ORIGINAL ARTICLE

Veterinary Research Forum. 2022; 13 (2) 209 – 214
doi: 10.30466/vrf.2020.122469.2881
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

A study on acute oral caffeine intoxication and its treatment strategies in domestic pigeons (*Columba livia domestica*)

Sina Sedghi[1], Najmeh Mosleh[1], Tahoora Shomali[2]*, Seyed Amir Hossein Dabiri[2]

[1] Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [2] Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article Info

Article history:

Received: 14 March 2020
Accepted: 11 August 2020
Available online: 15 June 2022

Keywords:

Caffeine
Intoxication
LD50
Pigeon

Abstract

Limited knowledge is available on acute intoxication with environmental toxicants in birds. This experimental study determines features of acute caffeine intoxication and clinical outcome of different treatments in pigeons. The experiment was performed in three phases. Toxicity tests were performed in phases one and two while phase three was dedicated to comparative evaluation of fluid therapy and activated charcoal with or without diazepam and/or propranolol on clinical outcome of birds. Calculated LD50 was 366 mg kg^{-1} although presence of regurgitation compromised the accuracy of LD50 application. The dose-response (death) curve was sharp with a slope of 8.41. Clinical signs included renal, neurological, gastrointestinal and respiratory symptoms that generally initiated in a few minutes and lasted for few hours. The approximate toxic dose (ATD) was 294 mg kg^{-1}. Serum and brain concentrations after administration of ATD followed a normal distribution in a range of 14.6 - 83.3 mg mL^{-1} and 1.04 - 7.81 µg g^{-1}, respectively. Fluid therapy and activated charcoal with or without propranolol did not affect the clinical outcome of intoxicated birds while adding diazepam and intensive therapy with all of these agents even worsened the situation. In conclusion, caffeine is a potential source of intoxication in pigeons with a fast onset of clinical signs and a sharp increase in death rates by increasing doses. Symptoms are similar to mammals with prominent neurological signs although the ATD and serum concentrations are relatively high. Intensive therapy with above mentioned drugs is not recommended. Most birds survive after resolution of transient clinical signs without any special treatment.

© 2022 Urmia University. All rights reserved.

Introduction

Birds are gaining increasingly more attractions as companion animals in many parts of the world. As the interest in this area expands by pet owners, veterinary clinicians face with new challenges related to diverse disease states in different avian species. Toxicoses comprise an unneglectable reason for pet bird referrals to veterinary clinics. Sharing the same environment and even human foods with pet birds increase the risk of potential toxicities in avian species, especially for inquisitive birds. Unfortunately, knowledge about potential sources of toxicants, the resultant clinical symptoms, prognosis, external and/or internal toxic doses and optimal treatment strategies in avian species is very limited as compared to other companion animals especially dogs and cats.

Caffeine (1,3,7-trimethyl xanthine) is a purine-like alkaloid which is most famous for its role as a psychostimulant ingredient of chocolate, tea and coffee. In 1984, Day and Dilworth reported the toxicity of cocoa shell meal in broilers.[1] Apart from ubiquitous natural sources of caffeine, a diverse list of energy drinks and over the counter pharmaceutical preparations that contain caffeine as single agent or in combination with other drugs increase the risk of exposure of pet birds to a concentrated source of caffeine. Caffeine toxicity in humans[2] and dogs[3,4] is well defined in literature where there are only few case reports of accidental chocolate toxicoses in some avian species including parrots[5] and a budgerigar.[6]

Birds are not usually keen to ingest large amounts of chocolate.[7] Chocolate also contains theobromine as another potentially toxic substance and therefore these

***Correspondence:**

Tahoora Shomali. DVM, PhD
Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
E-mail: tshomali@shirazu.ac.ir

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

reports are not conclusive about caffeine intoxication. On the other hand, extrapolating the knowledge on caffeine toxicity in humans and/or dogs to avian species does not seem plausible due to considerable anatomical and physiological differences that may affect toxicokinetic and even toxicodynamic profile of the toxicant and subsequently patient's response and outcome.

This experimental controlled study was aimed to provide knowledge on acute caffeine intoxication in domestic pigeons as a common pet bird which can also serve as a model for other avian species. The study is focused on determining LD50, dose-response curve as well as clinical symptoms and their time course, approximate toxic dose (ATD), serum and brain concentration of caffeine in birds showing clinical signs after intoxication at ATD and finally the clinical outcome of different treatment strategies.

