Fatal Ethylene Glycol Intoxication Secondary to Accidental Ingestion

CRISTINA FURNICA[1,2], ANTON KNIELING[1,2]*, SIMONA IRINA DAMIAN[1,2]*, MADALINA DIAC[2], SOFIA DAVID[1,2], DIANA BULGARU ILIESCU[1,2], ION SANDU[3,4], CATALIN JAN IOV[3]

[1] Grigore T. Popa University of Medicine and Pharmacy, 16 Universitatii Str., 700115, Iasi, Romania

[2] Institute of Forensic Medicine, 4 Bunavestire Str., 700455, Iasi, Romania

[3] Alexandru Ioan Cuza University of Iasi, ARHEOINVEST Interdisciplinary Platform, 22 Carol I Blvd., 700506, Iasi, Romania

[4] Romania Inventors Forum, 3 Sf. Petru Movila Str., 700089, Iasi, Romania

[5] Apollonia University of Iasi, 2 Muzicii Str. 700399, Iasi, Romania

Ethylene glycol intoxication is potentially fatal and associated with typical clinical, laboratory and histopathological findings. The authors present the case of a 57-year-old male with a history of chronic alcoholism and who accidentally ingested approximately 1 litter of antifreeze solution. The patient was discovered comatose in his house and addressed to the emergency department with a Glasgow coma score of 3, severe metabolic acidosis, acute renal failure, atrial fibrillation and liver dysfunction. Despite reanimation manoeuvres and haemodialysis for 2 h the patient deceased 5 h after hospital admission. Necropsy examination revealed a stomach with oedematous walls, mucosa erosions and signs of bleeding together with a disorganised, granular single kidney with unidentifiable corticomedullary border. Histopathological examination displayed typical findings in the kidney such as autolytic changes of the epithelium and abundant calcium oxalate crystals in the lumen of the proximal tubules. Ethylene glycol intoxication is frequent in our country and its metabolites glycoaldehyde, glycolic acid, glyoxylic acid and oxalic acid are responsible for the severe metabolic acidosis and formation of calcium oxalate crystals in various organs and leading to severe multiple organ dysfunction and death. Forensic pathologists should be aware of clinical and biological manifestations as well as of typical histopathological findings as ethylene glycol is commonly ingested accidentally or used in homicidal/autolytical attempts.

Keywords: ethylene glycol, intoxication, metabolic acidosis, calcium oxalate crystals, toxic metabolites

Ethylene glycol (ethane-1,2-diol) is an alcohol with the chemical structure CH2OH-CH2OH and a molecular weight of 62.7 g/mol. Its structure resembles ethyl alcohol, but with the addition of a hydroxyl group on each carbon [1]. It is a colourless, odourless and non-volatile liquid of wide industrial use as a synthetic intermediate, solvent component, de-icing solution, cleaner and antifreeze agent in association with methanol [2]. It is soluble in water and organic solvents, boils at 197°C and freezes at -40°C. Ethylene glycol is quickly absorbed second to oral ingestion (less than 30 min) and slower in case of inhalation. The toxic dose in adults is between 1 to 1.5 mL/kg (0.7 mL/kg in children) and the lethal dose of almost 100 mL in adults and 1.5 mL/kg in children [3]. Plasma concentrations >0.2 g/L are toxic, and > 2g/L are potentially fatal. It has a low affinity for plasma proteins due to its hydrosolubility, which favours a high rate of tissue distribution, mainly nervous. 80% of the absorbed ethylene glycol is metabolized in the liver with an elimination half-life of 3-8 h, which can be prolonged to 17 hours, by inhibiting the alcohol dehydrogenase (ADH). 20% is excreted unchanged by the kidneys with a half-time of 18-20 h [1, 3].

ADH is the fundamental enzyme of ethylene glycol metabolising pathway and the major determinant of its toxicity (fig. 1).

