

Acute Effects of N-Acetylcysteine on Total Antioxidant Capacity, Total Oxidant Capacity, Nitric Oxide Level and Gammaglutamyl Transpeptidase Activity in Rabbits

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Abstract

N-acetylcysteine (NAC), acetylated derivative of cysteine amino acid, is widely used mucolytic agent. In this study, it was aimed to investigate the acute effects of NAC on total antioxidant capacity (TAC), total oxidant capacity (TOC), nitric oxide (NO), albumin, globulin, glucose levels and gammaglutamyl transpeptidase (GGT) activity. Eleven New Zealand rabbits were used in the study. Blood samples from the rabbits were taken before the start of the experiment to determine control values. NAC (100 mg/kg) was injected to rabbits as intramuscularly. Blood samples were collected from vena auricularis of rabbits at 3rd, 6th and 9th h. While TAC levels were found high ($P<0.05$) at 6 and 9 h, TOC levels were found low ($P<0.05$) at 3rd, 6th and 9th h after NAC injection compared to the values before the experiment. NO levels were found low ($P<0.001$) at 6th and 9th h, GGT activities were found low ($P<0.05$) at 3rd and 6th h, total protein and albumin levels were found low ($P<0.05$) at 3rd h after NAC injection compared to values before the experiment. As a result, after intramuscular injection of NAC, plasma TAC levels of rabbits increased, while TOC and NO levels decreased. According to the results, it can be concluded that NAC may show antioxidant effect in a short time. Therefore, NAC could be a good alternative when the antioxidant system needs to be strengthened due to its beneficial properties.

Keywords: N-acetylcysteine, Total antioxidant capacity, Total oxidant capacity, Nitric oxide

Tavşanlarda N-Asetil Sisteinin Total Antioksidan Kapasite, Total Oksidan Kapasite, Nitrik Oksit Düzeyleri ve Gamaglutamil Transpeptidaz Aktivitesi Üzerine Akut Etkileri

Özet

Sistein amino asidinin asetilenmiş türevi olan N-asetilsistein (NAS) mukolitik olarak kullanılan bir moleküldür. Yapılan çalışmada tavşanlara verilen NAS'ın total antioksidan kapasite (TAK), total oksidan kapasite (TOK), nitrik oksit (NO), albumin, globulin, glukoz düzeyleri ve gama glutamil transpeptidaz (GGT) aktivitesi üzerine akut etkilerini araştırmak amaçlanmıştır. Çalışmada 11 adet Yeni Zelanda ırkı tavşan kullanıldı. Denemeye başlamadan önce kontrol değerlerini saptamak için kan numuneleri alındı. Daha sonra tek doz kas içi NAS (100 mg/kg) enjekte edildi ve enjeksiyondan 3, 6 ve 9 saat sonra kan numuneleri alındı. Deneme öncesine göre total antioksidan seviyesi 6. ve 9. saatlerde yüksek ($P<0.05$) bulunurken, total oksidan seviyesi NAS verildikten sonra 3., 6. ve 9. saatlerde düşük ($P<0.05$) saptandı. Nitrik oksit düzeyleri deneme öncesi ile karşılaştırıldığında, denemenin 6. ve 9. saatlerinde düşük ($P<0.001$) bulundu. GGT aktivitesi deneme öncesi değerlerine göre, 3 ve 6. saatte düşük ($P<0.05$) saptandı. Benzer şekilde total protein ve albumin düzeyleri deneme öncesi değerlerine göre denemenin 3. saatinde istatistiksel olarak düşük ($P<0.05$) saptandı. Sonuç olarak, tavşanlara intramuscular NAS enjeksiyonunun plazma TAK düzeyini artırırken TOK ve NO düzeylerini azalttığı bulundu. Bu sonuçlara göre NAS'ın kısa bir süre içinde antioksidan etki yapabileceği ve antioksidan sistemin güçlendirilmesine ihtiyaç duyulan durumlarda iyi bir seçenek olabileceği düşünülmektedir.

