Potravinarstvo Slovak Journal of Food Sciences

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 244-251
doi: https://dx.doi.org/10.5219/755
Received: 14 February 2017. Accepted: 15 March 2017.
Available online: 26 April 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

DOES APRICOT SEEDS CONSUMPTION CAUSE CHANGES IN HUMAN URINE?

Eva Tušimová, Anton Kováčik, Marek Halenár, Katarína Michalcová, Katarína Zbyňovská, Anna Kolesárová, Jana Kopčeková, Jozef Valuch, Adriana Kolesárová

ABSTRACT

Natural substances, such as amygdalin, used in alternative medicine gained high popularity. Common people as well as patients with different diseases have almost unlimited access to various natural supplements. To protect human health, it is very important to study effect of these substances. Amygdalin is a cyanogenic glucoside derived from seeds of rosaceous plants, for example seeds of bitter almonds (*Prunus dulcis*), or apricot, cherry, apple, peach, plum, etc. It is a natural product that owns antitumor activity, it has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes and produces a kind of antitussive and antiasthmatic effects. The present *in vivo* study was designed to reveal whether amygdalin in apricot seeds has got an effect on human urine composition, pH value and urine associated health status after six weeks of oral administration. The study group finally consisted of 34 healthy adult volunteers (21 females and 13 males). All participants were asked to consume 60 $mg.kg^{-1}$ body weight of bitter apricot seeds daily (approximately 3.0 $mg.kg^{-1}$ of amygdalin) during 6 weeks. During the experiment, three urine collections were carried out (first collection – at the beginning of the experiment; second collection – after 21 days; third collection – after 42 days). Quantification of urine calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), chlorides (Cl^-), urea and pH value after apricot seeds supplementation was performed. Statistical analysis of variance showed, that consumption of bitter apricot seeds during 42 days had a significant (p <0.01) effect on amount of calcium excreted in urine, though this decrease shifted its level from elevated mean value in control collection into normal physiological range. Significant changes were observed in urea (p <0.05) and phosphorus (p <0.01) levels in urine after apricot seed ingestion, but gender was also considered to be a source of their variation.

Keywords: amygdalin; apricot seed; urine; minerals; urea

INTRODUCTION

Fruit and vegetable contain a significant amount of biologically active substances able to lower a risk of any type of cancer or other civilization diseases **(Jakubcova et al., 2014; Mendelová et al., 2016)**. CN-containing glycosides and nitrilosides exist in thousands of plants including strawberries, alfalfa sprouts, spinach, pecans, and in particularly high concentration in apricot kernels and other seeds, thus responsible for their characteristically bitter taste. It should be noted that bitterness is an important sensory indicator of potently anti-inflammatory, yet potentially poisonous compounds (in excessive amounts) within a plant. Amygdalin and other glycosidic compounds were abundant in the diets of early humans, and are commonly eaten by animals in the wild; the dietary lack thereof may contribute to increased cancer rates observed not only in humans, but also domesticated animals **(Sokolsky and Wargovich, 2012)**. Amygdalin, D-mandelonitrile-β-D-glucoside-6-β-D-glucoside is a cyanogenic glucoside derived from seeds of rosaceous plants, for example seeds of bitter almonds (*Prunus dulcis*), or apricot, cherry, apple, peach, plum, etc. **(Holzbecher et al., 1984; Chwalek and Plé, 2004; Santos Pimenta et al., 2014; Lee and Moon, 2016)**. Amygdalin $C_{20}H_{27}NO_{11}$, is composed of one molecule of benzaldehyde, two molecules of glucose and one molecule of hydrocyanic acid **(Chang et al., 2006)**.

Amygdalin is a natural product that owns antitumor activity, less side effects and relatively low priced **(Song and Xu, 2014)**. Besides the antitumor activity, amygdalin has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes **(Zhou et al., 2012)**. It is also decomposed by the action of β-D-glucosidase to yield hydrocyanic acid which stimulates the respiratory center reflexively and produces a kind of antitussive and antiasthmatic effects **(Badr and Tawfik, 2010; Lv et al., 2005)**.