Materials and Methods

Birds. Seventy-five pigeons from both sexes were used in the study. All birds were in mature plumage at approximately the same age and sexually inactive with body weight of 290 ± 30.00 g. Pigeons were out-bred with different phenotypes and were provided by the same aviary in Shiraz, Iran. All birds were clinically examined at their arrival and spent an adaptation period of 14 days before initiation of the study. Birds were kept individually in mesh-floored cages with floor area of 4,800 cm^2 that were equipped with proper perches. The ambient conditions were kept constant during the study with a temperature of around 23.00 ˚C and 10/14 hr light/dark cycle. Birds had free access to tap water and pelleted pigeon feed (Tiba bird®, Kermanshah, Iran). Birds were fasted overnight (12 hr) before dosing at all phases of the experiment. No death was observed during the adaptation period. All procedures used in this study were approved by institutional ethical guidelines which are in accordance with EU Directive 2010/63/EU for animal experiments. The institutional Ethics Committee granted number was 97GCU1M163773.

Phase one: Acute oral toxicity assay, estimation of LD50 and LD50 slope test. Acute oral toxicity of caffeine in pigeons was determined based on the OECD guideline with the test number 223.[8] Pure (> 99.00%) caffeine anhydrous powder (Fluka Chemie AG., Buchs, Switzerland) was suspended in distilled water just before being administered via oral gavage. The concentration of the suspension was determined in a way that the dosing volume was kept at 10.00 mL kg^{-1} for all doses. An edible green color was added to distilled water to help detecting regurgitation. Five birds were randomly allocated to control group (received distilled water). Test group was consisted of 4 birds at stage 1 (onr bird per dose), 10 birds at stage 2 (one bird per dose) and also 10 birds in stage 3 (two birds per dose). At each stage, doses were administered simultaneously to birds. The initial estimate of LD50 (367 mg kg^{-1}) was based on caffeine LD50 in rats as determined by Adamson.[9] Calculation of doses at each stage was performed according to OECD guideline by determining low dose, high dose and step values. Birds were closely observed during first 2 hr after dosing and then every 2 hr for the first day followed by once-a-day monitoring until day 14. Regurgitation, signs of intoxication and remission, abnormal behavior, body weight, mortality and time to death were recorded. Gross pathological examination was performed on all birds at the end of the experiment or after death. A probit regression model (with the logarithm of dose as the independent variable) was used for statistical data analysis by using a Microsoft® Excel (version 15.0; Microsoft Corporation, Redmond, USA) workbook called SEquential DEsign Calculator (SEDEC). The LD50, LD50 slope and confidence intervals were calculated.

Phase two: Determination of ATD. Up and down method with two birds at each dose level was used to determine ATD of caffeine in pigeons. The procedure was performed in a step-wise pattern and started at 150 mg kg^{-1}. Calculation of other doses was performed by using a step value of 1.40. The monitoring intervals and administration route used in this test was the same as above (phase one). The endpoint for toxic effect was ataxia (especially inability to keep balance on perch) during the first 2 hr after drug administration. If two birds showed contradictory results, a third one was examined at the same dose level. When two birds showed the positive result (ataxia as mentioned above) without occurrence of death until the end of day 3, the dose was considered as ATD.

Determination of serum and brain concentrations of caffeine in birds exposed to ATD. Twenty birds were randomly assigned into treatment (n = 15) and control (n = 5) groups. Birds of treatment group received caffeine at ATD by oral gavage while distilled water was administered to the control birds. The birds were monitored and at the onset of ataxia, blood samples were collected from wing vein and then the bird was euthanized by decapitation after cervical dislocation. Time of sampling for birds of treatment group was ranged from 17 to 50 min post drug administration with a mean ± standard deviation of 30 ± 10 min. Birds of control group were also sampled at 30 min post distilled water administration. After clotting, sera were collected by centrifugation at 4000 rpm for 10 min. Harvested sera were kept at – 20.00 ˚C until analysis. Immediately after euthanasia, whole brain was dissected and kept at – 70.00 ˚C.

