Original Article

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Effects of Low-level Occupational Exposure to Ammonia on Hematological Parameters and Kidney Function

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Abstract

Background: Many workers, particularly those working in manufacture of fertilizers, explosives, rubber, pesticides, textiles, and employees of petrochemical industries are exposed to ammonia in their workplaces. Toxic responses of hematopoietic system and kidney following occupational exposure to this chemical have not been thoroughly investigated.

Objective: To determine the relationship between long-term occupational exposure to low levels of ammonia and hematological parameters and kidney function.

Methods: In this cross-sectional study, 119 randomly selected, male petrochemical workers and 131 office employees (comparison group) were examined. Urine and blood samples were taken from all participants for urinalysis, complete blood count (CBC), serum calcium level, and blood urea nitrogen (BUN) and plasma creatinine. Personal, environmental, and peak ammonia exposure were also measured.

Results: The median personal, environmental, and peak occupational exposure to ammonia were 0.23, 0.16, and 65.50 mg/m^3, respectively, among the exposed group. No significant difference was observed between the exposed and unexposed participants in terms of hematological parameters and urinalysis. Conversely, calcium and BUN, while within the normal range, were significantly higher in the exposed than in the comparison group.

Conclusion: Occupational exposure to low atmospheric concentrations of ammonia was associated with subtle, sub-clinical, pre-pathologic changes in kidney function. Possible long-term consequences and ramifications of these effects require further investigation.

Keywords: Ammonia; Hematology; Kidney diseases; Occupational exposure

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Introduction

Ammonia is a colorless, water-soluble, irritant gas, with a pungent, suffocating odor.[1] Global production of ammonia in 2017 was about 174 million tons. Major producers were China, India, Russia, and the USA.[2] Iran, as the 14th ammonia producer in the global market, is the manufacturer of 2% of the total world production, dominantly in two petrochemical complexes.[3]

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Ammonia is widely used in producing fertilizers (about 80%), explosives, rubber, pesticides, textile, dye, some household cleaning products, *etc.*[1,2] Exposure to ammonia may take place in different occupations. Loftus, *et al*, reported that workers of an agricultural community might be exposed to a concentration of 0.2–238.1 μg/m^3 of ammonia during their routine works.[4] Fedoruk, *et al*, observed that during surface cleaning works, airborne concentration of ammonia could reach up to 9.04 mg/m^3. Additionally, the peak exposure to ammonia may exceed 86.9 mg/m^3 when a strong cleansing agent containing 3% ammonia is applied.[5] Khan, *et al*,[6] estimated the maximum daily ground-level concentration of ammonia in a petrochemical industry at 67 μg/m^3. In a urea fertilizer factory, Rahman, *et al*,[7] measured a mean exposure level of 18.77 mg/m^3 for ammonia, exceeding the threshold limit value (TLV) of 17.38 mg/m^3 for this compound set by the American Conference of Governmental Industrial Hygienists (ACGIH).[8]

Epidemiological and experimental studies suggest a range of adverse effects reported following exposure to ammonia, mostly those related to respiratory and dermal problems, but rarely on hematotoxic and nephrotoxic potentials of ammonia.[9-11] *Neghab*, *et al*, suggest that low-level occupational ammonia exposure is associated with chronic irreversible and acute reversible decrements in lungs' functional capacity.[9] In another study, signs of neutrophilic airway inflammation and elevated C reactive protein levels were observed among hairdressers who had occupational exposure to high concentrations (3–61 mg/m^3) of ammonia.[12] Inflammatory reactions, as well as cell apoptosis and DNA damage, have also been reported in animal studies.[13] Renal tubular calcification and renal tubular epithelial cell proliferation, as a result of exposure to high concentrations of ammonia, have been reported in experimental animals.[14]

To the best of our knowledge, possible long-term effects of occupational exposure to low levels of ammonia on hematopoietic system, kidney function, and serum calcium levels have so far not been investigated. We therefore conducted this study to address these issues.