In the same time ADH is the therapeutic target in blocking ethylene glycol metabolising pathway by using ethylic alcohol as a powerful competitor for binding to the enzyme as its affinity is 67 times higher for ethanol compared to ethylene glycol [4]. The metabolic products of ethylene glycol are: glycoaldehyde, glycolic acid, glyoxylic acid, oxalic acid and calcium oxalate. The conversion of glycolic acid to glyoxylic acid constitutes the limiting step in the process. The accumulated glycolic acid

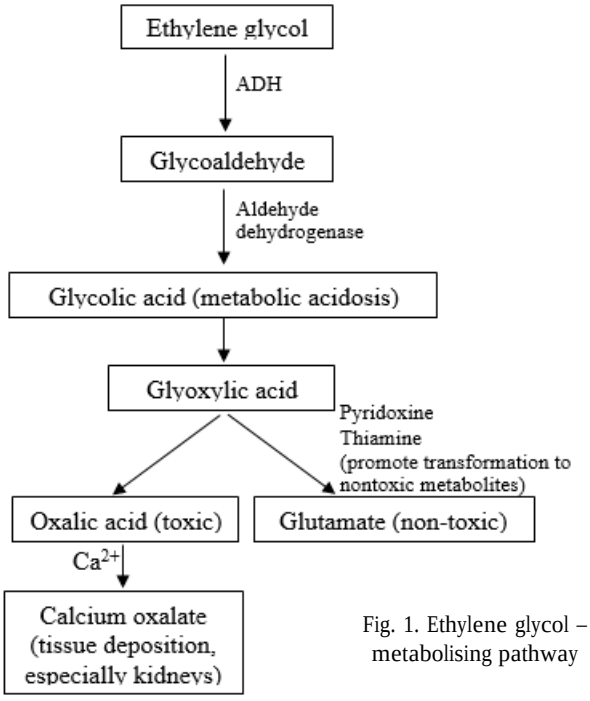

Fig. 1. Ethylene glycol – metabolising pathway

is an inducer of metabolic acidosis because of the high NAD (Nicotinamide adenine dinucleotide) ($C_{21}H_{27}N_7O_{14}P_2$) to NADH (nicotinamide adenine dinucleotide (NAD) + hydrogen (H)) ($C_{21}H_{29}N_7O_{14}P_2$) reduction ratio which causes the conversion of pyruvate to lactate [5, 6]. Glycoaldehyde, glyoxylate and its metabolite, oxalic acid, are the most toxic. The target organs in the ethylene glycol poisoning are: central and peripheral nervous system, kidneys, lung, heart, liver, muscles and retina. Crystals of

* *email: tony_knieling@yahoo.com; si_damian@yahoo.com*

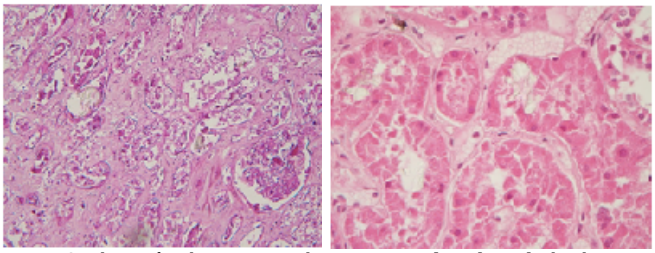

Fig. 2. Oxalate of calcium crystals precipitated in the tubular lumen (col HE 40X) (collection of IML Iassy)

calcium oxalate form in the blood and other tissues and their formation consumes calcium ions from the circulating blood leading to hypocalcemia. These crystals can precipitate in the tubular lumen of kidneys and lead to acute renal failure (ARF) [5, 7].

The experimental procedures were carried out in accordance with the mandatory principles of the Ethical Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi [8-14].

The aim of the current study is to present the clinical and biological manifestations and necropsy findings in a fatal case of ethylene glycol intoxication.