Sample preparation for high-performance liquid chromatography (HPLC) analysis. Whole brain was ground by a mortar and pestle and then 200 mg of brain tissue was mechanically homogenized in 10.00 mL of a

chloroform/isopropanol (85:15 v/v) solution. Then 1.20 g of ammonium bicarbonate and 100 µL of antipyrine, as internal standard (at a concentration of 400 µg mL^{-1} in 30.00% perchloric acid) was added to each sample. Samples were centrifuged at 5,000 rpm for 5 min at room temperature. The supernatant was collected and the solvent was dried at room temperature in an isolated place. The powder was kept at – 20.00 ˚C until injection to HPLC apparatus. At injection time, 300 µL of a solution containing HPLC grade water, isopropanol, acetonitrile and acetic acid (91: 4: 4: 1 v/v) was added to each sample. After centrifugation at 5,000 rpm for 6 min at room temperature, the supernatant was collected. For serum samples, 100 µL of internal standard (at a concentration of 40.00 µg mL^{-1} in 30.00% perchloric acid) was added to 500 µL of samples. After centrifugation at 12,000 rpm for 20 seconds at room temperature, supernatant was collected for HPLC analysis.

HPLC analysis. HPLC analysis was performed by a Knaur-HPLC system (Berlin, Germany) equipped with a UV-Vis-2500 detector and a Rheodyne injector (Model 7125; Rheodyne, Rohnert Park, USA). The injection volume was 50.00 µL. A Eurospher 100-5 C18 Column (150 × 4.60 mm; Knaur, Berlin, Germany) was used and separation was performed at 25.00 ˚C. The mobile phase was KH_2PO_4/acetonitrile (83:17 v/v, pH 3.50). The flow rate was 1.20 mL min^{-1} with a run time of 20 min. The detection wavelength was 274 nm with a retention time of 4.80 ± 0.20 min for caffeine and 11.50 ± 0.20 min for antipyrine. The concentration ranges of caffeine/ antipyrine which was used for linear regression analysis were 0.25 - 2.50 in brain samples and 0.78 - 100 for serum samples. The limit of detection and limit of quantification were 0.26 and 0.85 mg mL^{-1} for serum samples and 0.08 and 0.27 µg g^{-1} for brain samples, respectively. All reagents for HPLC analysis were purchased from Merck Company (Darmstadt, Germany). The HPLC method was adopted from Alvi and Hammami, with modifications.[10] Descriptive statistics and normality test (Shapiro-Wilk test) of data related to brain and serum caffeine concentrations were performed by SPSS software (version 22.0; IBM Corp., Armonk, USA).

Phase three: Comparative evaluation of different treatment procedures in birds with experimental caffeine intoxication. Thirty-six birds were randomly assigned into experimental and control groups (n = 6 each) and treated as follows: 1) Negative control (NC) group: birds received distilled water by oral gavage. Birds of groups 2-6 received caffeine suspended in distilled water at ATD by oral gavage. 2) Positive control (PC) or caffeine intoxication control: no treatment procedure was performed. 3) Treatment-1 (T1) group: fluid therapy was established by subcutaneous administration of Ringer's solution (IPPC, Tehran, Iran) at 10.00 mL kg^{-1} per site (four sites were used) each 90 min plus activated charcoal (Norit, Amersfoort, Netherland) at 2.00 g kg^{-1} by oral gavage every 6 hr until recovery or death.[11] 4) Treatment-2 (T2) group: In addition to fluid therapy and activated charcoal (as above), birds received diazepam (Caspian Tamin Pharmaceutical Co., Tehran, Iran) at 0.50 mg kg^{-1} by intravenous route (in wing vein) as per needed[12]. 5) Treatment-3 (T3) group: The protocol was the same as group T2, except that propranolol HCl (Tolidaru, Tehran, Iran) at the dosage of 0.04 mg kg^{-1} (by intravenous route) was administered instead of diazepam.[12] 6) Treatment-4 (T4) group: Birds of this group received all treatment options (fluid therapy, activated charcoal, diazepam and propranolol) as stated above. Birds were constantly monitored for 2 hr after administration of caffeine (or distilled water in NC group), then every 2 hr for the first day followed by once-a-day monitoring until day 3. Gross pathological evaluation was performed on dead birds as well as those remained alive at the end of third day. Administration of drugs was initiated immediately after appearance of clinical symptoms (especially ataxia).

