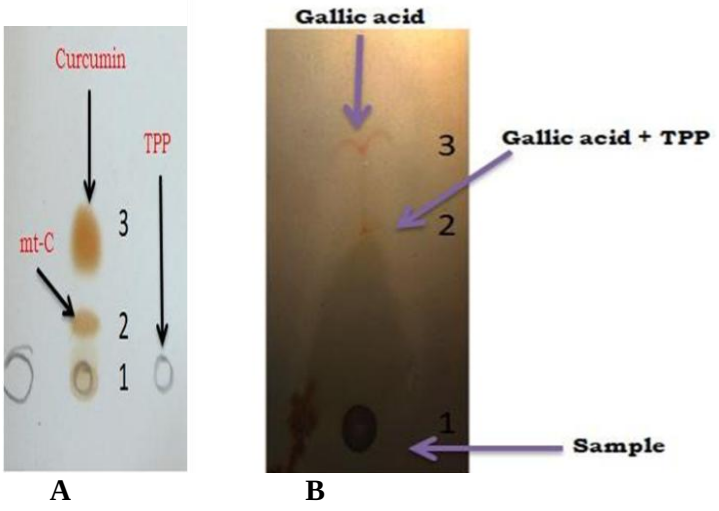

Figure 1: TLC result of mt-C (A) and mt-G (B).

The TLC plate (A) has three spots, the 1st spot indicates the sample, spot 2nd indicate curcumin that binds with the TPP i.e., mt-C and spot 3rd indicate curcumin. The molecular weight of spot 2nd is higher than spot 3rd because curcumin binds with TPP and its molecular weight increases, so its spot appeared in the middle region. Gallic acid is binds covalently with TPP which is hydrophobic in nature. The TLC (B) plate has three spots, the 1st spot indicate the sample and spot 2nd indicate gallic acid that binds with the TPP i.e., mt-G and spot 3rd indicate gallic acid. The molecular weight of spot 2nd is higher than spot 3rd

because gallic acid binds with TPP and its molecular weight increases, so its spot appeared in middle region (Figure 1).

3.2 Net body weight gain

After 14 days, mice treated with Bacteria (*E. coli*) the body weight was increased (A and B; p<0.01). Co-treatment of mice with bacteria and mt-C and mt-G, the adverse effect of bacterial toxicity reduced and decreased net body weight (Figure 2).

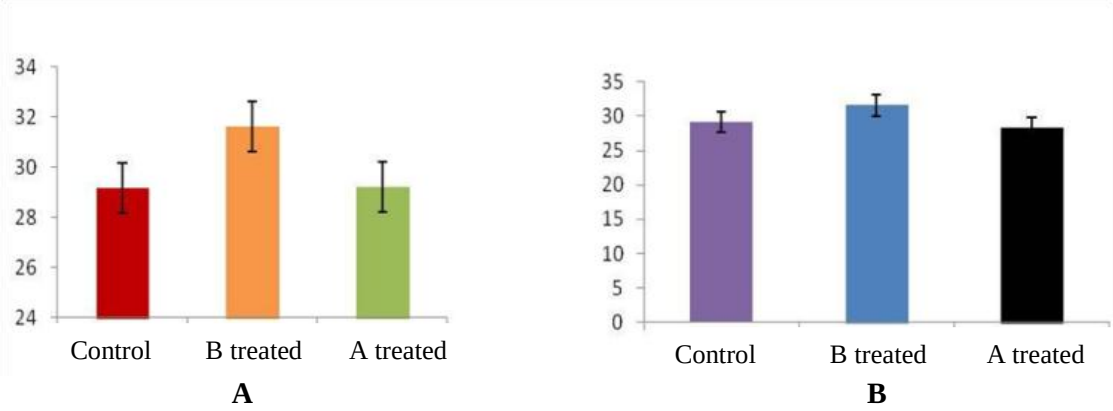

Figure 2: Effect of *E.coli*, and both antioxidant mt-C & mt- G treatment on mice, weight increased and decreased respectively. (A) p<0.01, p<0.001. In A. mt-C treated and B. mt-G treated, p<0.01, p<0.001(*E. coli* treated group versus mt- C and mt-G treated group).

Thus, the body weight is increased in *E. coli* treated group and the body weight is decreased more in mt-G treated group, so ameliorative effect of mt-G (p<0.001) is more effective than mt-C.

