Arch Clin Exp Med 2018;3(3):190-194. e-ISSN: 2564-6567

DOI:10.25000/acem.455516 Araştırma makalesi / Research article

Effects of α-lipoic acid on oxidative stress parameters in experimental hyperthyroidism

Deneysel hipertiroidizmde α-lipoik asidin oksidatif stres parametreleri üzerine etkileri

Adile Merve Baki [1], Abdurrahman Fatih Aydın [1], Pervin Vural [1], Merve Soluk Tekkeşin [2], Semra Doğru Abbasoğlu [1], Müjdat Uysal [1]

Abstract

Aim: To investigate the effects of α-lipoic acid (ALA) on prooxidant-antioxidant balance in liver tissue, as well as liver function tests in experimental hyperthyroidism.

Materials and Methods: For the evaluation of prooxidant-antioxidant balance, reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP), glutathione (GSH) levels, and superoxide dismutase, catalase and glutathione peroxidase activities were determined. Histopathological examinations were also performed. Hyperthyroidism was induced by the administration of L-thyroxine [T4, 12 mg/L] in drinking water for 10 weeks. The ALA [100 mg/kg/day; 0.2% (w/w) in diet] was administered in last 5 weeks of experimental period.

Results: Oxidative stress in liver tissue from hyperthyroid rats was accentuated. Significant increases in hepatic ROS, MDA, and PC levels were found. Additionally, increased FRAP and decreased GSH levels were observed. ALA treatment lowered the elevated serum free T3 and T4 levels and significantly decreased hepatic ROS, MDA and PC levels. Serum liver function tests in hiperthyroid rats before and after ALA treatment were not changed.

Conclusion: Our results indicate that ALA treatment was effective in the improvement of changes in prooxidant-antioxidant balance, and may be useful as supportive agent for the treatment of hypertyroidism.

Key words: Proxidant-antioxidant balance, hyperthyroidism, α-lipoic acid.

Öz

Amaç: Deneysel hipertiroidizmde α-lipoik asid (ALA)' in karaciğer dokusunda prooksidan-antioksidan denge üzerine etkileri ile karaciğer fonksiyon testlerinin incelenmesi.

Yöntem: Prooksidan-antioksidan dengenin değerlendirilmesi için, reaktif oksijen ürünleri (ROS), malondialdehit (MDA), protein karbonil (PC), total antioksidan kapasite (FRAP) ve glutatyon (GSH) düzeyleri ile süperoksit dismutaz, katalaz ve glutatyon peroksidaz aktiviteleri incelendi. Ayrıca, histopatolojik incelemeler yapıldı. Hipertiroidi tablosu oluşturmak için T4 (12 mg/L) 10 hafta boyunca içme suyunda uygulandı. ALA [100 mg/kg/gün; % 0.2 [w/w]; diyette] deney süresinin son 5 haftasında uygulandı.

Bulgular: Karaciğerde oksidatif stresin arttığı görüldü. Hipertiroidili sıçanlarda ROS, MDA, PK düzeylerinde anlamlı artış bulundu. Ayrıca FRAP düzeylerinde artış ve GSH düzeylerinde azalma görüldü. ALA tedavisi, artan serum serbest T3 ve T4 düzeylerini düşürdü ve karaciğerde ROS, MDA ve PK düzeylerini anlamlı olarak azalmasına neden oldu. ALA uygulaması öncesi ve sonrasında serum karaciğer fonksiyon testlerinde bir değişiklik görülmedi.

Sonuç: Sonuçlarımız, ALA tedavisinin prooksidan-antioksidan dengedeki değişikliklerin düzelmesinde etkili olduğunu ve hipertiroidi tedavisinde destekleyici ajan olarak yararlı olabileceğini göstermektedir.

Anahtar Kelimeler: prooksidan-antioksidan denge, hipertiroidizm, α-lipoik asid.

[1] Istanbul University, Istanbul Faculty of Medicine, Department of Biochemistry, Çapa, Istanbul, Turkey.