Patients with cancer use complementary and alternative medicine frequently, up to 90% within one year at least part of their therapy **(Gansler et al., 2008; Hunt et al., 2010)**. It is not surprising, that use of amygdalin is recently increasingly advocated as an anticancer therapy **(Milazzo et al., 2011)**. It can be applied by apricot seed or tablet ingestion, or by intravenous administration **(Moertel et al., 1982)**. Cyanide from amygdalin can be released by the action of beta-glucosidase or emulsin. Although these enzymes are not found in mammalian tissues, the human intestinal microflora appears to possess these or similar enzymes capable of effecting cyanide release resulting in human poisoning. For this reason amygdalin may be as much as 40 times more toxic by the oral route as compared with iv injection **(Casarett et al., 1980)**. It was reported, that after consumption of apricot seeds, cyanide can be produced in high enough level to cause a potential chronic toxicity problem **(Seigler, 1975)**, in another study **Seghers et al. (2013)** concluded that a daily intake of 70 apricot kernels during more than six weeks induces abnormal liver chemistry tests without other toxicity signs. However, in medicinal chemistry, there is a little distinction between a drug and poison, and the specific poisoning of cancer cells is also the basis of chemotherapy **(Sokolsky and Wargovich, 2012)**.

Urine may be a waste product, but it contains an enormous amount of information **(Delanghe and Speeckaert, 2014)** and it have been studied extensively **(Kirchmann and Pettersson, 1994; Karak and Bhattacharyya, 2011)**. Urine is composed of 91 – 96% water **(Heinonen-Tanski, 2007)**, and the remainder can be characterized into inorganic salts, urea, organic compounds and organic ammonium salts **(Putnam, 1971)**. Variation in its composition is caused by differences in physical exercise, environmental conditions, as well as water, salt and high protein intakes **(Rose et al., 2015)**.

The present *in vivo* study was designed to reveal whether amygdalin in apricot seeds has got an effect on human urine composition, pH value and urine associated health status after six weeks of oral administration.

MATERIAL AND METHODOLOGY

Chemicals

Bitter apricot seeds were provided by Trasco (Žiar n. Hronom, Slovakia). Thin Layer Chromatography (TLC) was performed for the analysis of amygdalin content in bitter apricot seeds used in our experiment. Grinded apricot seeds (2 g) were mixed with 10 mL of methanol in a vial and put into ultrasonic bath for 30 minutes at 55 °C. After cooling, 10 µL of solution was applied onto TLC plates Kieselgel UV 254 20 x 20 cm (Merck KGaA, Darmstadt, Germany). Mixture of n-butanol, acetic acid and water (95 : 5 : 25) was used as a mobile phase. Separation took about 5 hours at room temperature. After separation, amygdalin content was determined by UV densitometer CS – 9000 (Shimadzu, Japan) at 205 nm. An external standard was used (1% amygdalin solution in methanol). Crude protein content was performed according to **Kjeldahl (1883)**, fat content was determined using **Soxhlet** method for fat extraction **(1879)** and crude fibre by **Henneberg-Stohmann** method **(1860)**. Amount of starch was measured via polarimetry and total sugars by Luff-Schoorl titration. Organic content in apricot seeds is shown in Table 1.

Mineral content in apricot seeds was determined using atomic absorption spectroscopy and is shown in Table 2.

Volunteers

The study group finally consisted of 34 healthy adult volunteers (21 females and 13 males). Volunteers were recruited from the general population of Slovakia (Nitra district). Respondents were 23-65 years old, where the average age of women was 40.65 ±11.31 years and the average age of men was 36.91 ±9.98 years. All subjects involved in the study were informed of all risks, discomforts and benefits and written informed consent to participate in the study was provided to them. The study was performed between September and December 2015. The trial was approved by the Ethic Committee at the Specialized Hospital St. Zoerardus Zobor, n. o., protocol number 030809/2015. All participants were asked to consume 60 $mg.kg^{-1}$ body weight of bitter apricot seeds daily (approximately 3.0 $mg.kg^{-1}$ of amygdalin) during 6 weeks. Volunteers were instructed as follows: not to change their usual diet and habits (physical exercise etc.), to consume approximately one seed each hour, to chew it as thoroughly as possible and to drink a lot of water after each consumption.

Urine sample collection

During the experiment, three urine collections were carried out (first collection – at the beginning of the experiment; second collection – after 21 days; third collection – after 42 days). First morning sample of urine was collected from all volunteers into sterile tubes. Urine analysis was performed at the same day.

Analysis

Quantification of calcium (Ca), magnesium (Mg), phosphorus (P) and urea after apricot seeds supplementation was performed through commercial sets

Table 1 Organic content in apricot seeds (%).