3.3 Serum Hormone Change

A significant reduction levels of T3 and T4 were observed in serum mice treated with *E. coli* (p<0.001) while the treatment with mt-C and mt-G prevent decrease level of T3 and T4 in serum significantly (p<0.001), However the level of thyroid stimulating hormone (TSH) increased significantly in treated group (p<0.001) while treatment with mt-C and mt-G prevent increase the level of TSH in serum (p<0.01) significantly (A-B) (Figure 3).

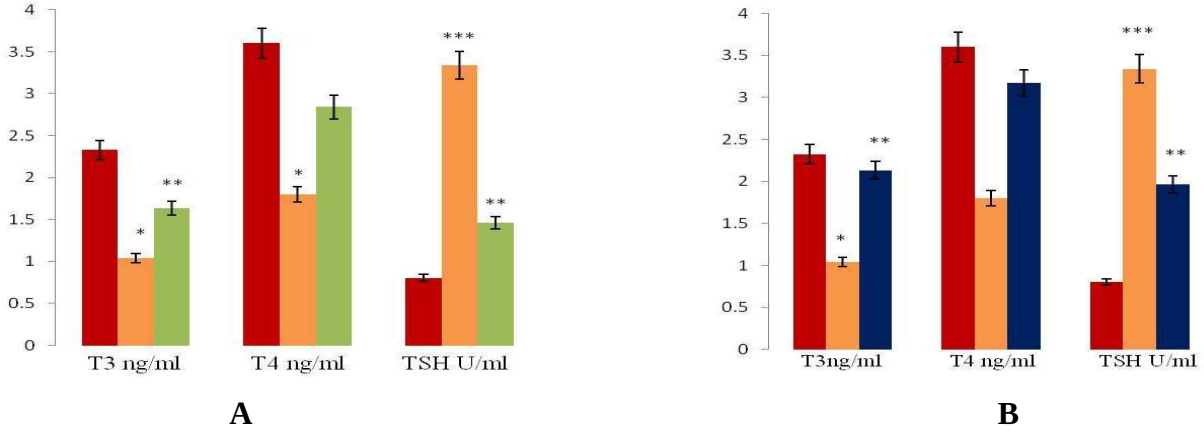

Figure 3: Effect of *E. coli* and mt-C & mt-G on mice serum, hormone levels in the group studied. T3, T4, TSH (Thyroid stimulating hormone). (A) ***p<0.001 (control vs treated group) and p<0.01 (control vs mt-C treated group). (B) ***p<0.001 (control vs treated group) and p<0.01 (control vs mt-G treated group). B treated- *E. coli* treated, A treated- mt-C treated and A treated- mt-G treated.

3.4 *LDH activity of thyroid gland induced by E. coli.*

For spectrophotometric based analysis of LDH isozymes, the LDH activity obtained from extracts tissue of normal mice. A & B treated with *E. coli* caused increase the activity of LDH isozymes. However, the LDH activity declines towards normal ($p<0.05$) tissues of bacteria affected mice co-treated with mt-C and mt-G. Treatment of normal mice with bacteria for 14 days caused increase LDH activity in thyroid gland as compared to control indicate tissue injury. As illustrated A & B, the level of LDH declined in bacteria mice treated along with mt-C and mt-G for 14 days ($p<0.05$) (Figure 4).