[2] Istanbul University, Institute of Oncology, Department of Pathology, Çapa, Istanbul, Turkey.

Ethics Committee Approval: The study wass approved by the local ethical authority.

Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır.

Conflict of Interest: No conflict of interest was declared by the author.

Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

Financial Disclosure: This study was supported by Scientific Research Projects Coordination Unit of Istanbul University. (Project number: 52916).

Finansal Destek: Bu çalışma İstanbul Üniversitesl Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından 52916 numaralı proje ile desteklenmiştir.

Geliş Tarihi / Received: 10.09.2018

Kabul Tarihi / Accepted: 26.11.2018

Yayın Tarihi / Published: 30.11.2018

Sorumlu yazar / Corresponding author:
Pervin Vural

Adres/Address: Istanbul Faculty of Medicine, Department of Biochemistry, Çapa, 34093, Istanbul, Turkey.

e-posta: pervinvural@yahoo.com

Tel/Phone: +90 212 4142188

Introduction

It is well known that a delicate balance exists between the rates of reactive oxygen species ([ROS) formation and their neutralisation. Protection of body against disturbances in prooxidant-antioxidant balance, is a multi-factorial process involving many cellular metabolic pathways. Increased ROS formation affects macromolecules such as lipids, proteins, DNA, and may alter their structure and function, resulting in cell damage [1]. Thyroid hormones (thyroxine and triiodothyronine, T3, T4) are necessary for the various physiological processes such as growth, differentiation, development and reproduction [1, 2].They are also necessary for the regulation of lipid and carbohydrate metabolism, and oxygen utility. In addition, thyroid hormones are impilicated in ROS formation in the cell by altering basal metabolism and respiratory chain reactions [2-4]. On the other hand, decreasing the availability of non-enzymatic antioxidants or increasing the expression of certain antioxidant enzymes thyroid hormones affect antioxidant mechanisms, thus alter prooxidant-antioxidant balance [5, 6]. Indeed, many clinical and experimental studies [7, 8] showed that oxidative stress develops in hyperthyroidism, and that the susceptibility to oxidative stress was different among tissues. Because of its rich mitochondrial content, and due to the important role in body metabolism and drug detoxification, the liver is among the most frequently studied tissues for evaluation of prooxidant-antioxidant balance.

Recently, it has been suggested that antioxidant therapy may be helpful for preventing the oxidative stress seen in hyperthyroidism and may provide support to the classical antithyroid treatment. For this purpose, various antioxidants such as vitamin E, curcumin, and quercetin have been used in hyperthyroidism and some favorable results have been obtained [7, 9-11]. α-Lipoic acid (ALA) is a mitochondrial coenzyme with significant antioxidant properties. ALA supports regeneration of many antioxidants such as glutathione (GSH), coenzyme Q10, vitamin E and C, repairs oxidized proteins, creates complexes with metal ions such as copper, manganese, and zinc, and prevents formation of ROS [12]. Because of these properties, it has been reported that ALA possess beneficial effects in various conditions related with accentuated oxidative stress [12-15]. To the best of our knowledge, the effects of of ALA in hyperthyroidism was not been investigated yet. Therefore, the aim of our study was to examine changes in prooxidant-antioxidant balance in liver tissue obtained from hyperthyroid rats, and to evaluate the effects of ALA on oxidative stress parameters, as well as liver function tests in experimental hyperthyroidism.

Material and methods

The investigation was performed on male Sprague-Dawley albino rats (weighing 250-350 g) purchased from Aziz Sancar Institute for Experimental Medical Research, Istanbul University, Turkey. Animals were housed in a light- and temperature-controlled room on a 12/12 hours light/dark cycle. All experiments met the guidelines the Animal Care and Use Committee of Istanbul University [Project No. 2014/111]. All chemicals were supplied from Sigma-Aldrich [St. Louis, MO, USA].