Anahtar sözcükler: N-asetil sistein, Total antioksidan kapasite, Total oksidan kapasite, Nitrik oksit



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INTRODUCTION

N-acetylcysteine (NAC) is the N-acetyl derivative of the L-cysteine amino acid. NAC is used in the treatment of respiratory tract diseases such as Chronic obstructive pulmonary disease (COPD), chronic bronchitis [1] and cardiovascular system diseases such as myocardial infarction and coronary failure [2]. It was also reported that it played a role in detoxification of toxic chemicals in the liver [3], septic shock induced by oxidative stress [4] and reduction of harmful effects of reactive oxygen types (hydroxyl radical and singlet oxygen) [5]. N-acetylcysteine plays a role in the synthesis of an important antioxidant molecule, known as reduced glutathione (GSH), by giving the cysteine residue [6,7]. Furthermore, since it carries thiol group in its structure and it is a sulfhydryl group donor, it binds or reduces free radicals non-enzymatically, helping to clean the medium from hydroxyl radical, the same way other thiols function [5].

NAC, which is the precursor of glutathione, plays a role in the increase of intracellular glutathione storage and in keeping the cytoplasmic reserves constant [6]. GSH is found in two forms: reduced (GSH) and oxidized (GSSH). The proportion of these two forms (GSH/GSSH) was reported as a marker for the antioxidative cellular capacity [8].

Circulating concentrations of different antioxidants or oxidants can be measured separately, but the measurements are time-consuming, labor-intensive, and costly and they require complicated techniques. Because the measurement of different antioxidant/oxidant molecules, is not practical and antioxidant/oxidant effects are additive, total antioxidant response (TAR) or total oxidant status (TOS) of a sample is measured and this is named as total antioxidant capacity [9] or total peroxide [10].

Nitric oxide (NO) is a neurotransmitter with a toxic effect, small molecules and a short half-life, which is soluble in lipids, and moves through cellular membranes easily and has a high reaction capacity [11,12]. NAC, when administered orally or intravenously, blocks the reabsorption of NO precursors, nitrate and nitrite in the kidneys and causes their excretion, lowering the NO levels in the blood [13]. NAC also inhibits the inducible nitric oxide synthase (iNOS) activity, which is one of the enzymes that provides for NO production [14].

Serum GGT (GGT; EC 2.3.2.2) activity increases as a result of liver damage or when the immune system is activated [15]. The main function of GGT is to hydrolyze gamma glutamyl peptide bonds, transferring them to a receptor. The most important substrate of this reaction is the glutathione. Another significant function of the GGT is to protect the intracellular glutathione levels and to control the NO production from GSNO (S-Nitrosoglutathione) [16].

Based on these findings, it was aimed to investigate the

effect of NAC on TAC, TOC, NO, albumin, globulin, glucose levels and GGT activity.

MATERIAL and METHODS

Eleven New Zealand Rabbits (Laboratory Animal Unit of the University of Kafkas, Kars, Turkey) of both sexes, aged between 7 and 9 months were used. Before the experimental procedure, consent for the study was taken from Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK: 2012-65). The body weight was 2.200-3.000 g. They were kept in cages at room temperature (22-25°C) with a 12:12 h light: dark cycle and fed a special pelleted rabbit diet as *ad libitum*. Before the experiment, blood samples were collected to obtain zero hours values. Then, animals were injected with single intramuscular dose of NAC, 100 mg/kg body weight (Asist®, Hüsnü Arslan İlaçları A.S, Istanbul, Turkey). Blood samples were taken from the marginal ear vein at 3rd, 6th and 9th h after injection into heparin treated tubes. Plasma was obtained by centrifugation at 3.000 rpm for 10 min and stored at -25°C until analyses.

Biochemical Analyses

All analyses were determined via spectrophotometer (PowerWave XS, BioTek, Instruments, USA)

Determination of total antioxidant and oxidant capacity: Total antioxidant capacities were determined colorimetrically using commercial kit (Rel Assay®, Gaziantep, Turkey) in plasma samples. Antioxidants in the sample reduce dark blue-green colored 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. Plasma total oxidant capacities were determined with commercial kit (Rel Assay®, Gaziantep, Turkey). Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are present in reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity is related to the total oxidant molecules present in the sample at 530 nm. Trolox and hydrogen peroxide standards were used for total antioxidant and total oxidant capacities [9,10].