Dry matter	Amygdalin	Crude protein	Fat	Crude fibre	Starch	Total sugars
95.9	5.8	22.8	39.7	28.5	2.3	6.3

Table 2 Dry matter (%) and mineral content ($mg.kg^{-1}$) in apricot seeds.

Dry matter	Ca	P	Mg	Na	K
95.9	1 774	4 700	2 050	642	5 925

DiaSys (Diagnostic Systems GmbH, Germany) on device Rx Monza (Randox Laboratories Ltd., United Kingdom). Amount of sodium (Na), potassium (K) and chlorides (Cl^-) was determined on device EasyLyte Plus (Medica Corporation, United States). Urine pH values were measured by pH meter 3200P (Agilent Technologies, United States).

Table 3 Analysis of variance calculated for parameters of human urine.

Source of variation / Parameter	Gender	Length of consumption	Interaction
Calcium	F = 0.4844	F = 5.810**	F = 3.061
Phosphorus	F = 4.171*	F = 3.350*	F = 3.938*
Magnesium	F = 0.908	F = 0.460	F = 0.082
Sodium	F = 0.304	F = 0.571	F = 0.930
Potassium	F = 1.639	F = 0.600	F = 2.390
Chlorides	F = 2.161	F = 0.399	F = 1.820
Urea	F = 6.224*	F = 4.379*	F = 0.019
pH	F = 2.118	F = 1.611	F = 0.184

Note. The level of significance was set at *p <0.05, **p <0.01, ***pP <0.001. Two-way ANOVA with gender and length of consumption as between-subject factors. See Material and methodology for details.

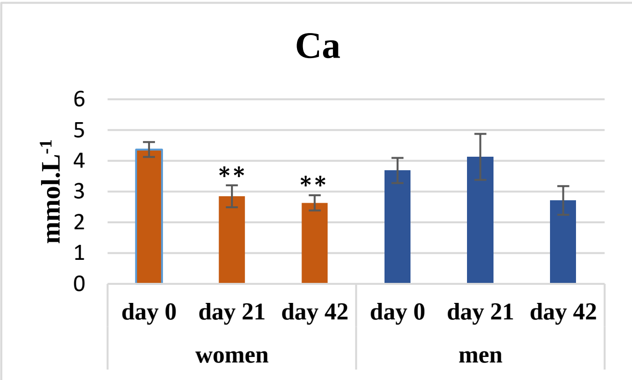

Figure 1 Urine levels of calcium in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

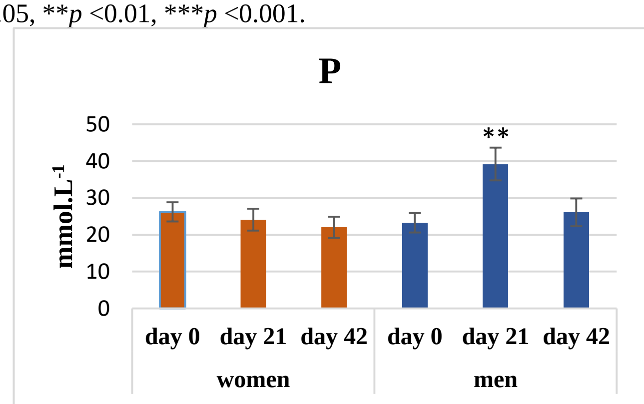

Figure 2 Urine levels of phosphorus in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

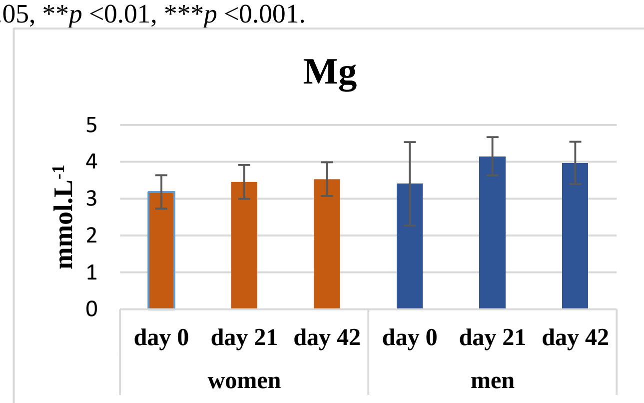

Figure 3 Urine levels of magnesium in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

Statistical analysis

Parameters of human urine at the beginning of experiment, after 21 and after 42 days of apricot seeds consumption were statistically evaluated and are given as means and SEM. All groups passed Shapiro-Wilk normality test and were compared by a within-subjects two-way analysis of variance (ANOVA), followed by Bonferroni post-tests when required using statistical software GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant if the probability of error was <5%.