Rats were randomly divided into four groups (6 rats per group) as control, ALA, T4 and T4+ALA. Rats of the control group were fed with standard diet ad libitum for 10 weeks. Rats in ALA group were fed initially with standard diet for 5 weeks. For 5 weeks following period, rats were fed with ALA [100 mg/kg/day; 0.2% (w/w)] supplemented diet. Tap water was given as drinking water in control and ALA groups. Hyperthyroidism (T4 and T4+ALA groups) was induced by administration of L-Thyroxine sodium pentahydrate (T4) in drinking water to a final concentration of 12 mg/L for 5 weeks. Three weeks after the administration of T4, free T3 (fT3) and free T4 (fT4) levels were determined in rats randomly chosen from the study groups. After completion of 5 weeks period, administration of T4 was continued for another 5 weeks (T4 and T4+ALA groups). Rats of T4+ALA group were fed with 0.2% (w/w) ALA containing diet for last 5 weeks.

At the end of study period (10 weeks), after 10-12 hours fasting period blood samples were obtained by cardiac puncture under pentobarbital anesthesia (50 mg/kg, i.p.) in plain tubes. Blood samples were centrifuged at 1500 × g (10 min, 4□C) to remove the serum. Liver tissue were removed immediately after blood collection, rinsed with ice-cold saline, blotted with filter paper. Liver tissue homogenizate (10%; w/v) was prepared in ice-cold 0.15 M KCl. Homogenates were centrifuged at 600 × g min to obtain supernatants where reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP), glutathione (GSH) and catalase (CAT) activity were measured. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined in postmitochondrial fraction obtained after cantifugation at 10,000 × g for 20 min of supernatants.

Determinations in serum

Serum fT3 and fT4 measurements were performed on a Elecsys automated analyzer (Roche Diagnostics, Mannheim, Germany). Serum alanine aminotransferase [ALT] and aspartate aminotransferase [AST] activities were determined by Cobas Integra 800 automated analyzer (Roche Diagnostics, Mannheim, Germany).

Determinations of ROS formation, lipid peroxidation and protein oxidation

ROS formation was measured fluorometrically [16]. After incubation with 2,7-dichlorodihydrofluorescein diacetate [DCFH-DA], the fluorescence of 2,7-dichlorofluorescein was read on a microplate fluorometer (Fluoroskan Ascent FL, Thermo Scientific Inc, USA)(excitation of 485 nm and emission of 538 nm). Lipid peroxidation was determined by measuring the levels of MDA by thiobarbituric acid test [17]. The absorbance was read at 535 nm against the blank. Molar extinction coefficient of 1.56×105 M-1.cm-1 was used for calculation of MDA levels. As a marker of protein oxidation PC levels were determined by the measuring of carbonyl groups reacting with 2,4-dinitrophenylhydrazine (DNPH)[18]. Molar absorbtion coefficient of 22,000 M−1 cm−1 at maximum absorbance (360 nm) was used for calculation of PC levels. For the spectrophotometrical measurements Ultraspec 3000 spectrophotometer (Pharmacia Biotech, Biochrom Ltd. Cambridge, UK) was used.

Determinations of non-enzymatic and enzymatic antioxidants

Ferric reducing antioxidant power (FRAP), reflecting total antioxidant status, was measured according to Benzie and Strain method [19]. Glutathione (GSH) levels were measured with 5,5-dithiobis-2-nitrobenzoate at 412 nm [20]. For catalase (CAT) activity measutement as a substrate hydrogen peroxide was used [21]. SOD activity measutement was based the effect of riboflavin-sensitized photooxidation of o-dianisidine [22]. For GSH-Px activity [23] measurement cumene hydroperoxide was used as substrate. Protein levels were determined using bicinchoninic acid [24].

Histopathologic evaluation

Liver tissues were fixed in 10% buffered formaldehyde, processed, and stained with hematoxylin and eosin (H&E) for histopathologic examination.

Statistical analysis

All statistical analyses were perfomed with IBM SPSS statistics for Windows (version 21; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± SD. Experimental groups were compared using ANOVA (post-hoc Tukey HSD) and Kruskal–Wallis (post hoc Mann-Whitney U) tests. A p value < 0.05 was considered to be statistically significant.