Determination of nitric oxide levels in plasma: Nitric oxide concentrations were determined with chemical method in plasma samples. Plasma samples were deproteinized with 10% zinc sulphate. Total NO (nitrate and nitrite) concentrations were determined colorimetrically by the acidic Griess reaction [17].

Determination of other biochemical parameters in plasma: Gamma glutamyl transpeptidase (GGT) activity, total protein, albumin and glucose levels in plasma were determined commercial kits via colorimetrically

(TML®, Ankara, Turkey). The globulin concentration was calculated by subtraction of the albumin value from the total protein value.

Statistical Analysis

The data for biochemical parameters were analyzed by ANOVA followed by post hoc Tukey test using SPSS Windows 10.0. All data were presented as mean \pm SE. Values were considered statistically significant if P value was less than 0.05.

RESULTS

After NAC injection, total antioxidant level was found higher at 6th and 9th h ($P < 0.05$), total oxidant capacity level was found lower at 3rd, 6th, and 9th h ($P < 0.05$) when compared to pre-experiment measurements. Nitric oxide levels were found statistically higher at 6th and 9th h when compared to pre-experimental measurements and the 3rd h of the experiment ($P < 0.001$). GGT activity identified in the study was statistically lower only 3rd and 6th h after NAC injection than before experiment ($P < 0.05$). Total protein and albumin levels found in the samples were lower on the 3rd h of the experiment compared to the pre-experiment levels ($P < 0.05$). No statistically significant variations were observed in globulin and glucose levels during the experiment (Table 1).

DISCUSSION

N-Acetylcysteine is reported as a molecule with antioxidant action due to the free thiol groups that exist in its easy cell-permeable structure and its role in reduced glutathione synthesis [18,19]. Effects of NAC on several toxic substances and disease conditions were studied [19-22]. However, there are not sufficient number of studies on acute effects of NAC on the antioxidant system and biomolecules without the existence of a disorder. Thus, it was

aimed to research the effects of NAC on antioxidant system and certain biochemical parameters in healthy rabbits and the duration of these actions in the present study.

In this study, intramuscular application of 100 mg/kg NAC demonstrated its effects generally after 3 h of application on most measured plasma parameters. It resulted in a statistical increase in TAC level after 3 h of the application and the increase lasted until the end of the experiment. In fact, the studies demonstrated that NAC displayed antioxidant properties and was effective. It was reported that the antioxidant action of NAC was through increasing liver blood flow, GSH levels and by removing free oxygen radicals [23,24].

In the present study, it was determined that there was a statistically significant decrease in TOC levels 3 hours after NAC application. This could be due to reactive oxygen type removal of NAC [25,26]. Bioactive aldehydes such as malondialdehyde (MDA) and hydroxy alkenal which are produced as a result of oxidation of lipid acids create cellular damage and cause an increase in oxidant molecule levels. Measurement of MDA levels is an important indicator to identify the cellular oxidant system when TOC level could not be determined. Altinoz et al. [27] investigated the effect of NAC against acrylamide toxicity in rats, it was reported that MDA levels increased as a result of acrylamide toxicity and MDA levels were reduced with NAC application [27]. Similarly, in a study studying the effect of NAC on myocardial ischemia-reperfusion damage, it was found that NAC reduced the MDA levels [28]. In another study, it was observed that lung and kidney MDA levels were lower in NAC administered group [29]. Results of the previous studies demonstrated that NAC caused reduction in MDA level, which is a product of lipid peroxidation and an indicator of oxidant capacity.