RESULTS

A total of 34 adult volunteers were used in the final data analysis, of which 21 (61.8%) were females and 13 (38.2%) were males. First sample collection (at the beginning of the experiment) was used as a control. Effect of gender and length of apricot seeds consumption on urine concentration of Ca, P, Mg, Na, K, Cl, urea and pH value is revealed by two-way ANOVA (Table 3). Urine concentration of calcium was affected by the length of consumption (p = 0.004). Level of phosphorus in urine was affected by gender (p = 0.044), by length of consumption (p = 0.039), but analysis of variance confirmed also their mutual interactions (p = 0.023). Urine concentration of urea was affected by length of consumption (p= 0.015), and by gender significantly (p = 0.014). Other following parameters were not significantly affected.

Figure 1 shows the concentrations of the urine calcium divided by the gender and length of consumption. We found statistically significant decrease of Ca content between first and second collection (p <0.01), and third collection (p <0.01) in the female group. In the male group, no significant differences were found.

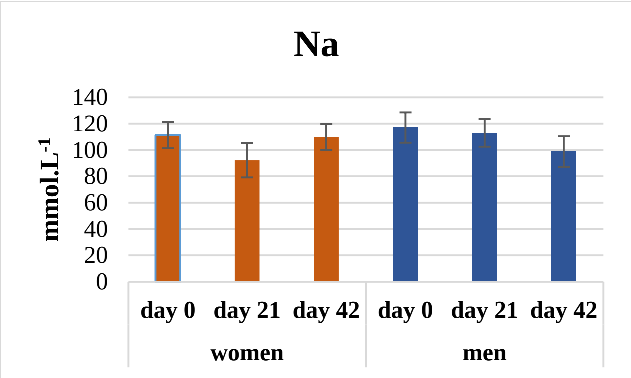

Figure 4 Urine levels of sodium in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

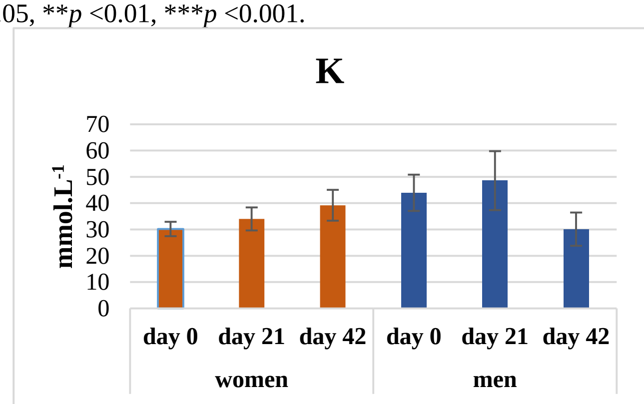

Figure 5 Urine levels of potassium in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

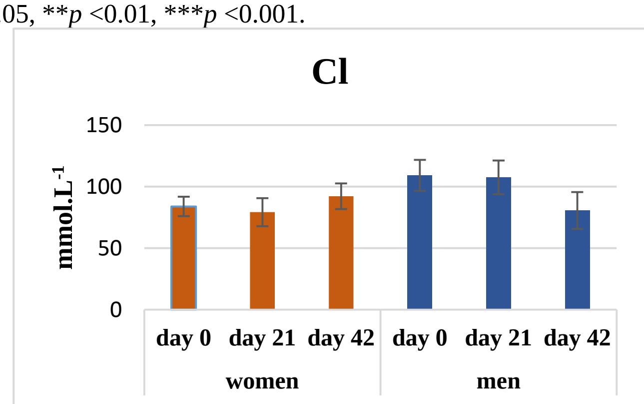

Figure 6 Urine levels of chlorides in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

