Journal of Applied Biological Sciences 5 (3): 1-5, 2011
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

# Effects of Caffeic Acid Phenethyl Ester on Plasma Homocysteine, Asymmetric dimethylarginine, Nitric Oxide Levels in Rats Constituted Hyperthyroidism

Funda KARABAĞ[1] Nâlan BAYŞU SÖZBİLİR[2]

[1]Uşak University, Faculty of Science and Literatures, Moleculer Biology Department, Uşak, TURKEY

[2]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Biochemistry, Afyon, TURKEY

*Corresponding Author
e-mail:fundakarabag@hotmail.com

**Received:** May 01, 2011
**Accepted:** May 10, 2011

**Abstract**

Thyroid hormones are important in regulating cardiac function and cardiovascular hemodynamic. Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts and has been used in folk medicine for many years.

**Design**

The aim of this study was to investigate the effects of caffeic acid phenethyl ester on may be endothelial dysfunction in rats with experimentally induced hyperthyroidism. This study is the first examining the effects of caffeic acid on these parameters.

**Material and Method**

We used fifty male Wistar albino rats in this study. The experimental animals were divided into five groups. The first group was control group. The second group was placebo group. The third group received L-tiroxine, the fourth group received caffeic acid and the fifth group received both caffeic acid and L-tiroxine. Plasma freeT3, freeT4, total cholesterol, HDL cholesterol and triglyceride levels were measured by autoanalyzer. Plasma homocysteine levels were measured by high-performance liquid chromatography (HPLC) with fluorescence detect. Plasma nitric oxide and asymmetric dimethylarginine concentrations were measured by ELISA. Statistical analysis was performed using one-way analysis of variance (ANOVA) test in SPSS 13.0 program.

**Findings**

Caffeic acid phenethyl ester, significantly decreased ($p<0.001$) free T3, free T4 and asymmetric dimethylarginine levels and significantly increased ($p<0.001$) nitric oxide, homocysteine, total cholesterol, HDL cholesterol and triglycerid levels.

**Result**

In conclusion; CAPE may show a protective effect against endothelial damage by increasing plasma nitric oxide levels and decreasing asymmetric dimethylarginine levels, in hyperthyroidism induced rats.

***Keywords:*** Hyperthyroidism, rat, asymmetric dimethylarginine, nitric oxide.

## INTRODUCTION

Thyroid hormone has relevant effects on the cardiovascular system (1). Many symptoms and signs recognized in patients with overt hyperthyroidism and hypothyroidism are due to the increased or reduced action of thyroid hormone on the heart and the vascular system, respectively, and the related hemodynamic derangements (2). The relationship between thyroid status and the cardiovascular system is not unidirectional. A body of data indicates that acute and chronic cardiovascular disorders may alter the metabolism of thyroid hormones (2).

Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS) (3) and is a substance that plays an important role in vascular homeostasis regulating vessel tonus (4). At the same time, it counteracts some vital steps in atherosclerosis such as platelet aggregation, leukocyte-endothelium interaction, and proliferation of vascular smooth muscle cells (4,5). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of endothelial NOS and decreases its production and bioavailability (6). ADMA is produced by methylation of arginine remnants during normal protein cycle in many tissues including vascular endothelium (4). It is metabolized to citrulline via dimethylaminohydrolase (4, 7).A lot of data have recently been published on the importance of ADMA in endothelial dysfunction as a cardiovascular risk factor (8).

Homocysteine is an amino acid formed from the metabolism of methionine, an essential amino acid derived from dietary protein. Increased levels of Hcy have been associated to increased overall mortality and to high mortality from cardiovascular disease (CVD) (9,10). All except one study (11) described some relation between vascular disease and Hcy levels. There is also evidence that Hcy can alter the coagulation system and the resistance of the endothelium to thrombosis (12). It also interferes with the antithrombotic and coagulation functions of nitric oxide (13).

Caffeic acid phenethyl ester (CAPE) is an active component of honey bee propolis extracts and has been used in folk medicine for many years. Caffeic acid phenethyl ester was known to have antioxidant, antiinflammatory, antimicrobial, immunomodulatory, and antineoblastic effects (14,15).