Khanna et al. [22] investigated the effect of NAC in cadmium toxicity on Leydig cells isolated from a 28 days old male mouse. In that study, it was argued that NAC

Table 1. Plasma TAC, TOC, NO, albumin, total protein, globuline, glucose levels and GGT activity in N-acetylcysteine given rabbit

Tablo 1. N-asetil sistein verilen tavşanlarda plazma TAK, TOK, NO, total protein, globulin ve glukoz düzeyleri ile GGT aktivitesi

Parameters	Before Experiment	After Experiment Hours			P
		3 rd	6 th	9 th	
TAC (mmol Trolox Eq/L)	0.4 \pm 0.03 ^b	0.5 \pm 0.04 ^{ab}	0.6 \pm 0.7 ^a	0.6 \pm 0.2 ^a	0.05
TOC (μ mol H ₂ O ₂ Eq/L)	7.6 \pm 0.3 ^a	6.7 \pm 0.3 ^b	6.5 \pm 0.3 ^b	6.4 \pm 0.3 ^b	0.05
NO (mikromol/L)	29.1 \pm 2.9 ^a	25.3 \pm 2.7 ^a	13.4 \pm 1.2 ^b	18.0 \pm 2.0 ^b	0.001
Albumin (mg/dL)	3.8 \pm 0.04 ^a	3.5 \pm 0.08 ^b	3.6 \pm 0.06 ^b	3.7 \pm 0.03 ^{ab}	0.05
Total Proteine (mg/dL)	6.0 \pm 0.10 ^a	5.5 \pm 0.1 ^b	5.4 \pm 0.08 ^b	5.5 \pm 0.1 ^b	0.05
Globuline (mg/dL)	2.2 \pm 0.1	1.9 \pm 0.08	1.9 \pm 0.1	1.9 \pm 0.2	Ns
Albumine/Globuline	1.8 \pm 0.1	1.9 \pm 0.1	2.0 \pm 0.1	2.2 \pm 0.3	Ns
Glucose (mg/dL)	90.1 \pm 1.3	89.6 \pm 1.8	91.7 \pm 1.9	91.7 \pm 1.5	Ns
Gamma Glutamyl transpeptidase (U/L)	9.5 \pm 0.3 ^a	7.2 \pm 0.5 ^b	7.7 \pm 0.5 ^b	8.2 \pm 0.5 ^{ab}	0.05

^{a,b} The groups in the same line labeled different letters are statistically significant ($P < 0.05$, $P < 0.001$), Ns: Non significant

application lowered cadmium induced oxidative stress in Leydig cell, decreased the formation of oxidant molecules and could be used as a potential protective agent.

In fact, the findings of the present study demonstrated that NAC rapidly decreased the oxidant capacity. The decrease in TOC level could be explained by two mechanisms. One could be associated with the antioxidant effect of NAC due to the sulfhydryl groups in its structure, the other could be the increase in antioxidant capacity induced by the increase in reduced glutathione synthesis.

A paramagnetic free radical, NO, is formed as a result of oxidation of L-arginine citrulline by nitric oxide synthase enzyme. After synthesis and its action, NO is rapidly neutralized by haemoglobin or superoxide anion and transformed into nitrites or nitrates^[30]. A statistical decrease in NO levels 6 hours after the experiment was identified in the present study. It was reported that intravenous NAC application reduced nitrate and nitrite excretion from kidneys both in healthy individuals and individuals who had heart surgery, decreasing bioavailability of NO. It was asserted that application of NAC and its metabolites (cysteine and glutathione) inhibits reabsorption of nitrite and nitrate by possibly inhibiting renal carbonic anhydrase (CA) activity or inorganic anion carriers such as bicarbonate, which provides alkalinity in urine^[13]. In the present study, NAC application lowered nitric oxide levels in the plasma. One reason for that could be the inhibition of the reabsorption of NO metabolites nitrate and nitrite in urine. It was also argued that the effect of NAC on NO could be via removal of superoxide anions and inhibition of NO synthase activity^[31]. In a study by Lee et al.^[29] conducted to examine the effect of NAC to reduce organ injuries in Sprague-Dawley rats in a series of empirical and clinical studies, it was demonstrated that NO levels were significantly lower in the NAC administered group. This result clearly demonstrates the effect of NAC on NO metabolism. In a study that investigated the effect of NAC on cyclophosphamide-induced cardiotoxicity in rats, it was recorded that NAC application prevented oxidative and nitrosative stress and promoted the protection of antioxidant enzyme activity^[32]. The changes observed in TAC, TOC, and NO levels in samples after NAC injection were generally similar with previous studies^[23,24,28,31].

