FABAD J. Pharm. Sci., 27, 197-203, 2002

RESEARCH ARTICLES

The Protective Effects of Cinnarizine and Nitrendipine on Naphthalene Induced Oxidative Stress in Mice Tissues

Şebnem Ş. ÇEÇEN*, Gülhan CENGİZ*, Göknur AKTAY**, Tülin SÖYLEMEZOĞLU*°

The Protective Effects of Cinnarizine and Nitrendipine on Naphthalene Induced Oxidative Stress in Mice Tissues

Summary : *In this study, the effects of nitrendipine and cinnarizine, dihydropyridine and diphenylpiperazine derivative calcium channel antagonists, respectively, on naphthalene-induced oxidative stress were investigated in mice. Nitrendipine (NIT; 50 mg/kg,i.p.) , cinnarizine (CIN; 200 mg/kg, i.p.) and a reference antioxidant N-acetylcysteine (NAC; 7 mmol/kg, i.p.) were administered one hour prior to the administration of naphthalene (NAP; 400 mg/kg, i.p.). Lactate dehydrogenase (LDH) , malondialdehyde (MDA) and cellular gluthathione (GSH) levels were determined as markers of oxidative stress. As a result, the two different derivatives of calcium antagonists, nitrendipine and cinnarizine may have different functions against NAP-induced toxicity depending upon the organo-specificity.*

Key Words: *Naphthalene; oxidative stress; N-acetylcysteine; nitrendipine; cinnarizine.*

Received : 4.11.2002
Revised : 17.2.2003
Accepted : 21.3.2003

Sinnarizin ve Nitrendipin'in Fare Dokusunda Naftalenle İndüklenmiş Oksidatif Stres Üzerindeki Koruyucu Etkileri

Özet : *Bu çalışmada, farelerde naftalenin oluşturduğu oksidatif stres üzerine, dihdropridin ve difenilpiperazin türevi kalsiyum kanal antagonistleri olan nitrendipin ve sinnarizinin etkileri araştırıldı. Nitrendipin (NIT;50mg/kg, i.p.), sinnarizin (CIN;200 mg/kg, i.p.) ve referans bir antioksidan olan N-Asetilsistein (NAC; 7 mmol/kg, i.p.), naftalen (NAP; 400mg/kg,i.p.) uygulamasından bir saat önce verildi. Oksidatif stresin göstergeleri olarak laktat dehidrogenaz (LDH), malondialdehit (MDA) ve hücresel glutatyon (GSH) düzeyleri ölçüldü. Sonuç olarak, iki değişik kalsiyum antagonist türevi olan, nitrendipin ve sinnarizinin organlarda farklı fonksiyonlara sahip olduğu düşünülerek, naftalenin neden olduğu toksisiteye karşı etkili oldukları söylenebilir.*

Anahtar kelimeler : *Naftalen; oksidatif stres; N-Asetilsistein; nitrendipin; sinnarizin.*

INTRODUCTION

Naphthalene (NAP) is widely used in various commercial and industrial applications. Cigarette smoke, automobile exhaust gases, and coal combustion products contain relatively large amounts of NAP[1]. Exposure to NAP results in the development of bronchiolar damage[2], cataracts[3], and hemolytic anemia[4] in humans and in laboratory animals. The toxic manifestations induced by NAP appear to involve the conversion of NAP to naphthoquinones and NAP epoxides. These metabolites are capable of circulating and becoming covalently bound to macromolecules in tissues in a dose and time dependent manner, which can cause more toxic mechanisms involving redox cycling and oxidative stress[5,6].

Lipid peroxidation (LPO) is considered as a universal mechanism of oxidative deterioration of membrane lipids induced by different factors and by many pathologic states, including ischemia, stress, various toxicoses, etc[7,8]. Recognition of the protective role played by GSH, N-acetylcysteine (NAC), a known antioxidant and free radical scavenger, counteracts oxidative stress and replenishes GSH[9,10]. Nucleophilic sulphydryl groups on cysteine side chains are re-

* Ankara University, Institute of Forensic Medicine, 06260, Dikimevi, Ankara, TURKEY.
** İnönü University, Faculty of Pharmacy, Department of Pharmacology, Malatya, TURKEY.
° Correspondence

active targets for epoxide and quinone metabolites of NAP.