It has been shown to inhibit 5-lipooxygenase catalyzed-oxygenation of linoleic acid and arachidonic acid and to potently induce the inflammatory cell apoptosis through a glucocorticoid receptor independent mechanism (16,17). Caffeic acid phenethyl ester effectively downregulates a variety of proinflammatory cytokines and mediators by inhibition of the transcription of the nuclear factor-Kappa (NF-κB) (18). At a concentration of 10 μM, CAPE completely blocks the production of reactive oxygen species (ROS) in human neutrophils and xanthine/ xanthine oxidase system (19).

This study was designed in rats with experimentally induced hyperthyroidism to investigate the protective effects of caffeic acid phenylethyl ester (CAPE) on may be formed endothelial dysfunction that based on thyroid hormons.

## MATERIALS AND METHODS

### Materials

#### *Animals*

Fifty male Wistar albino rats weighing 250-300 g were used in the experiments. They had continuous access to food and water and were housed under controlled conditions of temperature (21–23°C) and illumination (12 hr light: dark cycle) with light on from 8 a.m. to 8 p.m. daily.

#### *Experimental Design*

The animals were randomly divided into five groups of ten animals in each group. The animals in the experimental group I were received control rats received only vehicle. The animals in the experimental group II were placebo rats received only intraperitonially 0.5 ml sterile physiological saline for 4 weeks. The animals in the experimental group III were intraperitonially administered L -thyroxin (0.3 mg/kg) in 0.5 ml sterile physiological saline. The animals in the experimental group IV were CAPE intraperitonially administered at a dose of 10 μg/ kg per day for 4 weeks. The animals in the experimental group V received L-thyroxin (0.3 mg/kg) and CAPE (10 g/kg) for 4 weeks. The CAPE was administered at the same time each day. At the end of the experimental period, the animals were killed by cervical decapitation (20). The blood samples were collected after overnight fasting in test tubes with anticoagulant plasma fractions separated by centrifugation.

#### *Chemicals*

L-Thyroxine (Sigma-Aldrich, St. Louis, USA) was dissolved in 0.01 mol/ l NaOH (Merck & Co., Inc. Rahway, NJ, USA) and diluted with saline (21). CAPE (Sigma-Aldrich, USA) was dissolved in ethanol and further dilutions were made in saline.

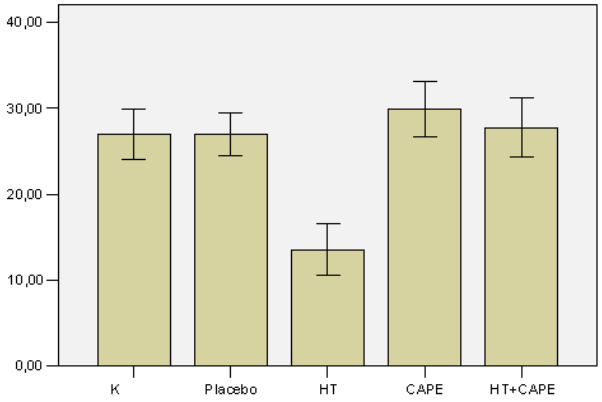
#### *Assay Prosedure*

Levels of plasma thyroid hormones were determined by chemiluminescence method in autoanalyzer. Levels of plasma lipids were determined by autoanalyzer. Levels of plasma plasma Nitric oxide concentrations were measured by ELISA(Cayman Colorimetric Assay Kit, USA ). Asymmetric dimethylarginine concentrations were measured by ELISA (Immun Diagnostik

Colorimetric Assay mause/rat Kit, Germany & England). Plasma homocysteine levels were measured by high-performance liquid chromatography (HPLC) with fluorescence detect.

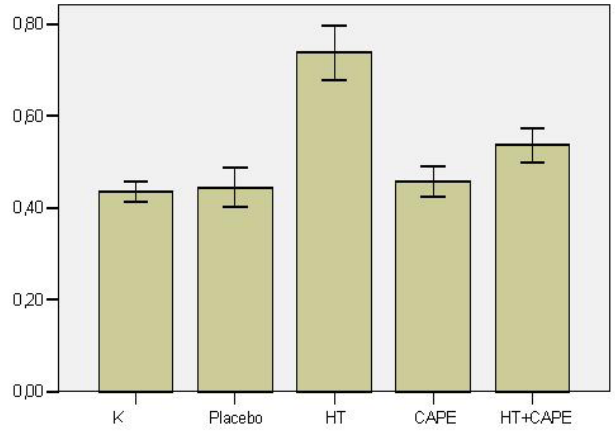
#### *Statistical Analysis*

Statistical analysis was performed using one-way analysis of variance (ANOVA) test in SPSS 13.0 (SPSS, Chicago, IL, USA) program. Data are presented as mean values S.D. A value of $P < 0.001$ was accepted as significant.