Results

Body (BW) and liver weights, and liver index

BW significantly decreased in experimental hyperthyroidism (T4 group). While liver weight did not changed, the liver index was found to be increased in this group. ALA treatment did not change body and liver weights in T4 treated rats. Only liver index decreased significantly after ALA treatment (Table 1).

Table 1. Efects of ALA on body and liver weights, as well as liver index study groups (mean± SD; n=6 each)

	Control	ALA	T4	T4+ALA
Body weight [g]	310.8±38	319.5±44.3	261.0±17.0[a]	307.3±17.3
Liver weight [g]	7.94±1.21	9.51±1.90	9.58±0.47	8.98±0.66
Liver index* [%]	2.5 ±0.16	2.95±0.22	3.68±0.29[a]	2.92±0.13[b]

α-lipoic acid (ALA), [a] p< 0.05 as compared with control; [b] p< 0.05 compared with T4 group, groups (6 rats of each): Control, ALA, T4 and T4+ALA, *Liver index = Liver weight × 100 / body weight

Serum thyroid function tests

T4 administration significantly increased serum fT3 and fT4 levels. ALA treatment resulted in a significant decrease in fT3 and fT4 levels in hyperthyroid rats (Table 2).

Table 2. Effects of ALA on serum fT3, fT4 levels, ALT and AST activities in study groups (mean± SD; n=6 each)

	Control	ALA	T4	T4+ALA
fT3 [pmol/L]	3.17±0.40	3.56±0.56	11.6±3.51[a]	5.81±1.78[a,b]
fT4 [pmol/L]	26.3±5.90	28.3±5.76	114.8±47.4[a]	50.7±21.5[a,b]
ALT [U/L]	44.6±6.50	55.8±16.7	58.5±10.1	63.0±18.6
AST [U/L]	117.0±16.8	111.8±16.9	119.1±25.8	132.8±28.3

α-lipoic acid (ALA), free T3 (fT3), free T4(fT4), alanine aminotransferase (ALT), aspartate aminotransferase(AST), thyroxine (T4), [a] p<0.05 as compared with control; [b] p<0.05 as compared with T4 group

Serum liver function tests

Serum ALT and AST activities did not change in hyperthyroid rats. No change were observed in ALT and AST activities after ALA treatment in comparisson with hyperthyroid rats (Table 2).

Hepatic prooxidant and antioxidant parameters

There were significant increases in hepatic ROS, MDA, PC levels in hyperthyroid rats (Figure 1). FRAP values significantly increased and GSH levels decreased. However, hepatic SOD, CAT and GSH-Px activities did not alter in hyperthyroid rats (Figure 2). ALA administration significantly decreased ROS, MDA and PC levels [Figure 1]. However, there were no significant differences in hepatic FRAP and GSH levels, SOD, CAT and GSH-Px activities after ALA administration to hyperthyroid rats (Figure 2).

Histopathological evaluation

Normal liver histological structure was seen in control and ALA groups (Figure 3). Necrotic and necrobiotic appearance in liver paranchymal cells, irregularity in lobular structure, vacuolar degeneration in some cells and sinusoidal congestion were observed in T4 group. In T4+ALA group, the liver showed similar appearance like the T4 group, but there was a decrease in parenchymal necrosis and a narrowing in the sinusoids, whereas vacuolar degeneration was not seen. In addition, several apoptotic cells were detected (Figure 3).

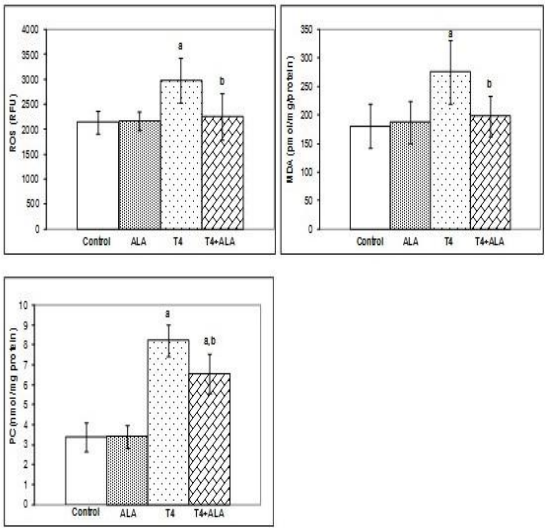

Figure 1. Effects of α-lipoic acid (ALA) on reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl (PC) levels in liver tissue in thyroxine (T4) administered rats (mean±SD; n=6 each).