Numerous experimental data strongly suggest that calcium antagonists also may be useful in oxidative hepatocellular injury[11], and renal injury[12] in addition to the treatment of cardiovascular disease[13], neurological and psychiatric disorders[14], opioid and ethanol dependence[15].

In a previous study, we showed that nitrendipine protects tissues against NAP-induced oxidative stress. In this study, pretreatment with nitrendipine significantly attenuated the oxidative effect of NAP in lungs following an acute dose, while it did not affect (or affected to a minor extent only) the level of malondialdehyde (MDA) in liver and kidney[16].

In the present study, NAP-induced oxidative stress was assessed by measuring MDA, GSH and LDH (lactate dehydrogenase) levels, and the protective effects of nitrendipine and cinnarizine were investigated to assess whether these calcium channel blockers have any different effects depending on their organospecificity. NAC was used as a reference antioxidant against NAP toxicity.

MATERIAL and METHODS

Animals

Mice used in this study were housed and cared for in accordance with Refik Saydam Hıfzısıhha Institute, Animal Care Unit, which applies the guidelines of the National Institutes of Health (NIH) on Laboratory Animal Welfare. Procedures involving animals and their care were conducted in conformity with international laws and policies and the studies on animals accepted by Ethic Council of Ankara University, Veterinary Faculty (28.9.1999, No: 7).

Local breed albino male mice, weighing 20-25 g, were housed in group cages under normal laboratory conditions (temperature 20-22°C, natural day-night cycle), and with free access to commercial chow and water. The food was withdrawn 12-16 h before the experiment.

Treatment

Animals were divided into five groups consisting of six mice each and exposed as follows: (I) control group received corn oil; (II) received 400 mg/kg NAP[17]; (III) received 7 mmol/kg NAC[18]; (IV) received 50 mg/kg nitrendipine[19]; (V) received 200 mg/kg cinnarizine[20]. All chemicals were prepared with corn oil and were given i.p. N-acetylcysteine, nitrendipine and cinnarizine were administered 1 h prior to the administration of NAP as described in group II. Mice were sacrified by an overdose of diethyl ether 4 hours after the last injections. Blood was withdrawn by intracardiac punction and then lungs, livers and kidneys were removed.

Lipid peroxidation in plasma

MDA, a decomposition product of LPO, was determined by measuring the thiobarbituric acid reactive substances (TBARs) in plasma and tissues. MDA levels in plasma were measured according to the method of Buege and Aust[21]. One ml of plasma was combined with 2.0 ml of trichloroacetic acid (TCA; 15% w/v)-thiobarbituric acid (TBA; 0.375 % w/v)-0.25 N HCl and centrifuged at 10 000 x g for 5 min. The supernatant was mixed with 20 µl butylated hydroxytoluene (BHT; 0.02% in 95% ethanol w/v) to prevent further oxidation and heated for 15 min in a boiling water bath. The precipitate was removed by centrifugation at 10 000 x g for 5 min after cooling under tap water. The absorbance of the plasma sample was read at 532 nm against a blank. The plasma TBA reactive products were expressed as nmoles of MDA/ml. 1,1,3,3-tetraethoxypropan was used as the standard for calibration of the curve.

Homogenization

Tissues of animals were immediately excised and chilled in ice-cold 0.9 % NaCl. After washing with 0.9% NaCl, 0.5 g of wet tissue was weighed exactly and homogenized in 4.5 ml of 0.25 M sucrose to obtain a 10 w/v% suspension in order to measure LPO

in tissues. 0.2 g tissue was homogenized with 8 ml 0.02 M Na_2EDTA to measure GSH level.

Lipid peroxidation in tissues

The method of Ohkawa et al.[22] as modified by Jamall and Smith[23] was used to determine MDA in tissue samples. MDA, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. The tissue extract supernatant was obtained by a two step-centrifugation first at 1000 xg for 10 min and then at 2000 xg for 30 min at 4 °C. 0.20 ml of supernatant was transferred to a vial and was mixed with 0.20 ml sodium dodecyl sulfate solution (SDS; 8.1%), 1.50 ml of acetic acid solution (CH_3COOH; 20%, v/v, adjusted to pH 3.5 with NaOH), and 1.50 ml of 0.8%(w/v) solution of TBA and the final volume was adjusted 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test sample and 10% trichloroacetic acid (TCA) were centrifuged at 1000 x g for 10 min. The absorbance of the supernatant was measured at 532 nm against tissue blank. Tissue blank was processed using the same experimental procedure except that the TBA solution was replaced with distilled water. 1,1,3,3-tetraethoxypropane was used as standard for the calibration curve. The tissue TBA reactive products were expressed as nmoles of MDA/g wet tissue.