Experimental part

Case presentation

A 57-year-old male with a history of chronic alcoholism, grade II obesity, essential arterial hypertension and chronic kidney disease (single kidney secondary to surgery) was discovered unconscientious in his house by his caregivers and referred by the ambulance to the emergency department. The caregivers estimated that he ingested approximately 1 L of antifreeze solution. On admission, the patient was comatose (Glasgow coma score 3) with severe metabolic acidosis (*p*H 6.86, alkaline reserve 5 mmol/L), nitrate retention syndrome (creatinine 3.49 mg%), atrial fibrillation alternating with sinus rhythm (50 beats/min) and liver dysfunction (aspartate amino-transferase 112 U/L, alanine aminotransferase 93 U/L). Toxicological evaluation indicated metabolic coma possibly due to ethylene glycol intoxication. Urinary catheterisation, intubation and mechanical ventilation were performed and the patient was hospitalised in the Intensive Care Unit (ICU). Urine analysis revealed the presence of calcium oxalate crystals, a finding which corroborated with the severe metabolic acidosis is positive for ethylene glycol intoxication. In the ICU, a central catheter was inserted in the right jugular vein and emergency haemodialysis was initiated. The patient became unstable after 2 h of haemodialysis with systolic blood pressure 55-60 mmHg under inotropic support. Haemodialysis was interrupted 2h and 30 min after initiation secondary to haemodynamic instability. Immediately after the patient developed a first cardiac arrest and was resuscitated. A second cardiac arrest occurred 3 h after haemodialysis initiation and did not respond to resuscitation manoeuvres. The patient expired approximately 5 h after presentation.

Necropsy was performed and revealed a stomach with oedematous walls, mucosa erosions, signs of bleeding and traces of a yellowish liquid. The single left kidney presented excessive perirenal fat, a bosselated surface and was difficult to decapsulate leading to parenchymal ruptures. On the section, the corticomedullary border could not be identified, the structure was disorganised and the cut surface slightly granular. Toxicological examination of fluid samples collected from the body did not reveal ethanol or ethylene glycol.

Histopathological examination of kidney fragments displayed segmental or total sclerosis of multiple glomeruli, inflammatory monocytic periglomerular infiltration, thickened arteriolar walls. Proximal tubules presented autolytic changes, intracytoplasmic deposits of a brown material and abundant crystals in the lumen, birefringent in polarized light and compatible with calcium oxalate (fig. 2). In the medullary, there were observed numerous tubules with cystic dilation, hyaline cylinders in the lumen, rare microcalcifications and advanced interstitial fibrosis. The renal capsule was thickened with micronodules associated to lympho-plasmocitary infiltrate.

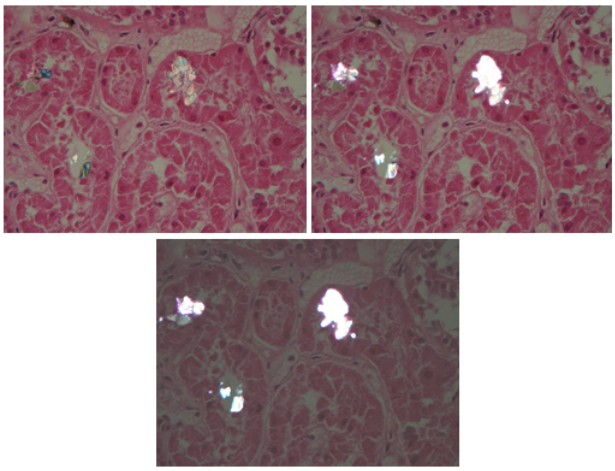

Fig. 3. Evolution of oxalate of calcium crystals precipitated in the tubular lumen (col HE 40X and polarized light) (collection of IML Iassy)

The histopathological examination was compatible with chronic glomerulonephritis and sustained the diagnosis of ethylene glycol intoxication by identifying calcium oxalate crystals in proximal tubules lumen.

Results and discussions

The toxicity of ethylene glycol is due to its metabolites rather than to the initial product itself. Once ingested, ethylene glycol is absorbed rapidly in the digestive tract, with lower absorption rates in the skin and lung, has low affinity for plasma proteins due to its water solubility, which favours a high rate of tissue distribution [1].

The suggestive triad of ethylene glycol intoxication is: central nervous system depression (CNS), ARF and metabolic acidosis with high-anion-gap [15, 16]. Hypocalcemia occurs in advanced stages and is secondary to the production of calcium oxalate [7].