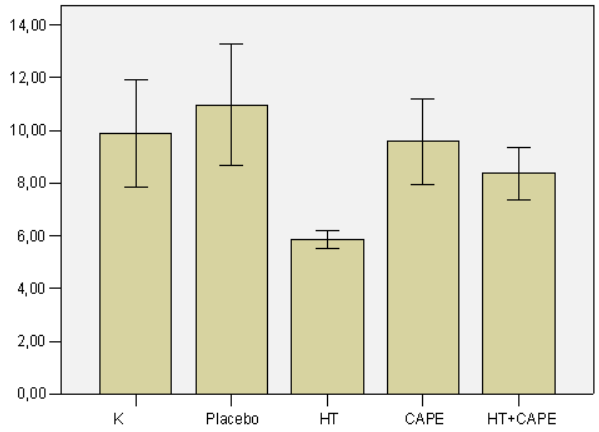

HT: hyperthyroidism group , CAPE: Caffeic acid group , CAPE+HT: Caffeic acid and hyperthyroidism group , K: Control

**Fig.1.** Homocyteine Laevels




HT: hyperthyroidism group , CAPE: Caffeic acid group , CAPE+HT: Caffeic acid and hyperthyroidism group , K: Control

**Fig.2.** ADMA Levels




HT: hyperthyroidism group , CAPE: Caffeic acid group , CAPE+HT: Caffeic acid and hyperthyroidism group , K: Control

**Fig.3.** NO Levels

**Table 1**

| | Control | Placebo | HT | CAPE | CAPE+HT | p |
|---|---|---|---|---|---|---|
| **fT4 (ng/dL)** | 2,49±0,07[c] | 2,68±0,13[c] | 25,20±0,46[a] | 2,73±0,08[c] | 14,39±0,14[b] | 0,000 |
| **fT3 (pg/ml)** | 2,33±0,09[c] | 2,55±0,13[c] | 11,63±0,35[a] | 2,40±0,10[c] | 5,35±0,07[b] | 0,000 |
| **Homocyteine (µmol/l)** | 26,94±1,28[a] | 26,98±1,08[a] | 13,56±1,30[b] | 29,86±1,41[a] | 27,75±1,51[a] | 0,000 |
| **ADMA ( µmol/l)** | 0,44±0,01c | 0,44±0,[0]2c | 0,74±0,03[a] | 0,46±0,01[c] | 0,54±0,02[b] | 0,000 |
| **NO (µM)** | 9,91±0,90[a,b] | 10,96±1,02[a] | 5,86±0,14[c] | 9,59±0,72[a,b] | 8,37±0,44[b] | 0,000 |
| **T.Colesterol (mg/dl)** | 57,40±1,76[a] | 57,70±2,29[a] | 26,40±1,57[c] | 58,30±2,42[a] | 47,40±1,18[b] | 0,000 |
| **HDL (mg/dl)** | 20,16±0,66[a] | 19,64±0,61[a] | 14,25±0,43[b] | 19,82±0,59[a] | 14,03±0,47[b] | 0,000 |
| **LDL (mg/dl)** | 19,70±1,82[a] | 20,69±1,62[a] | 3,65±0,72[c] | 21,39±1,74[a] | 15,74±1,20[b] | 0,000 |
| **TG (mg/dl)** | 89,70±2,40[a] | 80,70±2,47[a] | 58,50±2,47[b] | 87,50±3,36[a] | 85,40±4,34[a] | 0,000 |

(P<0,001), HT: Hyperthyroidism Group, CAPE: Caffeic acid Group, CAPE+HT: Caffeic acid and Hyperthyroidism group.
*a,b,c: the similar line: different character moved groups significantly*

## RESULTS

Plasma fT3, fT4 concentrations were significantly increased in rats with hyperthyroidism compared to controls, placebo, CAPE and "CAPE+HT" groups (p<0,001). Co-administration of CAPE with L thyroxine significantly decreased (p<0.001) the elevated fT3 and fT4 levels (table1). Plasma homocysteine concentrations were significantly decreased in rats with hyperthyroidism compared to controls, placebo, CAPE and "CAPE+HT" groups (p<0,001). Plasma homocystein levels obtained from "CAPE+hyperthyroidism" group were significantly (p<0,001) higher compared to hyperthyroidism group (fig1).