Gluthathione (GSH) in tissues

GSH was measured by Sedlak and Lindsay's method[24]. The purpose of this method is to measure nitromercaptobenzoic acid anion that gives intense yellow color at 412 nm resulting from the reaction of sulphydryl groups with Ellman's reagent. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled H_2O and 1.0 ml of 50% TCA. The tubes were centrifuged for 15 min at 3000 x g. Two ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer (pH: 8.9), 0.1 ml Ellman's reagent, (5,5'-dithiobis-2-nitro-benzoic acid) - (DTNB) was added, and the sample was shaken. The absorbance was recorded at 412 nm within 5 minutes after the addition of DTNB against a reagent blank with no homogenate. The tissue GSH levels were expressed as µmol/g wet tissue.

Lactate dehydrogenase (LDH) assay

Serum samples were analyzed for LDH with a Clonital diagnostic kit (Cat. No.:KC030). The method is based upon the conversion of pyruvate into L-lactate by the following reaction at 340 nm.

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{L - lactate} + NAD^+$$

A Schimadzu UV-1208 spectrophotometer was used for detection.

Statistical Analysis

Significant differences were determined by ANOVA, followed by the Student-Newman-Keul's post hoc test with the help of computer software (Instat).

RESULTS and DISCUSSION

Previous studies have demonstrated that NAP causes oxidative stress in experimental animals as evidenced by enhanced LPO and decreased GSH content in hepatic and pulmonary tissues. In the presence of depleted tissue GSH, the reactive metabolites of relatively low doses of NAP can bind tissue macromolecules covalently (e.g. lipids, proteins and nucleic acids) and this may result in cell injury[1]. Honda et al.[25] reported that the administration of NAP (200 mg/kg i.p) leads to a depletion in pulmonary GSH but does not affect LPO in mice. At higher doses (400 or 600 mg/kg, i.p) of NAP nephrotoxicity has also been shown to develop[17]. The current results clearly demonstrate that administration of NAP (at a single dose of 400 mg/kg, i.p.) induces LPO and depletes GSH in all tissues. Present results correlate well with previous findings[17,26]. Furthermore, NAP significantly enhanced the LPO level in plasma and LDH activity in serum (Figure 1 and 2). Many laboratories measure serum LDH activity as a "liver function test". It is well known that LDH is an ubiquitous intracellular en-

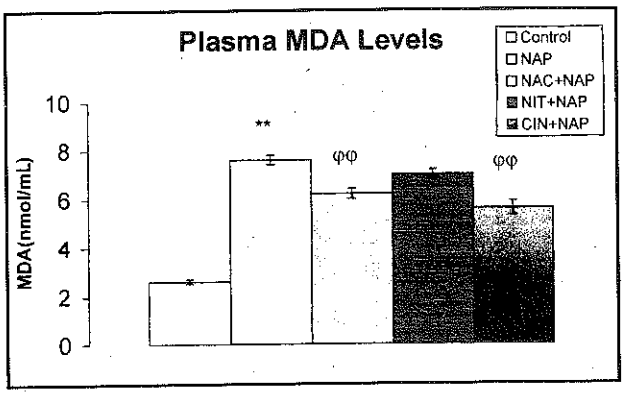

Figure 1. Effects of NAP, NAC+NAP, NIT+NAP and CIN+NAP on plasma MDA levels.
Results are obtained as the mean (±SEM) of nine mice per group.
** Significantly different from control (p<0.001)
φφ Significantly different from NAP (P<0.001)