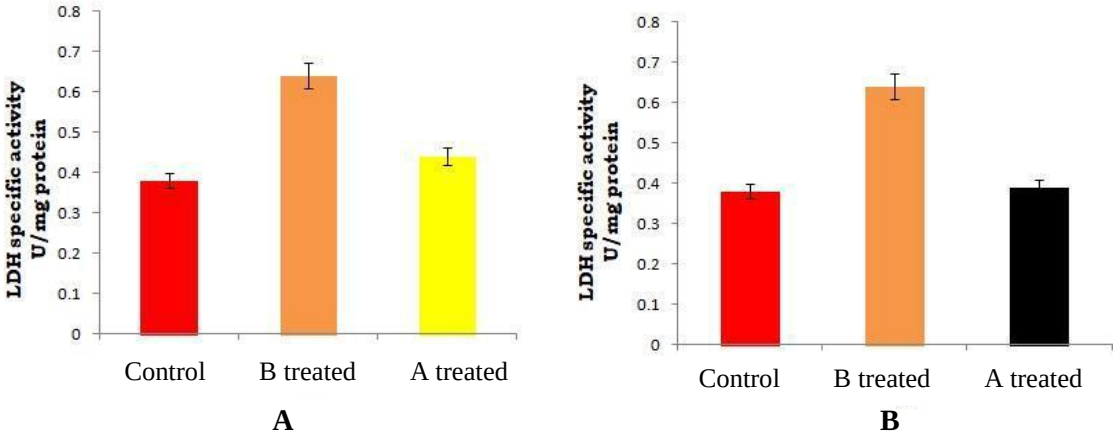

Figure 4: Effect of *E. coli* and mt-C & mt-G on the specific activity of lactate dehydrogenase in the thyroid gland. Data in panel represents mean± SD. B treated- *E.coli* treated, A treated- mt-C treated, B treated- *E. coli* treated, A treated- mt-G treated.

3.5 Lipid peroxidation assay

The level of lipid peroxidation measured in terms of MDA is one of the widely accepted assays for determining the level of oxidative damage and oxidative stress at the cellular level. Mice treated with bacteria caused a significant increase the level of MDA ($p<0.001$) in thyroid gland compared to control group. Co-treatment with mt-C and mt-G bacteria treated mice significantly decreased MDA level ($p<0.001$) in the thyroid gland (A & B) (Figure 5).

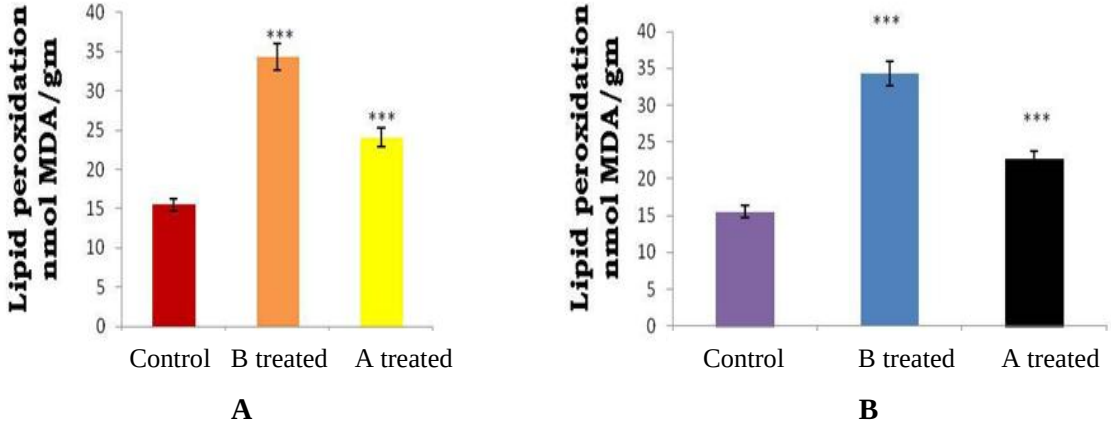

Figure 5: Effects of *E. coli* and mt-C, mt-G, LPO in the thyroid gland. Data in panel represents mean ± SD. (*$p<0.05$, **$p<0.001$, ***$p<0.001$) B treated- *E. coli* treated, A treated- mt-C treated, B treated- *E. coli* treated, A treated- mt-G treated.

3.6 Catalase

Catalase determine by level of H_2O_2 activity. The level of H_2O_2 is one of the widely accepted assays for determining the level of oxidative damage and oxidative stress at the cellular level. Treated mice with bacteria cause a significant increase H_2O_2 in thyroid gland compared to control group. Co-treatment of mt-C and mt-G with bacteria treated mice prevented the increase H_2O_2 in the thyroid gland (Figure 6).