In the present study, a statistical decrease was found in albumin levels after 3 and 6 h after the experiment but on the 9th h albumin level reached to normal limits. Similarly, a statistical decrease was identified in total protein levels 3 h after NAC injection. Findings of the present study differed from the results shown in other studies. In a study that investigated the effects of ursedeoxycholic acid, resveratrol and NAC in nonalcoholic hepatic lipodosis in rats, it was reported that joint administration of resveratrol and NAC brought glucose, albumin, MDA, GSH, triglyceride, low density lipoprotein and leptin levels back to normal^[33].

In the current study, following the NAC injection, no variations were observed in globulin and glucose levels when compared to pre-experiment measurements.

Gammaglutamyl trans peptidase is a ectopeptidase responsible for the degradation of glutathione in the gammaglutamyl cycle^[34]. In the present study, it was determined that GGT activity started to decrease 3 h after the experiment and reached normal levels on the 9th hour. This shows that NAC could be a key enzyme in the synthesis of glutathione and GGT.

Glutathione biosynthesis via gammaglutamyl cycle is important for maintaining GSH homeostasis and normal redox status. GGT also initiates the metabolism of glutathione S-conjugates to mercapturic acids by transferring the gamma-glutamyl moiety to an acceptor amino acid and releasing cysteinylglycine^[35]. If GGT activity is above normal, GSH is destroyed by GGT enzyme and this situation is extremely important for the antioxidant defense system. In fact, the decrease in GGT activity as a result of the injection was consistent with the information mentioned above.

As a result, after intramuscular injection of NAC, plasma TAC levels of rabbits increased, while TOC and NO levels decreased. According to the results, it can be concluded that NAC may show antioxidant effect in a short time. Therefore NAC could be a good alternative when the antioxidant system needs to be strengthened due to aforementioned properties.

REFERENCES

- Schermer T, Chavannes N, Dekhuijzen R, Wouters, E, Muris, J, Akkermans R, van Schayck O, van Weel C:** Fluticasone and N-acetylcysteine in primary care patients with COPD or chronic bronchitis. *Resp Med*, 103, 542-551, 2009. DOI: 10.1016/j.rmed.2008.11.003
- Adamy C, Mulder P, Khouzami L, Andrieu-Abadie N, Defer N, Candiani G, Pavoine C, Caramelle P, Souktani R, Le Corvoisier P, Perier M, Kirsch M, Damy T, Berdeaux A, Levade T, Thuillez C, Hittinger L, Pecker F:** Neutral sphingomyelinase inhibition participates to the benefits of N-acetylcysteine treatment in post-myocardial infarction failing heart rats. *J Mol Cell Cardiol*, 43, 344-353, 2007. DOI: 10.1016/j.yjmcc.2007.06.010
- Lasram MM, Lamine AJ, Dhoubi IB, Bouzid K, Annabi A, Belhadjmidia N, Ben Ahmed M, El Fazaa S, Abdelmoula J, Gharbi N:** Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats. *Life Sci*, 107, 50-58, 2014. DOI: 10.1016/j.lfs.2014.04.033
- Galley HF, Howdle PD, Walker BE, Webster NR:** The effects of intravenous antioxidants in patients with septic shock. *Free Radical Bio Med*, 23, 768-774, 1997. DOI: 10.1016/S0891-5849(97)00059-2
- Aruoma OI, Halliwell B, Hoey BM, Butler J:** The Antioxidant Action of N-Acetylcysteine - Its Reaction with Hydrogen-Peroxide, Hydroxyl Radical, Superoxide, and Hypochlorous Acid. *Free Radical Bio Med*, 6, 593-597, 1989. DOI: 10.1016/0891-5849(89)90066-X
- Skrzydłowska E, Farbiszewski R:** Protective effect of N-acetylcysteine on reduced glutathione, reduced glutathione-related enzymes and lipid peroxidation in methanol intoxication. *Drug Alcohol Depen*, 57, 61-67, 1999. DOI: 10.1016/S0376-8716(99)00040-X
- Moldeus P, Cotgreave IA, Berggren M:** Lung protection by a thiol-containing antioxidant: N-acetylcysteine. *Respiration*, 50, 31-42, 1986. DOI: 10.1159/000195086