Plasma ADMA concentrations obtained from hyperthyroidism group were significantly higher compared to other groups. Plasma ADMA concentrations obtained from "CAPE+hyperthyroidism" group were significantly (p<0,001) higher compared to control, placebo and CAPE groups and significantly (p<0,001) low compared to hyperthyroidism group (fig2). Plasma NO concentrations obtained from hyperthyroidism group were significantly (p<0,001) low compared to control, placebo, CAPE and "CAPE+hyperthyroidism" groups. Plasma NO concentrations obtained from "CAPE+hyperthyroidism" group were significantly higher (fig3). Plasma total cholesterol, HDL, LDL and triglycerid levels obtained from hyperthyroidism group were significantly lower (p<0,001) than other groups (table1). Plasma total cholesterol, HDL, LDL and triglycerid levels obtained from "CAPE+hyperthyroidism" group were significantly (p<0,001) higher (table 1).

## DISCUSSION

Thyroid hormone has various effects on the cardiovascular system and both hyperthyroidism and hypothyroidism cause or accelerate cardiovascular diseases. Hyperthyroidism causes a hyperdynamic cardiovascular state including a faster heart rate, increased left ventricular contraction and relaxation, and atrial fibrillation.

The aim of this study was to investigate the effects of caffeic acid phenethyl ester on may be endothelial dysfunction in rats with experimentally induced hyperthyroidism. Endothelial dysfunction is common in patients with apparent coronary atherosclerosis or patients with cardiovascular risk factors. Clinical studies revealed that endothelial dysfunction seems to be the possible cause of such complications (22). Elevated plasma concentrations of the endogenous nitric oxide synthase (eNOS) inhibitor asymmetric dimethylarginine (ADMA) represent a novel risk factor for the development of endothelial dysfunction and a predictor for all-cause and cardiovascular mortality. In the present study, increased plasma ADMA levels and decresed plasma NO levels were observed in the hyperthyroid group. Therefore, it is conceivable that an increase in ADMA in hyperthyroidism might represent a compensatory mechanism to decrease NO production.

The major findings of the present study are that: 1) ADMA levels are increased whereas NO levels are decreased in plasma from rats with experimentally induced hyperthyroidism. 2) there is a significant, direct relationship between free T4 and free T3 levels and plasma ADMA; and 3) there is also a significant, but

inverse, relationship between free T4 and free T3 levels and NO 4) Homocysteine and lipid levels are decreased in in plasma from rats with experimentally induced hyperthyroidism.5) A novel finding in the present study was Caffeic acid phenethyl ester, significantly decreased free T3, free T4 and asymmetric dimethylarginine levels and significantly increased nitric oxide, homocysteine total cholesterol, HDL cholesterol and triglycerid levels.

Our results are in accordance with Arıkan et al (23) who report a significant increase in ADMA levels and decreased NO levels of hyperthyroidism. The mechanisms involved in the rise in ADMA concentrations in the present study have not been defined Several lines of evidence indicate that ADMA is synthesized from the degradation of methylated proteins rather than from the methylation of free arginine. The specific enzyme protein arginine N-methyltransferase (protein methylase I) has been shown to methylate internal arginine residues in a variety of polypeptides. Thyroid hormone up-regulates protein methylase I activity (24), offering a putative mechanisms for elevated ADMA levels associated with hyperthyroidism.

However, one of the major effects of thyroid hormone is to increase mitochondrial respiration (25, 26) by many complex changes in the number and activity of mitochondrial respiratory chain components. Accelerated mitochondrial electron transport, brought about by a thyroid hormone-induced hypermetabolic state, results in the increased generation of superoxide at the site of ubiquinone (27). Superoxide radical can lead to the formation of many other reactive species, including hydroxyl radicals, which can readily start the free radical process of lipid peroxidation (28).