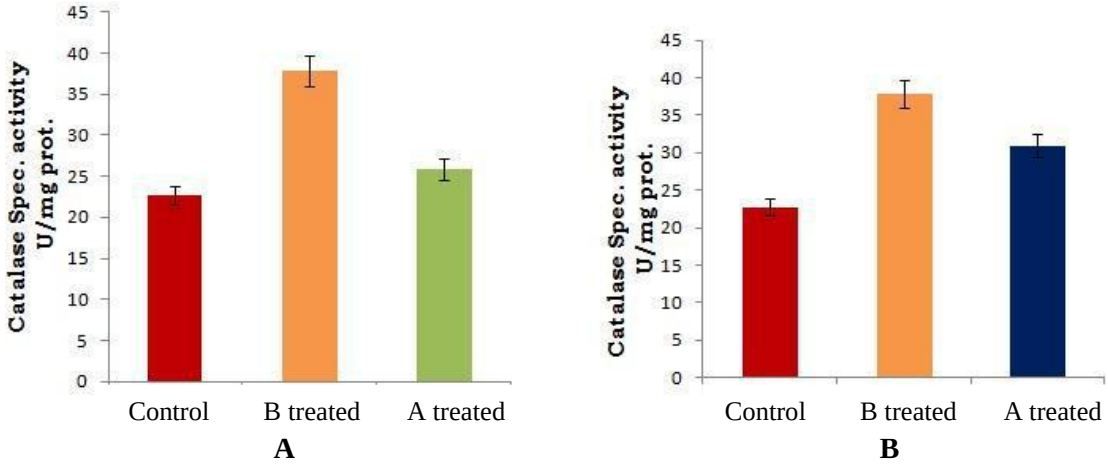

Figure 6: Effect of *E. coli* and mt-C & mt-G on the specific activity of catalase in the thyroid gland. Data in panel represents mean± SD. A. B treated- *E. coli* treated, A treated- mt-C treated, B. B treated- *E. coli* treated, A treated- mt-G treated.

3.7 SOD activity in thyroid gland of mice

Superoxide dismutase enzymes catalyze the superoxide (O_2-) into oxygen and hydrogen peroxide. Oxidative stress and mitochondrial dysfunction are known to produce superoxide anions. SOD is the first enzymes, neutralize superoxide anion free radical (O_2) generate during the oxidative stress of the cells. According to Fig.9: A & B, the level of active SOD significant increase in the thyroid gland ($p<0.01$). However, co-treatment of bacteria treated mice with mt-C and mt-G prevent this increased level of total SOD in the thyroid gland (A & B) (Figure 7).

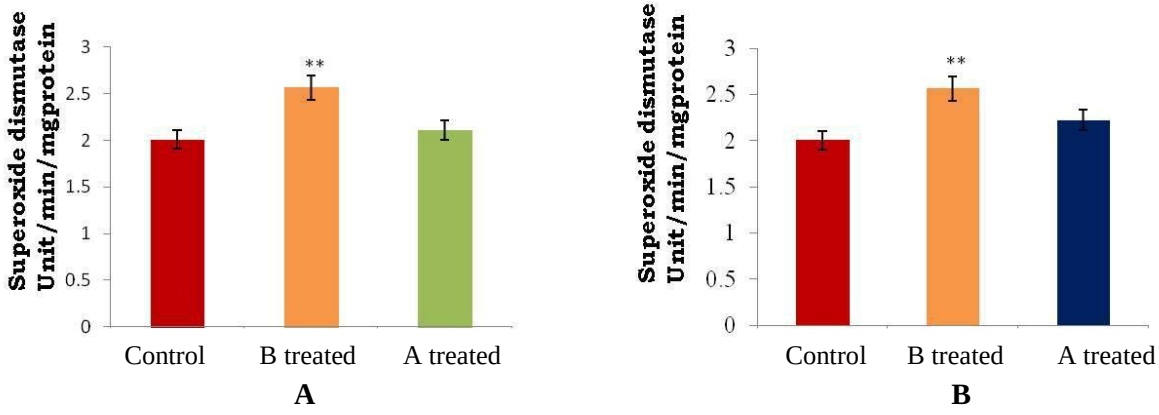

Figure 7: Effect of *E. coli* and mt-C & mt-G on the specific activity of superoxide dismutase on the thyroid gland of mice. Data panel represents mean± SD. A. B treated- *E. coli* treated, A treated- mt-C treated, B. B treated- *E. coli* treated, A treated- mt-G treated

4. Discussion

Mitochondria targeted curcumin and gallic acid is important for inhibition of toxicity against the generation of free radicals in the mitochondrial matrix (2, 3). In the present study is that antioxidant compounds delivered directly to mitochondrial matrix they attenuate free radicals with great efficacy.