Three clinical phases are described in ethylene glycol intoxication:

- neurologic phase (30 min– 12 h after ingestion);
- cardiopulmonary phase (12-36 h after ingestion);
- renal phase (24-72 h after ingestion).

After ingestion, there is an initial euphoric phase, as in alcoholic intoxication. Subsequently, nausea and vomiting appear, followed by CNS depression, associated with cerebral oedema and oxalosis. Several nervous symptoms may occur, such as confusion, hallucinations, convulsions, ataxia, nystagmus, coma, nuchal rigidity, tremors, hyporeflexia and tetany [17]. There may also be abdominal pain and hematemesis.

If the treatment in not initiated in the shortest delay, cardiovascular and pulmonary symptoms occur such as tachypnoea (compensation of metabolic acidosis), tachycardia, prolongation of QT interval, hypertension, congestive heart failure, cyanosis, pulmonary oedema, Kussmaul respiration. At approximately 16 h after ingestion,

metabolic acidosis is detected together with initial signs of ARF (increase of creatinine and urea levels). Metabolic acidosis is relatively refractory to sodium bicarbonate therapy [1, 3].

ARF occurs 24-73 h after ingestion secondary to tubular necrosis and manifests through haematuria, proteinuria, flank pain, oliguria and anuria [7]. In this time, cerebral oedema progresses and generates severe CNS depression, prolonged convulsions, and increased intracranial pressure that may potentially lead to herniation. Generalized convulsions are pathognomonic for ethylene glycol intoxication but localized convulsions may also appear same as myoclonic tetanic contractions secondary to hypocalcaemia. Myositis symptoms such as muscle tenderness occur at elevated creatine kinase levels. Transient cranial nerve paralysis (II, V, VI, VII, VIII, IX, X) was signalled in the literature 4-18 days after ingestion in case of inadequate, delayed or no treatment received [1].

The target organs in the ethylene glycol intoxication are the central and peripheral nervous system, kidneys, lung, heart, liver, muscles and retina with the following histopathological signs [1, 3, 15]:

- central nervous system: oedema, meningo-encephalitis, loss of Purkinje cells;
- kidney: dilatation of the proximal and distal tubules, interstitial oedema and intratubular deposit of calcium oxalate crystals;
- lung: oedema, interstitial pneumonitis;
- other organs: interstitial myocarditis with electrophysiological changes, steatosis, myositis, and deposits of calcium oxalate in the retina.

According to Grant et al. who examined a series of intoxicated children, the ophthalmoscopic examination revealed papillary oedema with diffuse borders and associated dilation of retinal vessels. Other patients exposed to ethylene glycol vapours presented with uncoordinated eye movements (nystagmus) of supranuclear origin with participation of vestibular nuclei [18].

The American Academy of Clinical Toxicology established the following diagnostic criteria for ethylene glycol intoxication [19]:

- ethylene glycol plasma concentration > 20 mg/dL (3 mmol/L);
- recent (hours) documentation or history of ingestion of toxic amounts of ethylene glycol (1-1.5 mL/kg);
- osmolar gap > 10 mOsm/kg.

According to the same Academy, a high clinical suspicion of ethylene glycol intoxication can be raised if at least two of the following criteria are present [19]:

- blood *p*H < 7.3;
- serum levels of bicarbonate <20 mEq/L (20 mmol/L);
- osmolar gap >10 mOsm/kg;
- presence of calcium oxalate crystals in urine.