Also, it could be hypothesized that hyperthyroidism would decrease dimethylarginine dimethylaminohydrolase (DDHA) activity through increased production of oxygen free radicals and increased lipid peroxidation (29).

Thus, oxidative stress in the vasculature should always stimulate ADMA production and/or inhbit ADMA degradation, in concentrations that significantly inhibit eNOS activity (30) or even uncouple the enzyme which would further increase superoxide production in a positive feedback fashion.

In the present study, we found homocysteine levels that rats with hyperthyroidism had a significantly lower compared with the control group. Both animal and human studies have demonstrated that hyperthyroidism with hight glomerular filtration rate, which in turn in closely related to plasma total homocysteine (31,32).

It has been demonstrated that CAPE has antioxidant, antiinflammatory, immunomodulatory, and anticarcinogenic properties (33).

The mechanisms behind the CAPE-induced reduction in thyroid hormone are not clear. Possibilities include CAPE induced modulation in deiodination system, which affects deiodinase activity through its antioxidant properties (34).

The present study was thus designed to investigate the efficacy of CAPE pretreatment on may be endothelial dysfunction in rats with experimentally induced hyperthyroidism.

Normalization of thyroid hormone levels with CAPE administration was associated with a significant tHcy and NO levels increase however ADMA levels decrease.

In conclusion; CAPE may show a protective effect on endothelial damage by increasing plasma NO levels and decreasing ADMA levels, in hyperthyroidism induced rats.

## REFERENCES


[1] Fazio S, Palmieri A., Lombardi G, Biondi B. 2004. Effects of Hormone on the Cardiovascular System. The Endocrine Society. 31-50.

[2] Morkin I, Ojamaa K .2001. Thyroid hormone and the cardiovascular system. N Engl J Med. 344: 501–509.

[3] Z. Zhang, Y. Y. Zou, Y. Zhou, H. Zhou, and Y. J. Li .2009. "The aggravatory effect of nicotine on Helicobacter pylori-induced gastric mucosa injury: role of asymmetric dimethylarginine," Journal of Clinical Gastroenterology, vol. 43, no. 3, pp. 261–266.

[4] M. T. Selcuk, H. Selcuk, A. Temizhan et al. 2008. "The effect of plasma asymmetric dimethylarginine (ADMA) level and L-arginine/ADMA ratio on the development of coronary collaterals," Turk Kardiyoloji Dernegi Arsivi, vol. 36, no. 3, pp. 150–155.

[5] U. Landmesser and H. Drexler, "The clinical significance of endothelial dysfunction," Current Opinion in Cardiology, vol. 20, no. 6, pp. 547–551.

[6] J. Leiper and P. Vallance.1999. "Biological significance of endogenous methylarginines that inhibit nitric oxide synthases,". Cardiovascular Research, vol. 43, no. 3, pp. 542–548.

[7] P. Vallance, A. Leone, A. Calver, J. Collier, and S. Moncada. 1992. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure, Lancet, vol. 339, no. 8793, pp. 572–575,

[8] H. Miyazaki, H. Matsuoka, J. P. Cooke et al.1999. "Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis," Circulation, vol. 99, no. 9, pp. 1141–1146.

[9] S. H. Mudd, F. Skovby, H. L. Levy, et al.1985. The natural history of homocystinura due to cystathionine β-synthase deficiency. American Journal of Human Genetics, vol. 37, no. 1, pp. 1–31.

[10] O. Nygard, J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad, and S. E. Vollset .1997. Plasma homocysteine levels and mortality in patients with coronary artery disease. New England Journal ofMedicine, vol. 337, no. 4, pp. 230–236.

[11] G. Alfthan, J. Pekkanen, M. Jauhiainen, et al. 1994. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis, vol. 106, no. 1, pp. 9–19.

[12] M. R. Malinow. 1994. Hjomocyst(e)ine and arterial occlusive diseases. Journal of InternalMedicine, vol. 236, no. 6, pp. 603– 617.

[13] J. S. Stamler and A. Slivka. 1996. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutrition Reviews, vol. 54, no. 1, pp. 1–30.