Materials and Methods

Participants

The current cross-sectional study was conducted in the largest ammonia production center of Iran, a petrochemical complex located in Assalouyeh, Bushehr, southern Iran. Using the census sampling method, 119 operational, repair and maintenance employees with a history of at least one year recent and ongoing exposure to ammonia were studied as the exposed group. A comparison group consisted of 131 office employees with no history of exposure to ammonia, was also included. The field workers and office employees were excluded if they had any past medical or family history of kidney or hematologic diseases. Additionally, they had no history of exposure to other toxic chemicals, were not drinkers, and did not regularly consume any drugs such as gentamicin, aspirin, cyclosporine, cimetidine, or non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin, ibuprofen, and diclofenac sodium, or other nephrotoxic drugs.[15]

The protocol of the study was approved by the university Ethics Committee. All participants signed an informed written consent form prior to the study.

Personal Data

The general characteristics of the participants including age (yrs), body mass index (BMI, kg/m^2), work experience (yrs, as a surrogate for the exposure), education

level (yrs), number of smokers/non-smokers (%), duration of being a smoker (yrs), number of cigarettes smoked per day, and marital status (%) were gathered by a data collection sheet.

Personal and Environmental Exposure Assessment

Overall, personal exposure assessment was carried out by collecting air samples from breathing zones of 45 workers in the autumn 2017, where they performed their routine daily tasks. In the meantime, 34 environmental samples were collected from contaminated points, where the exposed group was spending most of their daily work time (12 hrs). To assess the exposure of the comparison group, 10 personal samples were collected from their workplace. Ammonia was sampled and analyzed according to NIOSH Method 6016.[16]

Details of sampling procedures have been described elsewhere.[9] In brief, the interfering particles were removed using a cellulose ester membrane filter (pore size 0.8 µm, diameter 37 mm) (SKC Cat. No. 2253-01), placed in a special cassette. An adsorbent tube containing sulfuric acid-treated silica gel, (SKC Cat. No. 22610-06) was located in the circuit for absorption of ammonia. A personal sampling pump (SKC AirChek® XR5000) provided an air flow of 200 mL/min. The entire work shifts were sampled and at the end, ammonia samples were extracted by deionized water and analyzed by a chromatograph (Metrohm Switzerland, model 850).

A direct-reading instrument (Gas Alert Extreme, GAXT-A2-DL), with a dynamic range of 0 to 278 mg/m^3, was used to assess peak exposures when there was a leakage or a faulty system, resulting in a short-term high exposure situations among both operational, repair and maintenance employees who were responsible for handling such emergency situations.

The measured concentrations were compared to the adjusted TLV-TWA value by Brief and Scala model (reduction factor of 12 h: 0.5).[17] Therefore, the TLV-TWA and TLV-ceiling (TLV-C) were estimated at 8.69 and 24.34 mg/m^3, respectively.

Blood Sampling and Analysis

Fasting blood samples (10 mL) were collected from the participants by a medical expert, taken from the antecubital vein between 7:00 and 9:00 am. Simultaneously, urine samples were collected on site.[18] Each sample was transferred in two containers—a sterile tube containing ethylene-diamine-tetra-acetic acid (EDTA, as an anticoagulant agent) (5 mL), and a plain (5 mL) vacutainers to obtain serum samples for hematological and biochemical assays, respectively. The containers were then transferred to the laboratory in a cold box and processed within two hours of sample collection. A medical expert immediately centrifuged (400×g for 20 min) the plain vacutainer samples to separate serum. Samples were stored at -20 °C until analysis.[19]

Hematological Assays

A hematology cell counter (Nihon Kohden, Tokyo, Japan) was used to determine the hematological parameters including white blood cell count (WBC) and differential, red blood cell (RBC) count, hemoglobin (Hb) and hematocrit (HCT) levels, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW).

Biochemical Assays

An autoanalyzer (model BT 1500, Biotecnica Instrument Co, Rome, Italy) was used to determine the serum level of blood urea nitrogen (BUN), plasma creatinine, and serum calcium level.

Table 1: General characteristics of the exposed (n=119) and unexposed (n=131) groups. Values are either Mean (SD) or percentage.