One of the key clinical indications for the diagnosis of ethylene glycol intoxication is the metabolic acidosis with high-anion-gap [16]. The anion gap is the difference between sodium and the major cations (chlorine and bicarbonate), and is calculated using the formula anionic gap (AG) = $Na^+ + Cl^- + HCO_3^-$. The normal gap anion is determined by the presence of negatively charged proteins that are not measured (except albumin). The increase in gap anion is secondary to an increase in unmeasured serum anions or a decrease in cations such as calcium or magnesium. In case of ethylene glycol intoxication, calcium is consumed in order to form calcium oxalate crystals (monohydrate and dihydrate forms) that accumulate in multiple tissues and occur in up to 50% of cases [16]. Monohydrate crystals predominate and are specific to ethylene glycol intoxication [5]. Direct toxicity, cortical oedema, inhibition of mitochondrial activity in proximal renal tubular cells and the decrease in succinate dehydrogenase activity could be explained by monohydrate calcium oxalate crystals that are transported intracellularly. The histopathological diagnosis of ARF secondary to ethylene glycol intoxication relies on recognising typical destructive tubular lesions associated to extensive necrosis and the presence of calcium oxalate crystals in the lumen [20]. Except calcium oxalate formation, the consumption of calcium also leads to hypocalcemia manifesting through clinical and ECG signs.

The osmolar gap is a useful tool for the differential diagnosis of metabolic acidosis due to ethylene glycol intoxication with the one generated by non-toxic causes. Ethylene glycol is osmotically active both in its native form and through metabolites, and induces an increase in osmolarity. The osmolar gap is calculated by subtracting the serum osmolarity from the measured osmolarity. If the osmolar gap is greater than 10 mOsm/kg it is highly suggestive of intoxication by ethylene glycol or other alcohols. At a lethal concentration, the contribution of ethylene glycol to osmolarity is of 8 mOsm/kg. Ethanol overestimates the osmolar gap induced by ethylene glycol [21].

The intensity of the symptoms will depend on the ingested quantity and the time elapsed before starting treatment.

Qualitative determination of ethylene glycol in blood and urine involves the following steps [22]:

·dilution of 1 mL of blood/urine with 7 mL of distilled water;
-treatment of the solution with 1 mL of sodium tungstate;
-adding 1 drop of sulfuric acid 0.67N followed by filtration;
dilution of 1 mL of filtrate with 4 mL of distilled water;
- treatment with 0.25 mL periodic acid 0.1N;
- 10 min rest;
- adding 2 mL of Schiff reagent.

The presence of a purple colour indicates the presence of formic aldehyde derived from ethylene glycol.

Identification of ethylene glycol in gastric content and on offending bodies is performed after a prior filtration and vaporisation on a water bath until the solution increases consistency. The residue is then oxidized to oxalic acid using an equal solution of potassium dichromate 0.1N and sulfuric acid (heated on a water bath for 15 min). After cooling, the oxalic acid is extracted with ether [23].

Quantitative determination uses the following reagents: solution of periodic acid 0.01M and sulfuric acid 0.05M, solution of lead acetate 10%, solution of sodium bicarbonate 1N, solution of chromotropic acid (0.2 mL of chromotropic acid diluted in 2 mL of distilled water and mixted with 48 mL of sulfuric acid 13N), solution of sodium tungstate 10%, solution of sulfuric acid 0.039 N (0.1 mL), standard ethylene glycol solution (10μg/100mL). In a tube, there are added 1 mL of biologic fluid together with 8.5 mL of sulfuric acid 0.039 N and 0.5 mL of sodium tungstate 10%. The tube is then centrifuged at 2000 rotations/minute for 5 min. 0.5 mL of periodic acid is added to the supernatant. After 1 hour at room temperature, there are added 1 mL of lead acetate and 0.5 mL of sodium bicarbonate 1N. The tube is centrifuged again and afterwards, for 2 mL of supernatant there are added 2 mL of chromotropic acid. Both the probe and standard are kept for 30 min in boiling water. Ethylene glycol is then quantified using a spectrophotometer: ethylene glycol (10μg/100mL) = $(A_p/A_s) \times 100$ where A_p is the absorbance of the probe and A_s the absorbance of the standard solution [22].

Treatment of ethylene glycol intoxication typically involves 3 stages [1, 4, 19, 24]:

Reanimation

General guidelines for resuscitation should be followed including airway protection and fluid administration. Bicarbonate should be used with caution and in patients with severe acidosis (*p*H < 7.1), otherwise hypocalcemia and hypernatremia may be induced. Sodium bicarbonate helps to correct metabolic acidosis, increases the renal elimination of glycolic acid, and inhibits the precipitation of calcium oxalate crystals. Ionized calcium levels and QT interval should be monitored during infusion, as it may aggravate hypocalcemia. Hypocalcaemia is to be treated cautiously with glucuronate or calcium chloride, otherwise increased production of calcium oxalate and its deposition at the tissue level may be induced [1, 19].