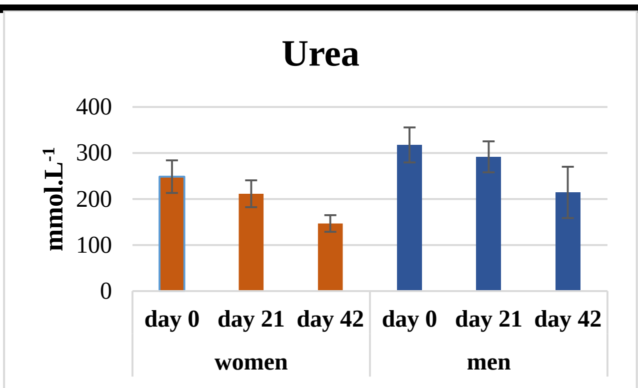

Figure 7 Urine levels of urea in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

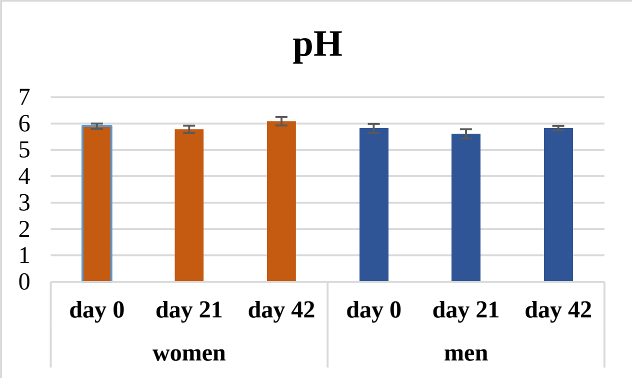

Figure 8 Urine levels of pH in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *p <0.05, **p <0.01, ***p <0.001.

The concentration of phosphorus in the male urine significantly (p <0.01) increased to the maximum in 21st day of experiment (Figure 2). In the female group, a slight decrease of P content was observed.

Figure 3 describes how apricot seeds consumption influenced level of urine magnesium. In the both genders, we didn't found significant differences, however we found a moderate increase of the observed parameter.

Content of the urine sodium shows the opposite tendency than previous parameter (Figure 4). Results show a slight decrease of Na in collections after 21, resp. 42 days of consummation with non-significant differences in the both genders.

Differences between potassium levels were statistically non-significant too, but we recorded considerable decrease of this parameter between male control collection (day 0) and male third collection. Opposite tendency, an increase, was observed in the female group, also statistically non-significant (Figure 5).

Urine concentration of chlorides showed very similar tendency (Figure 6). A slight decrease of Cl^- level occurred in the male group and slight increase occurred in the female group.

Content of urea in both groups had similar tendency (Figure 7). Decrease of urea level was recorded in the male group and in the female group too. Besides that, decrease in the female group between first and third collection was significant (p <0.05).

The last studied parameter, pH value, was shown to be the most stable (Figure 8). The analysis of variance of this parameter showed only negligible effect of apricot kernels consumption.

DISCUSSION

In past decades, amygdalin has been studied extensively from different points of view. It has been reported, that amygdalin is a natural product that owns antitumor activity **(Song and Xu, 2014)** has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes **(Zhou et al., 2012)** and produces a kind of antitussive and antiasthmatic effects **(Badr and Tawfik, 2010; Lv et al., 2005)**. Besides that, it has possible modulatory impact on cell viability **(Halenár et al., 2016)** and the steroid production in porcine ovaries *in vitro*, as **Halenár et al. (2015a, 2015b)** published in their study. **Michalcová et al. (2016)** reported, that natural substances present in apricot seeds may be involved in mechanisms of ovarian folliculogenesis of rabbits through inhibition of FSH levels. Amygdalin is also a potential antifibrotic agent that may have therapeutic potential for patients with fibrotic kidney disease **(Guo et al., 2012)**. **Kováčová et al. (2016)** observed a significantly lower values of primary osteons' vascular canals and secondary osteons in femoral bones of rabbits administered by amygdalin. Our previous *in vivo* study on rabbits showed no obvious effect of intramuscular administration of amygdalin (0.6 and 3.0 $mg.kg^{-1}$) on energy profile **(Tušimová et al., 2016a)**. Similarly, **Miller et al. (1981)** did not observe changes in blood chemistry of rats fed a diet containing 10% ground apricot kernels.