Results

Phase one: Clinical evaluations. Collectively, the clinical signs that were observed in birds due to caffeine administration can be summarized into four categories of renal (polyuria), neurological (ataxia as the most prominent sign accompanied by muscular tremors, agitation, hyperesthesia, rotation, inability to stand and finally tonic-clonic seizures), gastrointestinal (repeated deglutition, gagging and regurgitation, severe inappetence) and respiratory (aerophagia, coughing and most clearly rapid abdominal respiration) symptoms. The symptoms were generally initiated in a few minutes after caffeine administration and were lasted for a maximal time of few hours. Birds that did not die, gained full recovery. Body weight showed fluctuations during the study but birds that remained alive until the end of the experiment, usually showed a moderate weight loss (about 10.00%). Milder signs including hyperesthesia and repeated deglutition were present in birds that received caffeine at the lowest dose (52.00 mg kg^{-1}). Clinical signs were become more severe at higher doses and death was usually happened after a seizure. The death results of different doses are summarized in Table 1. Most deaths (7 out of 12 deaths) happened within the first 2 hr of caffeine administration. Four birds died at 10^{th} hour and only one death was observed 5 days after treatment. No death was observed in control birds. No significant lesion was observed in gross pathological examination of dead birds, the green color with caffeine residues were still present in crops of birds (but not lower parts of GI tract, except for one case) especially in higher doses. Birds that lived until the end of 14^{th} day showed occasional hemorrhage in gastrointestinal tract (gizzard) or swollen kidneys.

Table 1. Results related to death (n) outcome of caffeine administration in different doses (mg kg^{-1}) to pigeons in three stages of phase 1 of the study.

Stage 1			Stage 2			Stage 3		
Dose	**Tested**	**Died**	**Dose**	**Tested**	**Died**	**Dose**	**Tested**	**Died**
52.00	1	0	126	1	0	228	2	0
191	1	0	159	1	0	289	2	1
704	1	1	202	1	0	367	2	1
2595	1	1	257	1	0	466	2	1
	-	-	326	1	0	597	2	2
	-	-	413	1	1	-	-	-
	-	-	524	1	1	-	-	-
	-	-	665	1	1	-	-	-
	-	-	844	1	1	-	-	-
	-	-	1,070	1	1	-	-	-

Dose-response curve. As shown in Figure 1, the semi logarithmic dose-response (percentage of mortality) curve for caffeine in pigeons was sharp with a slope of 8.41 (95.00% confidence limits were 2.22 and 14.60). The calculated LD50 was 366 mg kg^{-1} with 95.00% confidence limits of 266 and 504 mg kg^{-1}. However, as instructed by the OECD guideline, presence of regurgitation can compromise the accuracy of LD50 determination. The only conclusion possible may be that the LD50 is above the lowest level at which no regurgitation or mortality occurred which was 125 mg kg^{-1} in the case of our study.

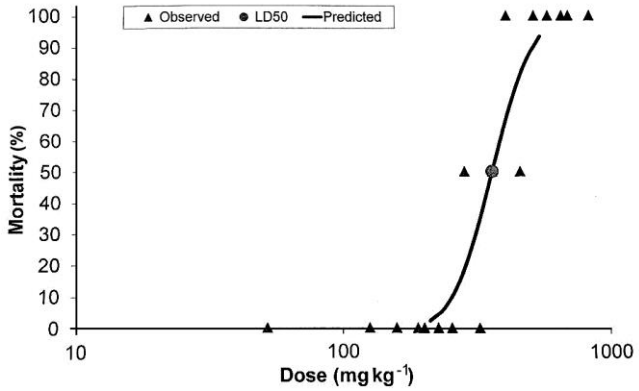

Fig. 1. Semi logarithmic dose-response curve of caffeine administration in pigeons.

Phase two. The ATD value was determined as 294 mg kg^{-1}. Brain and serum concentrations of caffeine followed a normal distribution (p = 0.20). Serum concentrations ranged from 14.6-83.30 mg mL^{-1} with 95.00% confidence interval for mean of 27.1 and 62.2 mg mL^{-1}. Brain concentration range was 1.04 - 7.81 µg g^{-1}. The 95.00% confidence interval for mean was 3.77 and 6.52 µg g^{-1}.