Variable	Exposed group	Unexposed group	p value
Age (yrs)	33.4 (5.0)	35.7 (6.4)	0.002
BMI (kg/m^2)	25.8 (4.3)	26.3 (3.4)	0.29
Work experience (yrs)	7.9 (4.7)	7.6 (3.8)	0.62
Smoking (%)			
Yes	3.4	6.1	0.04
No	96.6	93.9	
Length of smoking (yrs)	3.8 (3.0)	17.4 (14.7)	0.06
Number of cigarettes smoked (per day)	3.3 (1.3)	6.3 (5.8)	0.20
Education (yrs)	13.3 (2.9)	14.4 (3.1)	0.19
Marital status			
Single	19.3	12.2	0.16
Married	80.7	87.8	

Urinalysis

Urine samples were analyzed macroscopically, using the dipstick colorimetric method (Convergys® Urine Matrix test strips, Convergent Technologies, Coelbe, Germany) to determine qualitative parameters including glucose, protein, blood, nitrite, and urobilinogen.[20] Additionally, the samples were examined by a specialist for WBC, RBC, epithelial cells and casts under a microscope (Carl Zeiss standard 20 450,807 Binocular Transmitted Light Microscope, Carl Zeiss AG, Germany) at 40× magnification. In the urine sediment, 10 high-power fields (HPF) were counted and the counts were given as an average per HPF. A cut-off point of >2 cells per HPF was considered "abnormal" for the number of average RBC and WBC in the urine.[21] One specialist on a double-blind basis carried out all tests, after calibration of the instruments by standard methods.

Statistical Analysis

Data were analyzed using SPSS® for Windows® ver 19. *Student's t* test for independent samples to compare means between two groups of normally distributed data. Pearson χ^2 test or Fisher's exact test, where applicable, was used for comparison of frequency based data between the exposed and unexposed groups. Multiple linear regression was used to control for confounding variables. A p value <0.05 was considered statically significant.

Results

No statistically significant difference was observed between the exposed and unexposed groups in terms of studied demographic variables, except for age (p=0.002) and the prevalence of smokers (p=0.04) (Table 1). The exposure levels of the exposed and unexposed groups were far less than the adjusted TLV-TWA. The median environmental and peak ammonia exposure levels were 0.16 (IQR 0.02 to 0.48) and 65.50 (35.00 to 110.00) mg/m^3, respectively. The personal exposure level

Table 2: Mean (SD) hematological parameters in exposed (n=119) and unexposed (n=131) groups

Parameters	Exposed group	Unexposed group	p value	Normal range
WBC (×10³/μL)	6.74 (1.32)	6.95 (1.67)	0.44	4 to 11
Lymphocyte (%)	41.14 (8.53)	40.40 (7.60)	0.58	19 to 48
Monocyte (%)	3.23 (1.37)	3.20 (1.97)	0.92	1 to 7
Granulocyte (%)	55.60 (8.16)	56.37 (8.00)	0.57	40 to 74
RBC (×10⁶/μL)	5.01 (0.35)	4.97 (0.47)	0.66	4.5 to 4.9
Hb (g/dL)	15.47 (0.81)	15.18 (1.20)	0.07	14 to 18
HCT (%)	44.69 (2.18)	43.91 (3.24)	0.13	39 to 53
MCV (fL)	88.78 (5.88)	88.51 (6.33)	0.81	80 to 96
MCH (pg)	31.00 (2.22)	30.74 (3.02)	0.60	27 to 34
MCHC (g/dL)	34.62 (0.96)	34.74 (1.63)	0.58	32 to 37
RDW (%)	12.10 (0.33)	12.08 (0.48)	0.85	5.5 to 11.14

was 0.23 (0.14 to 0.45) for the exposed group; it was not detectable for the unexposed group. However, the mean peak exposure exceeded the adjusted TLV-C. No statistically significant difference was observed between the exposed and unexposed groups in terms of hematological parameters (Table 2). Similar results obtained after adjusting for age, BMI, marital status, smoking, work experience, and education.

The mean BUN and calcium levels were significantly ($p<0.03$) higher in the exposed compared with the unexposed group (Table 3). Nonetheless, in both groups, the mean values of all the measured variables were within the normal range. After adjusting for important confounders, a significant positive correlation was found between exposure to ammonia and BUN level (Table 4). Urinalysis results in the two study groups were not significantly different.

Discussion

The median personal (0.23 mg/m³) and environmental (0.16 mg/m³) exposure to ammonia did not exceed the adjusted TLV-TWA; the median peak exposures (65.50 mg/m³), however, exceeded the TLV-C. In general, the atmospheric concentrations of ammonia recorded in this study were lower than those reported in similar studies.