Gastric decontamination

Ethylene glycol is rapidly absorbed in the gastrointestinal tract so inducing vomiting and gastric lavage do not prove useful and cannot be applied in patients with altered state of consciousness. Activated charcoal is useful when administered immediately [25].

Metabolism inhibition

ADH inhibition is one of the traditional treatments in ethylene glycol intoxication. This approach can be empirically used when the diagnostic criteria of the American Toxicology Association are met.

The two ADH inhibitors that can be used are [26-28]:

·fomepizole (4-methylpyrazole), a powerful ADH inhibitor with no side effects. The loading dose is 15 mg/kg diluted in 100 mL of 0.9% saline or 5% glucose in 30 min mfollowed by a maintenance dose of 10 mg/kg every 12 hours for four doses. It should be taken into account that fomepizole is dialyzable and therefore the dose interval should be reduced with 4 hours during haemodialysis;

·ethanol acts as a competitive substrate for ADH because the enzyme has a 67 times higher affinity for ethanol than for ethylene glycol. In order to have a therapeutic effect, it is required that serum concentrations of 10 to 12.5 mg/L are reached for adequate enzymatic saturation. A loading dose of 0.6 to 0.7 g ethanol/kg is administered followed by a continuous infusion of 66 to 150 mg/kg/h. Ethanol is also highly dialyzable so the dose should be increased 2 to 3 times in patients undergoing haemodialysis. The ethanol infusion should be maintained until the ethylene glycol concentration decreases to < 3.2 mmol/L same as fomepizole administration.

Pyridoxine, thiamine and folic acid should also be administered as a substrate for conversion in non-toxic metabolites [3].

Haemodialysis is an excellent therapeutic alternative for the management of ethylene glycol intoxication. It eliminates both ethylene glycol and glycolic acid and satisfactorily controls lactic acidosis. The indications for haemodialysis are [19, 28]:

- deterioration of vital signs despite intensive care support (haemodynamic instability);
- significant metabolic acidosis (*p*H < 7.30);
- ARF or electrolyte imbalance that does not respond to conventional therapy;
- if ethanol and fomepizole cannot be administered.

A serum concentration of ethylene glycol >500 mg/L (8 mmol/L) is not a single criterion for haemodialysis, and in the absence of renal dysfunction and significant metabolic acidosis the use of fomepizole eliminates the need for haemodialysis.

Conclusions

Ethylene glycol is a highly toxic and accessible alcohol used for manufacturing common products whose accidental or intentional ingestion is a frequent cause of lethal intoxication. The diagnosis is suspected based on the association of severe neurological deterioration, metabolic acidosis with high-anion-gap and renal failure. The presence of calcium oxalate crystals in the urine is a positive sign for intoxication. Forensic pathologists should be aware of clinical and biological manifestations as well as of typical histopathological findings in order to make the accurate diagnosis even if the substance itself is no longer detected in the blood or other samples.

References

1.FORD, M.D., DELANEY, K.A., LING, L.J., ERICKSON, T., Clinical Toxicology, W.B. Saunders Company, Philadelphia, PA, 2001.

2.KNIELING, A. M.C. BULGARU ILIESCU, D., MANEA, C., DIAC M., CHISTOL, R.C., FURNICA, C. Methanol intoxication and basal ganglia necrosis, Rev. Chim. (Bucharest), **68**, no.5, 2017, p.1126.

3.OLSON, K.R., Poisoning and Drug Overdose, 6th ed. McGraw-Hill, New York, 2012.

4.JACOBSEN, D., MCMARTIN, K.E., J. Toxicol. Clin. Toxicol., **35,** no. 2, 1997, p. 127.