8. **Schmitt B, Vicenzi M, Garrel C, Denis FM:** Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. *Redox Biol*, 6, 198-205, 2015. DOI: 10.1016/j.redox.2015.07.012
9. **Erel O:** A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem*, 37, 112-119, 2004. DOI: 10.1016/j.clinbiochem.2003.10.014
10. **Erel O:** A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*, 38, 1103-1111, 2005. DOI: 10.1016/j.clinbiochem.2005.08.008
11. **Garthwaite J:** Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci*, 14, 60-67, 1991. DOI: 10.1016/0166-2236(91)90022-M
12. **Wang PG, Xian M, Tang X, Wu X, Wen Z, Cai T, Janczuk AJ:** Nitric oxide donors: chemical activities and biological applications. *Chem Rev*, 102, 1091-1134, 2002. DOI: 10.1021/cr000040l
13. **Tsikis D, Niemann J, Flentje M, Schwarz A, Tossios P:** N-Acetylcysteine (NAC) inhibits renal nitrite and nitrate reabsorption in healthy subjects and in patients undergoing cardiac surgery: Risk of nitric oxide (NO) bioavailability loss by NAC? *Int J Cardiol*, 177, 30-33, 2014. DOI: 10.1016/j.ijcard.2014.09.109
14. **Bergamini S, Rota C, Canali R, Staffieri M, Daneri F, Bini A, Giovannini F, Tomasi A, Iannone A:** N-acetylcysteine inhibits *in vivo* nitric oxide production by inducible nitric oxide synthase. *Nitric Oxide*, 5, 349-360, 2001. DOI: 10.1006/niox.2001.0356
15. **Rocksen D, Lilliehook B, Larsson R, Johansson T, Bucht A:** Differential anti-inflammatory and anti-oxidative effects of dexamethasone and N-acetylcysteine in endotoxin-induced lung inflammation. *Clin Exp Immunol*, 122, 249-256, 2000. DOI: 10.1046/j.1365-2249.2000.01373.x
16. **Lushchak VI:** Glutathione homeostasis and functions: Potential targets for medical interventions. *J Amino Acids*, 2012, Article ID: 736837-26, 2012. DOI: 10.1155/2012/736837
17. **Miranda KM, Espey MG, Wink DA:** A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5, 62-71, 2001. DOI: 10.1006/niox.2000.0319
18. **Sener G, Tosun O, Sehirli AO, Kacmaz A, Arbak S, Ersoy Y, Ayanoglu-Dulger G:** Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. *Life Sci*, 72, 2707-2718, 2003. DOI: 10.1016/S0024-3205(03)00187-5
19. **Cenesiz S, Ciftci G, Gulbahar MY, Yarim GF, Nisbet HO, Kabak Y, Nisbet C:** Investigation of the effects of N-acetyl cysteine and resveratrol to acute phase response in rats with experimentally induced arthritis. *Kafkas Univ Vet Fak Derg*, 18, 991-996, 2012. DOI: 10.9775/kvfd.2012.6900
20. **Cotgreave IA:** N-acetylcysteine: Pharmacological considerations and experimental and clinical applications. *Adv Pharmacol*, 38, 205-227, 1997. DOI: 10.1016/S1054-3589(08)60985-0
21. **Berk M, Malhi GS, Gray LJ, Dean OM:** The promise of N-acetylcysteine in neuropsychiatry. *Trends Pharmacol Sci*, 34, 167-177, 2013. DOI: 10.1016/j.tips.2013.01.001
22. **Khanna S, Mitra S, Lakhera PC, Khandelwal S:** N-acetylcysteine effectively mitigates cadmium-induced oxidative damage and cell death in Leydig cells *in vitro*. *Drug Chem Toxicol*, 39, 74-80, 2016. DOI: 10.3109/01480545.2015.1028068