[a]p<0.05 as compared with control; [b]p<0.05 as compared with T4 group.

Discussion

It is well known that there is a delicate balance between prooxidant and antioxidant systems in the body. The increase of free radicals and/or weakness of antioxidant system results in oxidative stress with subsequent organ damage. Through accelerating the basal metabolism and respiratory chain reactions, thyroid hormones increase generation of ROS in blood and various tissues with subsequent oxidative stress [2-4]. Hovewer, controversial results were obtained from many experimental and clinical studies [2-4, 8, 11, 25, 26]. A lot of factors such as severity of the hyperthyroidism, different susceptibility of various tissues to oxidative stress, and different methods used for the evaluation the prooxidant-antioxidant balance are involved this contradiction [2-4, 8, 11, 25, 26].

Many investigators have investigated the prooxidant-antioxidant balance in body fluids and various tissues of experimental hyperthyroid animals. Because many exogenous compounds are metabolised in liver, due to of its rich mitochondrial content and important role in body metabolism, the liver is among the most frequently studied tissues for the evaluation of prooxidant-antioxidant balance. Several investigators have reported increased levels of hepatic ROS [3, 27], MDA [9, 11, 25], diene conjugate [28], PC [3, 9, 27] and 8-hydroxy-deoxyguanosine [26] levels in rats with hyperthyroidism, however enzymatic and non-enzymatic antioxidants were found to be decreased [6, 25]. In our study, it was found that ROS, MDA and PC levels were also increased,

GSH levels were decreased, and there were no changes in antioxidant enzyme activities in liver. These findings are consistent with the findings obtained in other studies on the same subject [3, 9, 11, 25, 27, 28]. On the other hand, our study

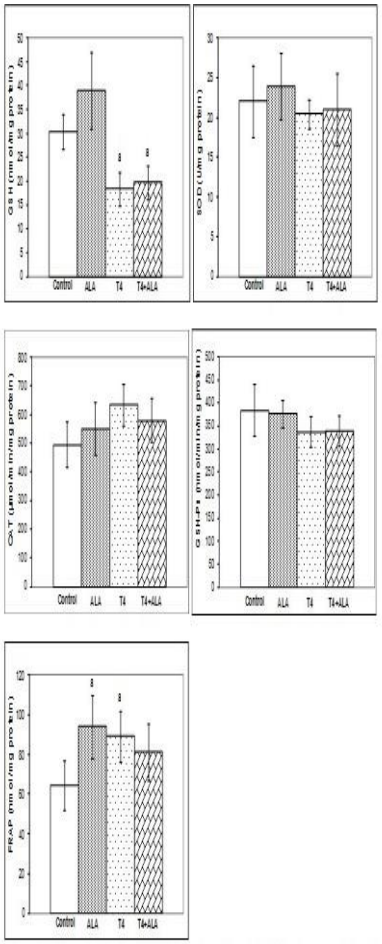

Figure 2. Effects of α-lipoic acid (AL) on glutathione [GSH] levels, superoxidase dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities and ferric reducing antioxidant power (FRAP) values in liver tissue in thyroxine (T) administered rats (mean±SD; n=6 each).
[a]p<0.05 as compared with control; [b]p<0.05 as compared with T4 group.

showed an increase in FRAP levels in the liver. As FRAP is an indicator of total antioxidant power in body fluids and tissues, increased value may be a compensatory mechanism against the oxidative stress developing in the liver in hyperthyroid state.