Table 3: Mean (SD) kidney function indicators and serum calcium in exposed (n=119) and unexposed (n=131) groups

Parameters	Exposed group	Unexposed group	p value	Normal range
BUN (mg/dL)	20.1 (4.4)	18.2 (4.9)	0.002	8 to 23
Creatinine (mg/dL)	1.1 (0.2)	1.2 (0.2)	0.08	0.9 to 1.6
Calcium (mg/dL)	9.8 (0.5)	9.7 (0.4)	0.03	8.6 to 10.6

Some researchers reported that about one-third of the samples collected from the air of urea fertilizer units contained more than 17.38 mg/m^3 of ammonia.[7,22] Use of new machinery, on time repair, proper maintenance, leakage control, and institution of engineering measures such as installing effective ventilation systems in the plant investigated in this study, might explain the reasons for this discrepancy.

No significant difference was noted between the exposed and unexposed participants in terms of hematological factors. Although the effects of *in vivo* administration of sub-lethal or lethal doses of ammonia on the hematopoietic system have been investigated by some researchers, possible effects of chronic low-level occupational exposure to ammonia on this organ have so far not been examined. Doig, *et al*, reported that acute exposure of swine to ammonia was not associated with significant hematological changes.[23] Similarly, Gustin, *et al*, reported that exposure of pigs to airborne concentrations of ammonia of 17.38, 34.76, and 69.53 mg/m^3 for six days had no significant effect on the differential leukocyte percentages and total WBC count, suggesting the absence of a stress, related to ammonia. Our findings were in line and consistent with these findings on hematological parameters. The mean BUN and serum calcium levels, although within the normal range, were significantly higher in the exposed workers than in their unexposed counterparts.

Association between exposure to ammonia and changes in the parameters of kidney function and serum calcium was further investigated by linear regression analysis. After adjusting for important confounders, only a significant positive correlation was found between exposure to ammonia and BUN level (Table 4). An average increase of 1.59 mg/dL was noted following 7.87 years of continuous exposure to ammonia. This increase could be viewed as a trivial, early, sub-clinical, pre-pathologic change. However, if in an optimistic estimation, we assume a linear trend for BUN increments over time, one would expect another 6.1 mg/dL of increase in BUN over the remaining work life of the employees; the mean BUN would then become a pathologic level of 26.2 mg/dL, exceeding the upper normal limit of 23 mg/dL.[24,25]

Table 4: Results of multiple linear regression after adjusting for age, BMI, marital status, smoking, work experience, and education

Parameters	Coefficient (95% CI)
BUN (mg/dL)	1.59 (0.43 to 2.75)
Creatinine (mg/dL)	-0.02 (-0.07 to 0.03)
Calcium (mg/dL)	0.11 (-0.01 to 0.22)

This situation would be further compounded by two additional factors—decreased body's ability to protect itself from the adverse effects of xenobiotics as a result of aging,[26] and structural changes in the kidney associated with aging making people more susceptible to toxic water-soluble xenobiotics.[27]

Inhalational absorption of ammonia could be the origin of elevated BUN since the inhaled ammonia is metabolized to urea in the liver.[28] In line with our obser-

TAKE-HOME MESSAGE

- Human biological responses to long-term occupational exposure to ammonia have not been thoroughly investigated.
- Exposure to ammonia did not exceed the adjusted TLV-TWA, although the mean value for peak exposures exceeded the TLV-C.
- No hematotoxic responses were observed.
- Calcium and BUN, while within the normal range, were significantly higher in the exposed than in unexposed group.

vations, Satpute, *et al*, reported that subacute exposure of rats to ammonium acetate is associated with renal necrosis and tubular degeneration.[29] Similarly, Rabkin, *et al*, noted that exposure to ammonia alters renal tubular cell growth and protein turnover in rabbits. These changes were noted when the proliferation of the primary proximal tubular epithelial cells was inhibited.[30]

A recent critical review on the toxicity of ammonia has provided evidence that ammonia can disturb many organs and cell types leading to dysfunction. Additionally, it concluded that hyperammonemia does lead to impaired kidney function and kidney injury.[31] High-protein diet and dehydration have been shown to affect BUN levels.[32,33] Additionally, in warm environment, dehydration might be a common physiological response leading to reabsorption of urea from kidney and elevated BUN.[34] Therefore, it may be argued that the elevated BUN levels observed in this study were not necessarily work-related; they would be the result of differences between the exposed and unexposed subjects in terms of other factors unstudied. While true, the following lines of circumstantial evidence indicate that the observed effect may well be attributed to exposure: Although the industry was located in a hot and humid place, the study was conducted during a cold season (fall), ruling out the effects of hot weather; the exposed and unexposed employees consumed a similar diet provided by the industry; after adjusting for potential confounders, a significant positive association was noted between the BUN level and exposure to ammonia, implying that the observed effect was work-related; the exposed and unexposed subjects are believed to have had similar baseline BUN levels at the commencement of their employment, as healthy young individuals with similar socioeconomic status; subjects were free from any pre-existing medical conditions, particularly, renal diseases and did not take any nephrotoxic drugs and had no exposure to nephrotoxic chemicals; the unexposed participants were significantly older and had a higher prevalence of smokers than the exposed individuals. Therefore, if the elevated BUN level was not work-related (due to ammonia exposure), one would expect that the level to be significantly higher in the control group than in the exposed subjects.