23. **Jones AL:** Mechanism of action and value of N-acetylcysteine in the treatment of early and late acetaminophen poisoning: A critical review. *J Toxicol*, 36, 277-285, 1998. DOI: 10.3109/15563659809028022
24. **Angulo P, Lindor KD:** Treatment of non-alcoholic steatohepatitis. *Best practice & research. Clin Gastroenterol*, 16, 797-810, 2002. DOI: 10.1053/bega.2002.0327
25. **Tsuji F, Miyake Y, Aono H, Kawashima Y, Mita S:** Effects of buccillamine and N-acetyl-L-cysteine on cytokine production and collagen-induced arthritis (CIA). *Clin Exp Immunol*, 115, 26-31, 1999. DOI: 10.1046/j.1365-2249.1999.00749.x
26. **Zhu J, Yin R, Shao H, Dong G, Luo L, Jing H:** N-acetylcysteine to ameliorate acute renal injury in a rat cardiopulmonary bypass model. *J Thorac Cardiovasc Surg*, 133, 696-703, 2007. DOI: 10.1016/j.jtcvs.2006.09.046
27. **Altinoz E, Turkoz Y, Vardi N:** The protective effect of N-acetylcysteine against acrylamide toxicity in liver and small and large intestine tissues. *Bratisl Lek Listy*, 116, 252-258, 2015. DOI: 10.4149/BLL_2015_049
28. **Bolcal C, Yildirim V, Doganci S, Sargin M, Aydin A, Kuralay E, Ozal E, Demirkilic U, Oz BS, Sayal A, Tatar H:** Do N-acetylcysteine, beta-glucan, and coenzyme Q10 mollify myocardial ischemia-reperfusion injury? *Heart Surg Forum*, 10, 222-227, 2007. DOI: 10.1532/HSF98.20061195
29. **Lee JH, Jo YH, Kim K, Lee JH, Rim KP, Kwon WY, Suh GJ, Rhee JE:** Effect of N-acetylcysteine (NAC) on acute lung injury and acute kidney injury in hemorrhagic shock. *Resuscitation*, 84, 121-127, 2013. DOI: 10.1016/j.resuscitation.2012.05.017
30. **Groves JT, Wang CC:** Nitric oxide synthase: models and mechanisms. *Curr Opin Chem Biol*, 4, 687-695, 2000. DOI: 10.1016/S1367-5931(00)00146-0
31. **Boga M, Discigil B, Ozkisacik EA, Guncun U, Badak MI, Dikicioglu E, Yenisey C, Meteoglu I:** The combined effect of iloprost and N-acetylcysteine in preventing spinal cord ischemia in rabbits. *Eur J Vasc Endovasc Surg*, 31, 366-372, 2006. DOI: 10.1016/j.ejvs.2005.10.027
32. **Mansour HH, Hafez HF, Fahmy NM, Hanafi N:** Protective effect of N-acetylcysteine against radiation induced DNA damage and hepatic toxicity in rats. *Bioch Pharmacol*, 75, 773-780, 2008. DOI: 10.1016/j.bcp.2007.09.018
33. **Ali MH, Messiha BA, Abdel-Latif HA:** Protective effect of ursodeoxycholic acid, resveratrol, and N-acetylcysteine on nonalcoholic fatty liver disease in rats. *Pharm Biol*, 1, 1-11, 2015. DOI: 10.3109/13880209.2015.1060247
34. **Levasseur R, Barrios R, Elefteriou F, Glass DA, Lieberman MW, Karsenty G:** Reversible skeletal abnormalities in gamma-glutamyl transpeptidase-deficient mice. *Endocrinology*, 144, 2761-2764, 2003. DOI: 10.1210/en.2002-0071
35. **Zhang H, Forman HJ, Choi J:** Gamma-glutamyl transpeptidase in glutathione biosynthesis. *Methods Enzymol*, 401, 468-483, 2005. DOI: 10.1016/S0076-6879(05)01028-1