There are just a few studies of blood chemistry changes and even fewer studies of urine composition changes after amygdalin administration *in vivo.*

Statistically significant decrease of calcium content between first and second collection (p <0.01), and also between first and third collection (p <0.01) was found in the female group. However, this decrease may be considered as a positive effect, since the reduction of excreted calcium shifted its level from elevated mean value in control collection (4.36 mmol.L^{-1}) into normal physiological range (1.2 – 3.7 mmol.L^{-1}) according to **Vasudevan et al. (2011)**. In the male group, no significant differences were found. Two-way analysis of variance showed very significant effect of length of apricot seeds consumption on urine calcium (p = 0.004) in presented study. Urine calcium level can be also decreased by very low protein intake **(Calloway and Margen, 1971)**.

The concentration of phosphorus in the male urine significantly (p <0.01) increased to the maximum in 21st day of experiment. In the female group, a slight decrease of P content was observed, though all the values were in the reference range (20 – 50 mmol.L^{-1}) **(Vasudevan et al., 2011)**. According to ANOVA results, amount of P in urine was significantly affected by gender (p = 0.044), length of consumption (p = 0.039) and by interaction of these two factors (p = 0.023). In very low protein diets phosphorus levels in urine were shown to be increased **(Calloway and Margen, 1971)**.

Sodium and potassium are major solutes excreted in urine **(Rose et al., 2015)**. In very low protein diets, urine potassium level can be increased and magnesium concentration in urine is not affected **(Calloway and Margen, 1971)**. Our results did not show any significant differences in sodium, potassium, magnesium and chlorides levels between collections either in males or in females.

Nitrogen in urine is predominantly in the form of organic nitrogen and mostly in the form of urea **(Beler-Baykal et al., 2011)**. Urea is the most predominant constituent making up over 50% of total organic solids, and it is produced through the metabolism of protein **(Rose et al., 2015)**. Urinary nitrogenous components increase with levels of protein in the diet; a positive correlation between urinary nitrogen and protein intake was found to be 0.91 **(Magee et al., 2004)**. Normal urea concentration range from 167 mmol.L^{-1} to 583 mmol.L^{-1} **(Vasudevan et al., 2011)**. Results of our study showed concentrations of urea within this range except for the last collection in females (146 mmol.L-^{1}) with decreasing tendency also in males. The difference in urea concentration between control and third collection in female group was found significant (p <0.05). Two-way analysis of variance showed significant effect of length of apricot seeds consumption (p = 0.015), but gender was also considered to be a source of variation (p = 0.014). **Vasudevan et al. (2011)** reported, that urea level decrease may be caused by low protein intake, prolonged fasting, but also by liver failure. However, our previous *in vivo* study on rabbits showed no obvious effect of amygdalin administration (0.6 and 3.0 mg.kg^{-1}) on hepatic profile after **(Tušimová et al., 2016b)**.

Value of pH in our study varied from 5.7 to 6.1, which is only slightly lower in comparison to values that **Rose et al. (2015)** observed. According to their results, the pH of fresh urine was largely neutral with a median of pH 6.2 with a range of pH values of 5.5 – 7.0. Our results showed no significant differences in pH values between urine collections in comparison to control collection, even there is a moderate increase in urine pH in both groups. Changes in urinary pH can be caused by numerous factors. It is reduced by high protein intake through meat and dairy products as well as through alcohol consumption **(Kanbara et al., 2012)** and increased by higher consumption of potassium and organic acids in vegetables and fruit **(Rose et al., 2015)**.

CONCLUSION

Natural substances, such as amygdalin, used in alternative medicine gained high popularity. Common people as well as patients with different diseases have almost unlimited access to various natural supplements. To protect human health, it is very important to study effect of these substances.

In summary, consumption of bitter apricot seeds (60 mg.kg^{-1} of body weight) during 42 days had a significant effect on amount of calcium excreted in urine, though this decrease shifted its level from elevated mean value in control collection into normal physiological range. Significant changes were observed in urea and phosphorus levels in urine after apricot seed ingestion, but gender was also considered to be a source of their variation. Although the detailed mechanisms behind the action of amygdalin remain undefined, we hypothesize that this natural substance may moderately affect mineral and nitrogen management and possibly acid-base balance of the body. However, future studies are required to investigate the mechanisms by which amygdalin affects regulatory systems in human organism.

REFERENCES

Badr, J. M., Tawfik, M. K. 2010. Analytical and pharmacological investigation of amygdalin in *Prunus armeniaca L.* kernels. *Journal of Current Pharmaceutical Research*, vol. 3, no. 9, p. 2134-2137.