Phase three. No clinical symptoms or gross pathological lesions were observed in birds of NC group and all of them were alive at the end of experiment without a change in their body weight. Birds in PC group showed obvious clinical signs. Neurological symptoms especially ataxia and agitation as well as anorexia were most prominent signs. One bird ended up dead (17.00%) in this group. Live birds showed about 10.00% decrease in body weight at the end of the experiment. Treatments in T1, T2 and T3 groups were not associated with a considerable change in clinical signs and these groups also showed weight decrease ranged from 8.00 - 12.00%. Fifty percent of birds in T2 group died during the experiment where death rate in T1 and T3 group was the same as PC birds (17.00%). The worst outcome was related to T4 group, where only one bird stayed alive until the end of the experiment (about 83.00% death). The live bird showed a drastic weight loss (25.00%). Birds of PC group and treatment groups (T1 - T4) had no appreciable lesion in necropsy.

Discussion

As the first finding of our study, we observed that caffeine at relatively low doses (52.00 mg kg^{-1}) can induce transient but noticeable clinical signs in pigeons. This finding can give us a clue on the importance of different caffeine sources as potential toxicants in pigeons. For instance, an average cup of coffee contains 40.00-150 mg of caffeine,[13] therefore if caffeine is considered as the only toxicant in coffee (which is too simplistic), less than one half to about 1/10 of a cup of coffee is needed for a 300 g pigeon to show clinical signs of intoxication. It is unlikely for a bird voluntarily to consume this much of coffee. Every cup of black tea contains 32.00 – 36.00 mg of caffeine[14] that excludes this beverage as an important source for caffeine intoxication in pigeons. However, it should be considered that there are more concentrated sources of caffeine which may be accessible to pet birds. For instance, caffeine pills contain between 100 to 200 mg caffeine per tablet and a 100 mg pill contains the amount of caffeine close to LD50 dose in a 300 g pigeon. Dried tea leaves and especially Nescafe™ powder also usually have high caffeine concentrations (13.00 mg g^{-1} of green tea, 10.00 mg g^{-1} of Indian tea and 36.00 mg g^{-1} for Nescafe™ powder). [15] Another finding of our study was the high slope of dose-response curve of caffeine-induced deaths in pigeons which demonstrated that a trivial increase in caffeine dose can appreciably increase the dead cases number.

Although the clinical signs of caffeine intoxication vary among human patients, they can be categorized into gastrointestinal, psychological/neurological, metabolic, musculoskeletal, pulmonary, renal and cardiovascular symptoms. [2] Dogs also show similar signs. [3] It seems that the signs that we observed in pigeons (neurological, respiratory, renal and gastrointestinal signs) have high similarities to those observed in humans and dogs. We could not evaluate cardiovascular signs in pigeons due to the fact that birds were severely over-reactive and the process could lead to more stress. [7]

As previously stated, clinical signs of intoxication had a fast onset. This can be related to fast absorption of caffeine especially in starved birds. Caffeine has a weak basic nature and favors a lipophilic state in more basic environments that facilitates its passage from lipid bilayer of cells and consequently its absorption. [16] Normally the pH of crop is about 6.00 whereas the stomach chambers are more acidic[17]; therefore, caffeine absorption from crop may be considerable. We observed the accumulation of caffeine in crops without reaching the intestines of most birds in post-mortem examination that is in accordance with the idea that the absorption was through crop with resultant fast increase in serum concentration and appearance of clinical signs.

Determination of serum and brain concentrations can be helpful in definitive diagnosis as well as prognosis of caffeine intoxication. In our study, we found mild clinical signs in birds that received caffeine at 52.00 mg kg^{-1} and the ATD for appreciable neurological signs was 294 mg kg^{-1} in pigeons. Administration of caffeine at ATD was associated with serum concentrations of 14.6-83.30 mg mL^{-1} and brain concentrations with 1.04 - 7.81 μg g^{-1} range. The reason behind relatively wide range of serum and brain concentration of caffeine results from the fact that the onset of ataxia (when the samples were collected) was somewhat different in different birds (about 30 min interval between the birds with fastest and slowest response) post caffeine administration. This implies that birds have shown a same clinical sign at different times with a wide range of serum or brain concentrations which can be related to the individual variations. Sampling at fixed time intervals post caffeine administration for pharmacokinetic assay could be associated with more homogenous concentrations which was not considered in the present study.

In humans, doses around 7.00 - 10.00 mg kg^{-1} can be considered as high sub-lethal doses that can result in obvious clinical signs, although the responses can be different in patients[2]. Consistently, blood caffeine levels above 80.00 - 100 μg mL^{-1} are lethal in humans. [18] Therefore, it seems that pigeons have relatively low sensitivity to caffeine intoxication compared to humans.