Macroscopic and microscopic analyses of urine parameters did not show any significant differences between the exposed and unexposed groups. Although given the low concentrations of ammonia, overt toxicity is not expected, the investigators did not find any human or animal studies in which urine parameters had been evaluated in response to low-level ammonia exposure.

The mean serum calcium was significantly higher in cases than in the comparison group (Table 3). Although raised serum calcium levels, as a result of exposure to chemicals, is not a peculiar finding and chemicals such as aluminum, lithium, potassium, vitamin A, vitamin D, thiazides, and antacid are known to increase the calcium level,[35] in multivariate analysis (Table 4), we found that the increase in the calcium level observed in our study was due to the effect of potential confounders; when the effect of confounding variables was controlled, the statistical difference between mean calcium levels of the exposed and unexposed employees vanished.

In conclusion, the findings of the present study showed that occupational exposure to low airborne concentrations of ammonia was associated with subtle, subclinical, pre-pathologic changes in kidney function. Possible long-term pathologic consequences and ramifications of these effects deserve further investigations.

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Conflicts of Interest: None declared.

Reference

1. Dalefield R. Chapter 20 - Smoke and Other Inhaled Toxicants. In: Dalefield R, ed. *Veterinary Toxicology for Australia and New Zealand*. Oxford, Elsevier, **2017**:361-72.
2. International Fertilizer Association (IFA). Short-Term Fertilizer Outlook 2017 – 2018: Production & International Trade and Agriculture Services. Zürich (Switzerland): IFA, **2017**:5-6.
3. Public Relations Department of National Petrochemical Company. Current situation and prospects of the world market for ammonia and urea: I.R. Iran. Tehran: National Iranian Petrochemical Company, **2017**.
4. Loftus C, Yost M, Sampson P, *et al.* Ambient Ammonia Exposures in an Agricultural Community and Pediatric Asthma Morbidity. *Epidemiology* 2015;**26**:794-801.
5. Fedoruk MJ, Bronstein R, Kerger BD. Ammonia exposure and hazard assessment for selected household cleaning product uses. *J Expo Anal Environ Epidemiol* 2005;**15**:534-44.
6. Khan AR, Al-Awadi L, Al-Rashidi MS. Control of ammonia and urea emissions from urea manufacturing facilities of Petrochemical Industries Company (PIC), Kuwait. *Journal of the Air & Waste Management Association* 2016;**66**:609-18.
7. Rahman MDH, Bråtveit M, Moen BE. Exposure to Ammonia and Acute Respiratory Effects in a Urea Fertilizer Factory. *Int J Occup Environ Health* 2007;**13**:153-9.
8. American Conference of Governmental Industrial Hygienists (ACGIH). TLVs and BEIs. kemper meadow drive , Cincinnati, OH, **2015**:124-6.
9. Neghab M, Mirzaei A, Kargar shouroki F, *et al.* Ventilatory disorders associated with occupational inhalation exposure to nitrogen trihydride (ammonia). *Ind Health* 2018;**56**:427-35.
10. Perkins MW, Wong B, Tressler J, *et al.* Adverse respiratory effects in rats following inhalation exposure to ammonia: respiratory dynamics and histopathology. *Inhal Toxicol* 2017;**29**:32-41.
11. Gustin P, Urbain B, Prouvost JF, Ansay M. Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: interaction with endotoxins. *Toxicol Appl Pharmacol* 1994;**125**:17-26.
12. Nemer M, Sikkeland LIB, Kasem M, *et al.* Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study. *Occup Environ Med* 2015;**72**:428-34.
13. Cheng CH, Yang FF, Ling RZ, *et al.* Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (Takifugu obscurus). *Aquat Toxicol* 2015;**164**:61-71.
14. National Research Council, Committee on Acute Exposure Guideline Levels. Acute exposure guideline levels for selected airborne chemicals, National Academies Press, **2008**.
15. Winder C, Stacey NH. *Occupational Toxicology*. 2nd ed. Taylor & Francis, **2004**.
16. National Institute of Occupational Safety & Health. NIOSH Manual of Analytical Methods. Ammonia by IC 6016: NIOSH, **1996**.
17. Verma DK. Adjustment of occupational exposure limits for unusual work schedules. *AIHAJ* 2000;**61**:367-74.
18. Neghab M, Jalilian H, Taheri S, *et al.* Evaluation of hematological and biochemical parameters of pesticide retailers following occupational exposure to a mixture of pesticides. *Life Sci* 2018;**202**:182-7.
19. Jalilian H, Neghab M, Tatar M, Taheri S. Respiratory and Dermal Symptoms and Raised Serum Concentrations of Biomarkers of Oxidative Stress among Pesticide Retailers. *Int J Occup Environ Med* 2018;**9**:194-204.
20. Roberts JR. Urine Dipstick Testing: Everything You Need to Know. *Emergency Medicine News* 2007;29:24-7.
21. Lamchiagdhase P, Preechaborisutkul K, Lomsomboon P, *et al.* Urine sediment examination: A