In recent years, it has been suggested that antioxidant therapy may be helpful in preventing oxidative stress in hyperthyroidism. For this purpose, various antioxidants such as vitamin E, curcumin, and quercetin have been used in hyperthyroidism and some favorable results have been reported [7, 9-11]. ALA is a mitochondrial coenzyme with powerful antioxidant properties. ALA has a beneficial effect in various conditions related with induced oxidative stress. Indeed, many investigators have reported that ALA is effective in ameliorating of various pathologies related with impaired prooxidant-antioxidant balance such as atherosclerosis, metabolic syndrome, diabetes mellitus, and diabetic neuropathy [12-15]. As far as we know, the effectiveness of ALA has not been investigated yet in patients with hyperthyroidism and experimental hyperthyroidism as well. Five weeks durated ALA treatment decreased significantly fT3 and fT4 levels. The suppressing effect of ALA on thyroid hormones levels in the literature was detected for the fist time in our study. The reason of fT4 and fT3 decreasing effect of ALA may be due to the inhibition of thyroid peroxidase and 5'-deiodinase enzymes. As a radical scavenger, by reducing H_2O_2 [which is necessary for function of thyroid peroxidase] ALA may also reduce the activity of thyroid peroxidase in hyperthyroid conditions. ALA administration to the hyperthyroid

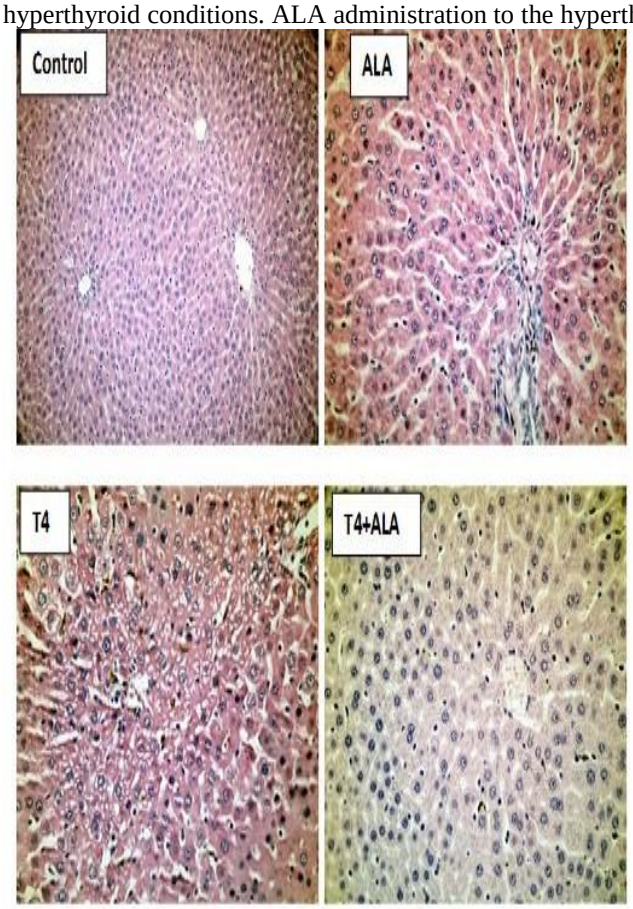

Figure 3. Histopathological findings in the liver tissues of hyperthyroid rats treated with α-lipoic acid (ALA) (H&E × 200).

rats decreased the elevated prooxidant markers in the liver, but not altered the antioxidant system.

Our findings indicate that: a] prooxidant state developed in the liver in experimental hyperthyroidism; b] ALA treatment has improving effect on hyperthyroid state by decreasing the elevated serum fT3, fT4, and by regulatig the prooxidant-antioxidant balance in the liver.

As a conclusion, ALA may be effective supportive agent in the treatment of hyperthyroidism.