[14] Koltuksuz U, Irmak MK, Karaman A,Uz E,Var A, Özyurt H, et al. 2000. Testicular nitric oxide levels after unilateral testicular torsion/detorsion in rats pretreated with caffeic acid phenethyl ester. Urol Res; 28: 360–363

[15] Çelik O, Türköz Y, Hasçalik S, Hascalik M, Cigremis Y, Mizrak B, et al .2004. The protective effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in rat ovary. Eur J Obset Gyn R B;117(2):183–188.

[16] Sud'ina GF, Mirzoeva OK, Puskareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD. 1993. Caffeic acid phenethyl ester as a lipooxygenase inhibitor with antioxidant properties. FEBS Lett; 329: 21–24.

[17] Watabe M, Hishikawa A, Takayanagi N, Nakaki T. 2004. Caffeic acid phenethyl ester induces apoptosis by inhibition of NF kappa B and activation of Fas in human breast cancer MCE 7cells. J Biol Chem;279:6017–6026.

[18] Natarajan K, Singh S, Burke TR, Grunberger JrD, Aggarwal BB. 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-Kappa B. Proc Natl Acad Sci USA;93: 9090–9095.

[19] İlhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O.1999. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. Eur J Cardiothorac Surg;16(4):458–463.

[20] Yilmaz HR, Uz E, Yucel N, Altuntas I, Ozcelik N. 2004. Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. J Biochem Mol Toxicol; 18:234–8.

[21] Shinohara R, Mano T, Nagasaka A. 2000. Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. J Endocrinol; 164: 97–102.

[22] Li Y, Chen H, Tan J, Wang X, Liang H, Sun X. 1998. Impaired release of tissue plasminogen activator from the endothelium in Graves' disease—indicator of endothelial dysfunction and reduced fibrinolytic capacity. Eur J Clin Invest; 28: 1050–1054.

[23] Arikan E, Karadag CH, Guldiken S .2007. Asymmetric dimethylarginine levels in thyroid diseases. J Endocrinol Invest. Mar;30(3):186-91.

[24] Amur SG, Shanker G, Pieringer RA. 1984. Regulation of myelin basic protein (arginine) methyltransferase by thyroid hormone in myelinogenic cultures of cells dissociated from embryonic mouse brain. J Neurochem 43: 494–498.

[25] Roodyn DB, Freeman KB & Tata JR. 1965. The stimulation by treatment in vivo with tri-iodothyronine or amino acid incorporation into protein by isolated rat-liver mitochondria. Biochemical Journal 94 628–641.

[26] Nishiki K, Ericinska M, Wilson DF & Cooper S. 1978. Evaluation of oxidative phosphorylation in hearts from euthyroid, hypothyroid and hyperthyroid rats. American Journal of Physiology 235 C212–C219.

[27] Turrens JF & Boveris A. 1980. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochemical Journal 191 421–427.

[28] Chance B, Sies H & Boveris A.1979. Hydroperoxide metabolism in mammalian organs. Physiological Reviews 59 527–605.

[29] Videla LA, Sir T, Wolff C. 1988. Increased lipid peroxidation in hyperthyroid patients: suppression by propylthiouracil treatment. Free Radic Res Commun 5: 1–10.

[30] Cooke JP (2000) Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol; 20:2032–7.

[31] Wollosen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. 1999. Plasma homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney int; 55: 1028-1035.

[32] Kreisman SH, Hennessey JV. 1999. Consistent reversibl elevations of serum creatinine levels in severe hypothyroidism. Ark İntern Med; 159: 79-82.

[33] Gurel A, Armutcu F, Sahin S, Sogut S, ÖzyurtH, Gülec M, Kutlu NO, Akyol O. 2004. Protective role of α-tocopherol and caffeic acid phenethyl ester on ischemia–reperfusion injury via nitric oxide and myeloperoxidase in rat kidneys. Clin Chim Acta; 339: 33–41.

[34] Ahmed M. Mohamadin, Lamiaa N. A. Hamma, Mohamed Fath El-Bab, Hala S. Abdel Gawad. 2006. Attenuation of Oxidative Stress in Plasma and Tissues of Rats with Experimentally Induced Hyperthyroidism by Caffeic Acid Phenylethyl Ester. Basic & Clinical Pharmacology & Toxicolog; 100: 84–90.