The results, mice treated with *E. coli* showed significant decreased T3, T4 levels and increased level of TSH. Consequently, the low hormonal level affects the metabolic needs of the body (4). The present results revealed an increased T3, T4 and decreased TSH level in treated with *E. coli*, curcumin and gallic acid. Decline T3 and T4 levels due to *E.coli* treatment is indicator of oxidative stress on thyroid follicle,

because thyroid follicle has certain poly unsaturated fatty acid and rich supply of oxygen radicals to susceptible of lipid peroxidation. *E. coli* secretes enterotoxin substance intimin. Intimin binds with GPCR that stimulates cAMP in the thyroid gland and increased the level of TSH to follow the hypothalmo-hypophysial-thyroid axis (5, 6).

In diseased condition, cells required more energy supply for their growth, anaerobic metabolism of glucose is switched and pyruvate transforms to lactic acid (7). LDH-1 is the specific LDH isoenzymes expressed more in hypothyroid condition and its activity show, T3, T4, and TSH levels in serum (8, 9). This was probably due to an increase LDH activity as the result of *de novo* synthesis or due to anaerobic metabolism needed to encounter the tissue metabolism (10). The results are consistent with an earlier report where a sublethal dose of *E. coli* has shown significant increase LDH level damaged the tissue of thyroid gland. The result of the present investigation has shown a linear view between LDH leakage and MDA concentration in tissue extracts exposed to *E. coli*, indicating that lipid peroxidation might be one of the markers mediating the tissue toxicity by the bacteria increased the level of lipid peroxidation (9). In the present investigation, a linear correlation between the level of MDA concentration and LDH leakage in all tissue of different groups of mice was observed results showed that lipid peroxidation may also have an important role in modifying LDH activity. Increased lactate production indicates a potential marker for investigating toxin induced cell stress. We found that *E.coil* increased lactate production in thyroid tissue extract. Scientist reported that mt-C and mt-G inhibit the LDH level in the thyroid of mice after exposure (11, 12). Probably by the normal level of mitochondrial oxidative phosphorylation was evidence of the results by the decreased expression and activity of LDH in tissues extract of bacterial treated mice.

Thyroid hormones regulates by the oxidant and antioxidant balance to protect cells (13). It is proposed that enhanced ROS level, induced by thyroid hormone deficiency results oxidative stress, in the liver, skeleton muscles and heart with lipid peroxidation (14). The basic biochemistry of antioxidant enzymes, superoxide dismutase is catalysis of superoxide anion (O_2-) to hydrogen peroxide (H_2O_2) and prevents the formation of toxic radicals. However, H_2O_2 is a powerful oxidant that eliminates free radicals from the cell and protects from oxidative damage of lipid, protein and DNA. Catalase (CAT) detoxifies the H_2O_2 and glutathione peroxidase (GPx) and the bacterial toxins are their capability to induced hypothyroidism in mice (4). Hypothyroidism in mice induced oxidative stress results increased free radical formation and reduced antioxidant defense mechanism (9, 15). In the present study, Lipid peroxidation was evaluated as oxidative stress marker of lipid peroxidation in thyroid gland of bacterial treated mice and the data revealed a significant elevation in MDA level in the tissue extract of bacterial treated mice. Bacterial toxin induced ROS production that caused lipid peroxidation (14). The results shows that mt-C and mt-G treatment inhibit the MDA production and thus protect the mice from *E. coli* induced toxicity. Scientist reported that mt-C and mt-G treatment restores the MDA levels (16, 17). The viruses, bacteria cause free-radical damage to healthy cells and deplete glutathione. Glutathione depletion has been correlated with lower immune function and increased vulnerability to infection due to the liver reduced ability to detoxify. (18). The results of present investigation support the idea that a natural outcome of inhibition of SOD is a decline in the capturing of superoxide.

5. Conclusion

The result of present study shows that bacteria induce thyroid gland toxicity in mice and co-administration of mt-G and mt-C restores the toxicity. Among mt-C and mt-G, mt-G is more powerful antioxidant. This investigation also revealed some new ideas and creativeness which is very useful for the mankind. As this study contains all those parameters and ideas that can be made useful in the society and gives the data about the beneficial and importance of the mt-curcumin and mt-gallic acid.

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