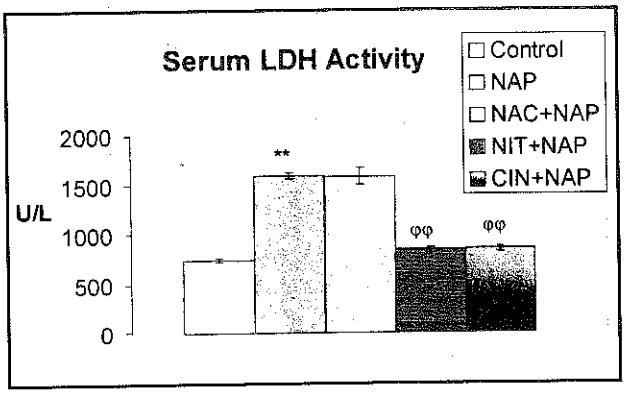

Figure 2. Effects of NAP, NAC+NAP, NIT+NAP and CIN+NAP on serum LDH levels.
Results are obtained as the mean (±SEM) of nine mice per group.
** Significantly different from control (p<0.001)
φφ Significantly different from NAP (P<0.001)

zyme used as a marker of cardiac and liver injury. It has been suggested that increased serum LDH also may reflect renal disorders, dermatitis, rheumatoid arthritis, alveolar proteinosis and pneumocystis pneumonia[27,28,29]. In these conditions, LDH may be released from damaged cells. This information explains the elevation of LDH levels in NAP treated animals in this study.

Because of their therapeutic benefits the sulphydryl groups containing antioxidants such as NAC are widely used. It is well known that NAC is used as an antidote in various intoxications such as acetaminophen toxicity by inducing the increase of GSH levels in tissues[30]. Intraperitoneal administration of NAC together with NAP, although it did not markedly change serum LDH levels (Figure 2), resulted in significant decreases in plasma, lung, liver (p<0.001) and kidney (p<0.01) MDA levels (Figure 1, 3) and significantly increased lung and liver GSH

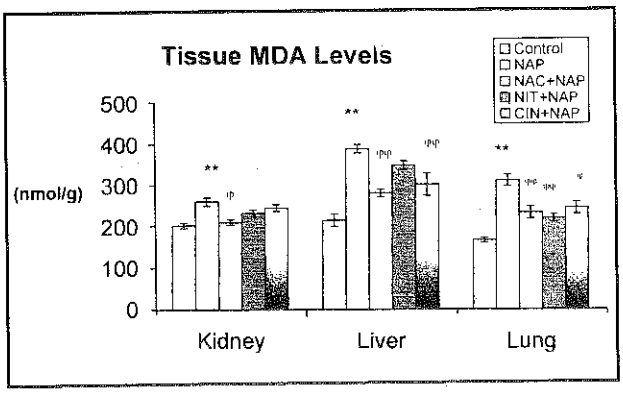

Figure 3. Effects of NAP, NAC+NAP, NIT+NAP and CIN+NAP on tissue MDA levels.
Results are obtained as the mean (±SEM) of nine mice per group.
* Significantly different from control (p<0.01)
** Significantly different from control (p<0.001)
φ Significantly different from NAP (P<0.01)
φφ Significantly different from NAP (P<0.001)

(p<0.01) (Figure 4) when compared to the group administered NAP alone. The reason why a decrease in serum LDH level did not occur for NAC treatment is unclear (Figure 2). Measurement of specific isoenzymes in serum may help to explain our results.

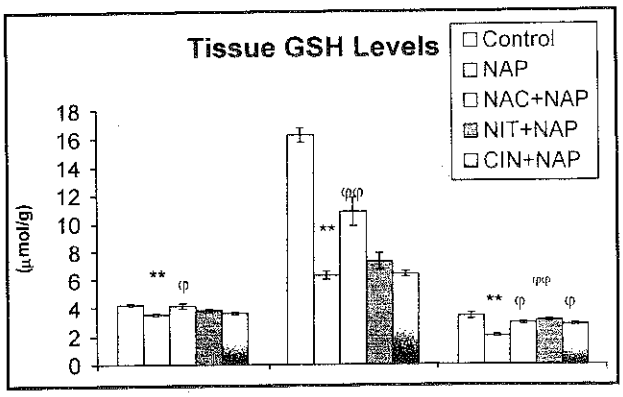

Figure 4. Effects of NAP, NAC+NAP, NIT+NAP and CIN+NAP on tissue GSH levels.
Results are obtained as the mean (±SEM) of nine mice per group.
** Significantly different from control (p<0.001)
φ Significantly different from NAP (P<0.01)
φφ Significantly different from NAP (P<0.001)