As the final phase of our study, we evaluated the outcome of different treatment strategies in pigeons intoxicated with ATD of caffeine. We found that fluid therapy and activated charcoal administration with or without propranolol does not affect the clinical outcome of intoxicated birds, while adding diazepam to fluid therapy and activated charcoal unexpectedly increased the death rates of intoxicated pigeons and an intensive therapy with all of these agents even worsened the situation. We cannot exactly describe the reason for these findings; however, it has been shown that caffeine intoxication can result in hypotension and tachycardia[2] and diazepam may have precipitated these cardiovascular problems. On the other hand, the high death rate in the intensive therapy group may be related to more stress imposed to birds during drug administration.

In conclusion, caffeine can be considered as a potential source for intoxication of pigeons with a fast onset of clinical signs and a sharp increase in death rate by increasing doses, which necessitates preventive measures to limit the access of birds to concentrated caffeine sources. The clinical signs of acute caffeine intoxication in pigeons are similar to mammals with prominent neurological symptoms although the ATD and serum concentrations are higher in pigeons than humans. Intensive therapy with above mentioned drugs is not recommended for intoxicated birds and most birds can survive after resolution of transient clinical signs without any special treatment.

Acknowledgments

This work was supported by School of Veterinary Medicine, Shiraz University, Shiraz, Iran (grant No. 97GCU1M163773).

Conflict of Interests

Authors declare no conflict of interests.

References

1. Day EJ, Dilworth BC. Toxicity of jimson weed seed and cocoa shell meal to broilers. Poult Sci 1984; 63(3): 466-468.
2. Willson C. The clinical toxicology of caffeine: A review and case study. Toxicol Rep 2018; 5: 1140-1152.
3. Peterson ME, Talcott PA. Small animal toxicology. 3rd ed. Philadelphia, USA: WB Saunders 2012; 220-221.
4. Tawde SN, Puschner B, Albin T, et al. Death by caffeine: presumptive malicious poisoning of a dog by incorporation in ground meat. J Med Toxicol 2012; 8(4): 436-440.
5. Gartrell BD, Reid C. Death by chocolate: a fatal problem for an inquisitive wild parrot. N Z Vet J 2007; 55(3): 149-151.
6. Varol K. Chocolate intoxication in a budgerigar. Erciyes Üniversitesi Veteriner Fakültesi Dergisi 2019; 16(1): 73-77.

7. Lightfoot TL, Yeager JM. Pet bird toxicity and related environmental concerns. Vet Clin North Am Exot Anim Pract 2008; 11(2): 229-259.
8. OECD Guidelines for the testing of chemicals, section 2, test No. 223: Avian acute oral toxicity test, 2016: 1-28.
9. Adamson RH. The acute lethal dose 50 (LD50) of caffeine in albino rats. Regul Toxicol Pharmacol 2016; 80: 274-276.
10. Alvi SN, Hammami MM. Validated HPLC method for determination of caffeine level in human plasma using synthetic plasma: application to bioavailability studies. J Chromatogr Sci 2011; 49(4): 292-296.
11. Gupta RC. Veterinary toxicology: basic and clinical principles. 2nd ed. Massachusetts, USA: Academic Press 2012; 1366-1367.
12. Samour J. Avian medicine. 3rd ed. Missouri, USA: Mosby Ltd. 2016; 22 , 406.
13. Tran NL, Barraj LM, Bi X, et al. Trends and patterns of caffeine consumption among US teenagers and young adults, NHANES 2003-2012. Food Chem Toxicol 2016; 94: 227-242.
14. Camargo MCR, Toledo MCF. HPLC determination of caffeine in tea, chocolate products and carbonated beverages. J Sci Food Agric 1999; 79(13): 1861-1864.
15. Srdjenovic B, Djordjevic-Milic V, Grujic N, et al. Simultaneous HPLC determination of caffeine, theobromine and theophylline in food, drinks and herbal products, J Chromatogr Sci, 2008; 46(2): 144-149.
16. Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man, Handb Exp Pharmacol 2011; (200): 33-91.
17. Vermeulen B, De Backer P, Remon JP. Drug administration to poultry. Adv Drug Deliv Rev 2002; 54(6): 795-803.
18. Kerrigan S, Lindsey T. Fatal caffeine overdose: two case reports. Forensic Sci Int 2005; 153(1): 67-69.