Beler-Baykal, B., Allar, A., Bayram, S. 2011. Nitrogen recovery from source-separated human urine using clinoptilolite and preliminary results of its use as fertilizer. *Water Science and Technology*, vol. 63, no. 4, p. 811-817. https://doi.org/10.2166/wst.2011.324 PMid:21330732

Calloway, D. H., Margen, S. 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. *The Journal of Nutrition*, vol. 101, no. 2, p. 205-216. PMid:5540441

Casarett, L. J., Doull, J., Klaassen, C. D., Amdur, M. D. 1980. Casarett and Doull's Toxicology. 2nd ed. New York : Macmillan Publishing. p. 328. ISBN 978-80-0023-304-04.

Chang, H. K., Shin, M. S., Yang, H. Y., Lee, J. W., Kim, Y. S., Lee, M. H., Kim, J., Kim, K. H., Kim, C. J. 2006. Amygdalin Induces Apoptosis through Regulation of Bax and Bcl-2 Expressions in Human DU145 and LNCaP Prostate Cancer Cells. *Biological and Pharmaceutical Bulletin*, vol. 29, no. 8, p. 1597-1602. https://doi.org/10.1248/bpb.29.1597 PMid:16880611

Chwalek, M., Plé, K. 2004. Convenient syntheses of isomaltose derivatives from amygdalin. *Tetrahedron Letters*, vol. 45, no. 24, p. 4749-4753. https://doi.org/10.1016/j.tetlet.2004.04.057

Delanghe, J., Speeckaert, M. 2014. Preanalytical requirements of urinalysis. *Biochemia Medica*, vol. 24, no. 1, p. 89-104. https://doi.org/10.11613/BM.2014.011 PMid:24627718

Gansler, T., Kaw, C., Crammer, C., Smith, T. 2008. A population-based study of prevalence of complementary methods use by cancer survivors: a report from the American Cancer Society's studies of cancer survivors. *Cancer*, vol. 113, no. 5, p. 1048-57. https://doi.org/10.1002/cncr.23659 PMid:18680170

Guo, J., Wu, W., Sheng, M., Yang, S., Tan, J. 2013. Amygdalin inhibits renal fibrosis in chronic kidney disease. *Molecular Medicine Reports*, vol. 7, no. 5, p. 1453-1457. PMid:23525378

Halenár, M., Medveďová, M., Maruniaková, N., Kolesárová, A. 2015a. Ovarian hormone production affected by amygdalin addition in vitro. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, special no. 2, p. 19-22.

Halenár, M., Medveďová, M., Maruniaková, N., Kolesárová, A. 2015b. Assessment of a potential preventive ability of amygdalin in mycotoxin-induced ovarian toxicity. *Journal of Environmental Science and Health B*, vol. 50, no. 6, p. 411-416. https://doi.org/10.1080/03601234.2015.1011956 PMid:25844862

Halenár, M., Tušimová, E., Nynca, A., Sadowska, A., Ciereszko, R., Kolesárová, A. 2016. Stimulatory effect of amygdalin on the viability and steroid hormone secretion by porcine ovarian granulosa cells *in vitro*. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 5, special no. 1, p. 44-46.

Heinonen-Tanski, H., Sjöblom, A., Fabritius, H., Karinen, P. 2007. Pure human urine is a good fertiliser for cucumbers. *Bioresource Technology*, vol. 98, no. 1, p. 214-217. https://doi.org/10.1016/j.biortech.2005.11.024 PMid:16413181

Henneberg, W., Stohmann, F. 1860. *Beitrage zur Begrundung einer rationeller Futterung der Weiderkauer*. Braunschweig : Schmetschke und Sohn. 315 p.

Holzbecher, M. D., Moss, M. A., Ellenberger, H. A. 1984. The cyanide content of laetrile preparations, apricot, peach and apple seeds. *Journal of Toxicology. Clinical Toxicology*, vol. 22, no. 4, p. 341-347. https://doi.org/10.3109/15563658408992565 PMid:6098693

Hunt, K. J., Coelho, H. F., Wider, B., Perry, R., Hung, S., Terry, R. Ernst, E. 2010. Complementary and alternative medicine use in England: results from a national survey. *International Journal of Clinical Practice*, vol. 64, no. 11, p. 1496-1502. https://doi.org/10.1111/j.1742-1241.2010.02484.x PMid:20698902