As previously stated, the thiol groups of endogenous GSH have importance in calcium transport across cell membranes. Depletion of GSH has been reported to destroy intracellular calcium equilibrium and transport systems. Disturbance of cellular calcium homeostasis generally leads to cell death. A number of recent studies have demonstrated that some calcium channel blockers protect the tissues against oxidative stress[31,32]. Lipid peroxides can diminish membrane fluidity, hence, increase nonspecific permeability to ions especially Ca^{2+}. Increasing evidence indicates that calcium channel blockers in lipophilic structure may destroy the lipid peroxide chains by attacking the lipid parts of the membrane[13]. Therefore, if the cell damage is caused by free radicals and/or by an increase in the calcium amount, it is possible that calcium channel blockers protect the tissue against the growing toxicity.

In a previous study[16], it was shown that a dihydropyridine derivative of calcium channel blocker, nitrendipine, prevented NAP-induced toxicity in lung compared to the NAP+NAC injected group. In this study, nitrendipine treatment also induced a remarkable decrease in serum LDH activity ($p<0.001$) (Figure 2), which was increased by NAP administration. While the alterations in MDA and GSH levels in the liver were not found significant statistically, there is a significant increase in GSH level ($p<0.001$) (Figure 4) and decrease in MDA levels in lung in the same group (Figure 3). Indeed, previous studies have demonstrated that dihydropyridine derivative calcium channel blockers bind weakly to liver tissues[11]. In our study, a greater responsiveness was observed to the protective effect of nitrendipine in lung tissue compared to other tissues.

In a previous study, it was shown that a piperazine derivative calcium channel blocker, cinnarizine, prevents LPO in rat liver homogenate[33]. A remarkable decrease was observed in MDA levels in lung ($p<0.01$) and liver ($p<0.001$) in our study (Figure 3). Similar to results obtained with nitrendipine administration, lung GSH levels were increased ($p<0.01$) (Figure 4) whereas serum LDH activity was decreased ($p<0.001$) with cinnarizine injection (Figure 2), but no effect was observed in liver GSH levels (Figure 4). It has been reported that nitrendipine and cinnarizine exert their hepatoprotective effects either by preventing hepatic microsomal oxidative metabolism or by resisting the metabolism of NAP into covalently bound product(s) or its major methanol-soluble metabolites such as 1-naphthol and 1,2-dihydro-1,2-dihydroxynaphthalene[17,34]. At least two classes of calcium channels exist in the liver, one is thought to be sensitive only to dihydropyridines and the other sensitive to all three classes[11].

Polyaromatic hydrocarbons are metabolized to their toxic quinone derivatives followed by the generation of free radicals and depletion of tissue GSH by the cytochrome P-450 system[17]. The liver because of its high cytochrome P-450 content, has a greater ability to generate potentially toxic NAP metabolites and could explain why NAP treatment damages the liver more severely and the lung relatively but not the kidney.

The Clara cells found in lungs are readily susceptible to oxidative damage caused by NAP due to its capability to transform chemicals into toxic intermediates. GSH depletion occuring as a consequence of oxidative stress, is a critical step in the beginning of the events leading to acute Clara cell cytotoxicity. Plopper et al.[35] reported that NAP, which is a cytochrome P-450 activated cytotoxicant, depletes intracellular GSH which is an early event that precedes initial signs of cellular damage in Clara cell cytotoxicity. Subsequently, if the intracellular GSH concentration drops below a certain threshold within the whole cell, the initial phase of injury described above becomes irreversible.

An increase in MDA levels due to NAP in kidneys was significantly prevented by the administration of NAC. However the decrease in MDA levels observed in kidneys of animals which received nitrendipine and cinnarizine were not significant. These results are well proportioned with the GSH levels measured in kidneys (Figure 4). It seems likely that NAC, having sulphydryl groups, provides more effective pre-

vention in kidneys compared to the calcium channel blockers used in this study.