comparison between the manual method and the iQ200 automated urine microscopy analyzer. *Clin Chim Acta* 2005;**358**:167-74.

22. Ali BA, Ahmed HO, Ballal SG, Albar AA. Pulmonary function of workers exposed to ammonia: a study in the Eastern Province of Saudi Arabia. *Int J Occup Environ Health* 2001;**7**:19-22.
23. Doig PA, Willoughby RA. Response of swine to atmospheric ammonia and organic dust. *J Am Vet Med Assoc* 1971;**159**:1353-61.
24. Aono T, Matsubayashi K, Kawamoto A*, et al.* [Normal ranges of blood urea nitrogen and serum creatinine levels in the community-dwelling elderly subjects aged 70 years or over--correlation between age and renal function]. *Nihon Ronen Igakkai Zasshi* 1994;**31**:232-6. [in Japanese]
25. Kim H, Lee S, Choue R. Metabolic responses to high protein diet in Korean elite bodybuilders with high-intensity resistance exercise. *J Int Soc Sports Nutr* 2011;**8**:10.
26. Risher JF, Todd GD, Meyer D, Zunker CL. The elderly as a sensitive population in environmental exposures: making the case. *Rev Environ Contam Toxicol* 2010;**207**:95-157.
27. Musso CG, Oreopoulos DG. Aging and physiological changes of the kidneys including changes in glomerular filtration rate. *Nephron Physiol* 2011;**119** Suppl 1:p1-5.
28. Murray RK, Granner DK, Mayes PA, Rodwell VW. *Harper's Illustrated Biochemistry*. McGraw-Hill, **2014**.
29. Satpute R, Lomash V, Hariharakrishnan J*, et al.* Oxidative stress and tissue pathology caused by subacute exposure to ammonium acetate in rats and their response to treatments with alpha-keto-glutarate and N-acetyl cysteine. *Toxicol Ind Health* 2014;**30**:12-24.
30. Rabkin R, Palathumpat M, Tsao T. Ammonium chloride alters renal tubular cell growth and protein turnover. *Lab Invest* 1993;**68**:427-38.
31. Dasarathy S, Mookerjee RP, Rackayova V*, et al.* Ammonia toxicity: from head to toe? *Metab Brain Dis* 2017;**32**:529-38.
32. Kim H, Lee S, Choue R. Metabolic responses to high protein diet in Korean elite bodybuilders with high-intensity resistance exercise. *J Int Soc Sports Nutr* 2011;**8**:10.
33. Jujo K, Minami Y, Haruki S*, et al.* Persistent high blood urea nitrogen level is associated with increased risk of cardiovascular events in patients with acute heart failure. *ESC Heart Fail* 2017;**4**:545-53.
34. Mashiko T, Umeda T, Nakaji S, Sugawara K. Effects of exercise on the physical condition of college rugby players during summer training camp. *Br J Sports Med* 2004;**38**:186-90.
35. Delaney K, Ling L, Erickson T, Ford M. *Clinical Toxicology*. Saunders, Philadelphia, **2001**.