References

1. Ichiki T. Thyroid hormone and atherosclerosis. Vascul Pharmacol. 2010;52:151-6.
2. Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E, et al. Thyroid hormones, oxidative stress, and inflammation. Mediators Inflamm. 2016;2016:6757154.
3. Venditti P, De Rosa R, Di Meo S. Effect of thyroid state on susceptibility to oxidants and swelling of mitochondria from rat tissues. Free Radic Biol Med. 2003;35:485-94.
4. Villanueva I, Alva-Sánchez C, Pacheco-Rosado J. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. Oxid Med Cell Longev. 2013;2013:218145.
5. Das K, Chainy GB. Thyroid hormone influences antioxidant defense system in adult rat brain. Neurochem Res. 2004;29:1755-66.
6. Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, et al. A comparative study of the oxidative profile in Graves' disease, Hashimoto's thyroiditis, and papillary thyroid cancer. Biol Trace Elem Res. 2010;138:107-15.
7. Subudhi U, Das K, Paital B, Bhanja S, Chainy GB. Alleviation of enhanced oxidative stress and oxygen consumption of L-thyroxine induced hyperthyroid rat liver mitochondria by vitamin E and curcumin. Chem Biol Interact. 2008;173:105-14.

8. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. Endocrinology. 1987;121:2112-8.
9. Subudhi U, Chainy GB. Expression of hepatic antioxidant genes in l-thyroxine-induced hyperthyroid rats: regulation by vitamin E and curcumin. Chem Biol Interact. 2010;183:304-16.
10. Panda S, Kar A. Annona squamosa seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: possible involvement of quercetin. Phytomedicine 2007;14:799-805.
11. Panda S, Kar A. Antithyroid effects of naringin, hesperidin and rutin in l-T4 induced hyperthyroid rats: possible mediation through 5'DI activity. Pharmacol Rep. 2014;66:1092-9.
12. Gorąca A, Huk-Kolega H, Piechota A, Kleniewska P, Ciejka E, Skibska B. Lipoic acid - biological activity and therapeutic potential. Pharmacol Rep. 2011;63:849-58.
13. Cimolai MC, Vanasco V, Marchini T, Magnani ND, Evelson P, Alvarez S. α-Lipoic acid protects kidney from oxidative stress and mitochondrial dysfunction associated to inflammatory conditions. Food Funct. 2014;5:3143-50.
14. Suh JH, Moreau R, Heath SH, Hagen TM. Dietary supplementation with [R]-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex. Redox Rep. 2005;10:52-60.
15. Yang RL, Li W, Shi YH, Le GW. Lipoic acid prevents high-fat diet-induced dyslipidemia and oxidative stress: a microarray analysis. Nutrition 2008;24:582-8.
16. Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med. 1999;27:612-6.
17. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302–10.
18. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357-63.
19. Benzie IFF, Strain JJ. The ferric reducing ability of plasma [FRAP] as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-6.
20. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
21. Worthington V. Catalase. In: Worthington Enzyme Manual: Enzymes and related biochemicals. NJ Worthington Biochem Corp. 1993:77-80.
22. Mylroie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol. 1986;82:512-20.
23. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.
24. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem. 1985;150:76-85.
25. Uysal M, Koçak-Toker N, Doğru-Abbasoğlu S. Carnosine protection againist liver injury. In: Preedy VR, editör. Imidazole Dipeptides: Chemistry, Analysis, Function and Effects. The Royal Society of Chemistry, Cambridge. 2015. pp: 510-527.
26. Andican G, Gelişgen R, Civelek S, Seven A, Seymen O, Altuğ T, et al. Oxidative damage to nuclear DNA in hyperthyroid rat liver: inability of vitamin C to prevent the damage. J Toxicol Environ Health A. 2004;67:413-20.
27. Kumar N, Kar A, Panda S. Pyrroloquinoline quinone ameliorates l-thyroxine-induced hyperthyroidism and associated problems in rats. Cell Biochem Funct. 2014;32:538-46.
28. Pamplona R, Portero-Otín M, Ruiz C, Bellmunt MJ, Requena JR, Thorpe SR, et al. Thyroid status modulates glycoxidative and lipoxidative modification of tissue proteins. Free Radic Biol Med. 1999;27:901-10.