5.JACOBSEN, D., HEWLETT, T.P., WEBB, R., BROWN, S.T., ORDINARIO, A.T., MC-MARTIN, K.E., Am. J. Med., **84**, no. 1, 1988, p. 145.

6.SEHEULT, J., FITZPATRICK, G., BORAN, G., Clin. Chem. Lab. Med., **55,** no. 3, 2017, p. 322.

7.MORFIN, J., CHIN, A., N. Engl. J. Med., **353,** no. 24, 2005, e21.

8.TOADER, E., TOADER, T., Revista Romana de Bioetica, **10**, no. 3, 2012, p. 66.

9.TOADER, E., Revista Romana de Bioetica, **8**, no. 2, 2010, p. 157.

10.GUDRUMAN, A.D., BIBIRE, N., TANTARU, G., APOSTU, M., VIERIU, M., DORNEANU, V., Rev Chim (Bucharest), **64**, no. 4, 2013, p. 393.

11.VIERIU, M., BIBIRE, N., PESTE, G., DORNEANU, V., POTORAC, L., Rev Chim (Bucharest), **64**, no. 3, 2013, p. 298.

12.RUSU, G., LUPUSORU, C.E., TARTAU, L.M., POPA, G., BIBIRE, N., LUPUSORU, R.V., CRISTOPOR, A.C., NECHIFOR, M., Farmacia, **63**, 2, 2015, p. 206.

13.ANDRITOIU, C.V., ANDRITOIU, V., CUCIUREANU, M., NICA-BADEA, D., BIBIRE, N., POPA, M., Romanian Journal of Morphology and Embryology, **55**, 3, 2014, p. 835.

14.DOBRIN, R., CIOBICA, A., TOADER, E., POROCH, V., Rev. Chim. (Bucharest), **67**, no. 9, 2016, p. 1778.

15.KRAUT, J.A., Clin. Toxicol (Phila), **53**, no. 7, 2015, p. 589.

16.GABOW, P.A,. Kidney. Int., **27**, no. 2, 1985, p. 472.

17.IMAM, Y.Z., KAMRAN, S., KARIM, H., ELALAMY, O., SOKRAB, T., OSMAN, Y., DELEU, D., Medicine (Baltimore), **93,** no. 10, 2014, e62.

18.GRANT, W.M., Toxicology of the Eye 3rd ed. Charles C. Thomas Publisher, Springfield, IL, 1986.

19.BARCELOUX, D.G., KRENZELOK, E.P., OLSON, K., WATSON, W., J. Toxicol. Clin. Toxicol., **37,** no. 5, 1999, p. 537.

20.POMARA, C., FIORE, C., D'ERRICO, S., RIEZZO, I., FINESCHI, V., Clin. Toxicol (Phila), **46**, no. 4, 2008, p. 322.

21.EDER, A.F., MCGRATH, C.M., DOWDY, Y.G., TOMASZEWSKI, J.E., ROSENBERG, F.M., WILSON, R.B., WOLF, B.A., SHAW, L.M., Clin. Chem., **44**, no. 1, 1998, p. 168.

22.BRUJA, N., IONESCU-VISAN, I., Diagnosticul de laborator in intoxicatiile acute, Ed. Militara, Bucuresti, 1987.

23.BANCIU, D., OARDA, M., Intoxicatiile acute, Ed. Medicala, Bucuresti, 1964.

24.BRENT, J., Drugs., **61**, no. 7, 2001, p. 979.

25.VALE, J.A., J. Toxicol. Clin. Toxicol., **35**, no. 7, 1997, p. 711.

26.BRENT, J., MCMARTIN, K., PHILLIPS, S., N. Engl. J. Med., **340**, no. 11, 1999, p. 832.

27.MCMARTIN, K., JACOBSEN, D., HOVDA, K.E., Br. J. Clin. Pharmacol., **81**, no. 3, 2016, p. 505.

28..CHENG, J.T., BEYSOLOW, T.D., KAUL, B., J. Toxicol. Clin. Toxicol., **25**, no. 1-2, 1987, p. 94.

Manuscript received: 14.01.2017