CONCLUSION

The present results suggest that the dihydropyridine derivative calcium channel blocker nitrendipine has more effective antioxidant activity in the lung than the piperazine derivative calcium channel blocker cinnarizine, and that cinnarizine protects the liver against NAP-induced toxicity more effectively. It is well known that the calcium channels sensitive to piperazine and dihydropyridine derivatives are localized in different organs and in different proportions[11]. These properties may account for the different effectiveness of calcium channel blockers. Three antioxidants examined caused beneficial changes in oxidative stress markers. However, the details of their mechanisms of action and the reasons for different responsiveness of different tissues to these compounds are still under evaluation.

ACKNOWLEDGEMENT

This research was supported by TUBITAK 1999 S109 (SBAG-AYD-267).

REFERENCES

1. Buckpitt AR, Castagnoli N, Nelson SD, Jones AD, Bahnson LS. Stereoselectivity of naphthalene epoxidation by mouse, rat , and hamster pulmonary, hepatic, and renal microsomal enzymes, *Drug Metab. Dispos,*. 15, 491-496, 1987.
2. Warren DL, Brown D, Buckpitt AR. Evidence for cytochrome P-450 mediated metabolism in the bronchiolar damage by naphthalene, *Chem. Biol. Interact,*. 40, 287-303, 1982.
3. Lubek BM, Kubaw S, Basu PK, Wells PG. Cataractogenicity and bioactivation of naphthalene derivatives in lens culture and in vivo, *Lens Eye Toxic.Res.*, 6, 203-209, 1989.
4. Smith MT, Evans CG. Inhibitory effect of superoxide generating quinones on superoxide dismutase, *Biochem. Pharmacol.*, 33, 3109-3110, 1984.
5. Bagchi M, Bagchi D, Balmoori J, Ye X, Stohs S J. Naphthalene induced oxidative stress and DNA damage in cultured macrophage y774A.1 cells, *Free Radic. Biol. Med.*, 25, 137-143, 1998.
6. Vuchetich PJ, Bagchi D, Bagchi M, Hassoun EA, Tang L, Stohs SJ. Naphthalene-induced oxidative stress in rats and the protective effects of vitamin E succinate, *Free Radic. Biol. Med.*, 21(5), 577-590, 1996.
7. Kagan V, Serbinova E, Novikov K, Ritov V, Kozlov Y, Stoytchev T. Toxic and protective effects of antioxidants in biomembranes, *Arch.Toxicol.*, 9, 302-305, 1986.
8. Meerson F, Kagan V, Kozlov Yu, Belkina L, Arkipenko Yu. The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart, *Basic Res. Cardiol*, 77, 465-485,1982.
9. Benrahmaune M, Therond P, Abedinzadeh Z. The reaction of superoxide radical with N-Acetylcysteine, *Free Radic. Biol. Med.*, 29: 775-782, 2000.
10. Hoffer E , Shenker L, Baum Y, Tabak A. Paraquat-induced formation of leukotriene B4 in rat lungs: Modulation by N-acetylcysteine, *Free Radic. Biol. Med,*. 22 (3), 567-572, 1997.
11. Thurman RG, Apel E, Badr M, Leamasters JL. Protection of liver by calcium entry blockers, *Ann. N. Y. Academy Sci.*, 560, 757-770, 1989.
12. Bauer JH, Reams GP. Effects of calcium antagonists on renal function, *Ann. N. Y. Academy Sci.*, 560, 785-782, 1989.
13. Sanjana PH, Pandit PR, Deshmukh YA, Rambhad GS. Calcium channels and drugs, *JAPI.*, 45(6), 464-471,1997.
14. Bidzinski A, Jankowska E, Pucilowski O. Antidepressant-like action of nicardipine, verapamil and hemicholinium-3 injected into the arterior hypothalamus in the rat forced swim test, *Pharmacol. Biochem. Behav,*. 36, 795-798, 1990.
15. Little HJ, Dolins SJ, Whittington MA. Possible role of calcium channels in ethanol tolerance and dependence, *Ann. N. Y. Academy Sci.*, 560, 465-466, 1989.
16. Aktay G, Cengiz G, Söylemezoğlu T. Protective effect of nitrendipine on naphthalene-induced oxidative stress, *J. Fac.Pharm. Gazi.*, 17(1), 19-24, 2000.
17. O'Brien AF, Smith LL, Cohen GM. Differences in naphthalene-induced toxicity in the mouse and rat, *Chem. Biol. Interactions.*, 55, 109-122, 1985.
18. Kröger H, Dietrich A, Ohde M, Lange R, Ehrlich W, Kurpisz, M. Protection from acetaminophen -induced liver damage by the synergistic action of low doses of the poly(ADP-ribose) polymerase-inhibitor nicotinamide and the antioxidant N-acetylcysteine or the amino acid L-methionine, *Gen. Pharmacol.*, 28(2), 257-263, 1997.
19. Koleva M, Kastelova A, Staneva-Stoytcheva, D, Stoytchev, Ts. Combined effect of propranolol with nifedipine or with diltiazem on rat liver monooxygenase activities, *Toxicol. Lett.*, 105, 153-161, 1999.
20. Czarnecka E, Kubik-Bogucka E. Effects of calcium antagonists on central actions of ethanol: Comparative studies with nifedipine, verapamil and cinnarizine, *Alcohol and Alcoholism*, 28(6), 649-655, 1993.
21. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods in Enzymology.*, 52, 303-310, 1978.

22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem.*, 85, 351-358, 1979.
23. Jamall IS, Smith JC. Effects of cadmium on gluthathione peroxidase, superoxide dismutase and lipid peroxidation in the rat heart: A possible mechanism of cadmium cardiotoxicity, *Toxicol. Appl. Pharmacol.*, 80, 33-42, 1985.
24. Sedlak J, Lindsay RH. Estimation of total protein-bound and non-protein sulphydryl groups in tissue with Ellman's reagent, *Anal. Biochem.*, 25, 192-205, 1968.
25. Honda, T, Kiyozumi M, Kojima S. Alkyl naphthalene XI. ulmonary toxicity of naphthalene, 2-methylnaphthalene and isopropylnaphthalene in mice, *Chem. Pharm. Bull.*, 38(11), 3130-3135, 1990.
26. Germansky M, Jamall I, S. Organ-specific effects of naphthalene on tissue peroxidation, gluthathione peroxidases and superoxide dismutase in the rat, *Arch.Toxicol.*, 61, 480-483, 1988.
27. Thompson PW, Jones DD. Serum lactic dehydrogenase as a marker of joint damage in rheumatoid arthritis, *Ann. Rheum. Dis.*, 46, 263-268, 1987.
28. Mukai H, Noguchi T, Kamimura K, Nishioka K, Nishiyama S. Significance of elevated serum LDH (lactate dehydrogenase) activity in atopic dermatitis, *J. Dermatol.*, 17, 477-481, 1990.
29. Hoffman RM, Rogers RM. Serum and lavage lactate dehydrogenase isoenzymes in pulmonary alveolar proteinosis, *Am. Rev. Respir. Dis.*, 143, 42-46, 1991.
30. Flanagan RJ, Meredith TJ. Use of N-acetylcystein in clinical toxicology, *Am. J. Med.*, 91, 131-139, 1991.
31. Casini AF, Maellaro E, Pompella A, Ferrali M, Comporti M. Lipid peroxidation, protein thiols and calcium homeostasis in bromobenzene-induced liver damage, *Biochem. Pharmacol,*. 36(21), 3689-3695, 1987.
32. Bellomo G, Orrenius S. Altered thiol and calcium homeostasis in oxidative hepatocellular injury, *Hepatology.*, 5, 876-882, 1985.
33. John GW, Shrivastava R, Chevalier A, Pognat JF, Massingham R. An in vitro investigation of the relationships between potency, lipophilicity, cytotoxicity and chemical class of representative calcium antagonist drugs. *Pharmacol. Res.*, 27, 253-261, 1993.
34. Matucci R, Bennardini F, Sciammarella ML, Baccaro C, Stendardi I, Franconi F, Giotti A. [3 H]- Nitrendipine binding in membrans obtained from hypoxic and reoxygenated heart, *Biochem. Pharmacol.*, 36, 1059-1063, 1987.
35. Plopper CG, Van Winkle VL, Fanucchi MV, Malburg SRC, Nishib, SJ, Ehang A, Buckpitt AR.Early events in naphthalene induced acute Clara cell toxicity.II. Comparison of gluthathione depletion and histopathology by airway location, *Am. J. Respir. Cell. Mol. Biol.*, 24, 272-281, 2001.