Jakubcova, Z., Zeman, L., Mares, P., Mlcek, J., Jurikova T., Dostalova, L., Mrazkova, E., Mrkvicova, E., Balla, S., Sochor, J. 2014. Effect of chamomile supplements to feeding doses on antimicrobial parameters in poultry. *Potravinarstvo*, vol. 8, no. 1, p. 228-232. https://doi.org/10.5219/383

Kanbara, A., Miura, Y., Hyogo, H., Chayama, K., and Seyama, I. 2012. Effect of urine pH changed by dietary intervention on uric acid clearance mechanism of pH-dependent excretion of urinary uric acid. *Nutrition Journal*, vol. 11, no. 1, p. 39. https://doi.org/10.1186/1475-2891-11-39 PMid:22676161

Karak, T., Bhattacharyya, P. 2011. Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality. *Resources, Conservation and Recycling*, vol. 55, no. 4, p. 400-408. https://doi.org/10.1016/j.resconrec.2010.12.008

Kirchmann, H., Pettersson, S. 1994. Human urine – chemical composition and fertilizer use efficiency. *Fertilizer Research*, vol. 40, no. 2, p. 149-154. https://doi.org/10.1007/BF00750100

Kjeldahl, J. 1883. New method for the determination of nitrogen in organic substances. *Zeitschrift für analytische Chemie*, vol. 22, no. 1, p. 366-383. https://doi.org/10.1007/BF01338151

Kovacova, V., Omelka, R., Sarocka, A., Sranko, P., Adamkovicova, M., Toman, R., Halenar, M., Kolesarova, A., Martiniakova, M. 2016. *Potravinarstvo*, vol. 10, no. 1, p. 393-399. https://doi.org/10.5219/625

Lee, H. M., Moon, A. 2016. Amygdalin Regulates Apoptosis and Adhesion in Hs578T Triple-Negative Breast Cancer Cells. *Biomolecules & therapeutics*, vol. 24, no. 1, p. 62-66. https://doi.org/10.4062/biomolther.2015.172 PMid:26759703

Lv, W. F., Ding, M. Y., Zheng, R. 2005. Isolation and quantitation of amygdalin in apricot kernel and *Prunus Tomentosa Thunb.* by HPLC with solid phase extraction. *Journal of Chromatographic Science*, vol. 43, no. 7, p. 383-387. https://doi.org/10.1093/chromsci/43.7.383 PMid:16176653

Magee, E. A., Curno, R., Edmond, L. M., and Cummings, J. H. 2004. Contribution of dietary protein and inorganic sulfur to urinary sulfate: Toward a biomarker of inorganic sulfur intake. *The American Journal of Clinical Nutrition*, vol. 80, no. 1, p. 137-142. PMid:15213040

Mendelová, A., Mendel, L., Czako, P., Mareček, J. 2016. Evaluation of carotenoids, polyphenols content and antioxidant aktivity in the sea buckthorn fruit juice. *Potravinarstvo*, vol. 10, no. 1, p. 59-64. https://doi.org/10.5219/551

Michalcová, K., Halenár, M., Tušimová, E., Kováčik, A., Chrastinová, Ľ., Ondruška, Ľ., Jurčík, R., Kolesárová, A. 2016. Blood plasma levels of anterior pituitary hormones of rabbits after apricot seed exposure *in vivo*. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 1241-1252. https://doi.org/10.5513/JCEA01/17.4.1838

Milazzo, S., Ernst, E., Lejeune, S., Boehm, K., Horneber, M. 2011. Laetrile treatment for cancer. *The Cochrane Database of Systematic Reviews*, no. 2, p. CD005476. https://doi.org/10.1002/14651858.cd005476.pub3

Miller, K. W., Anderson, J. L., Stoewsand, G. S. 1981. Amygdalin metabolism and effect on reproduction of rats fed apricot kernels. *Journal of Toxicology and Environmental Health*, vol. 7, no. 3-4, p. 457-467. https://doi.org/10.1080/15287398109529994 PMid:7288898

Moertel, C. G., Fleming, T. R., Rubin, J., Kvols, L. K., Sarna, G., Koch, R., Currie, V. E., Young, C. W., Jones, S. E., Davignon, J. P. 1982. A clinical trial of amygdalin (Laetrile) in the treatment of human cancer. *The New England Journal of Medicine*, vol. 306, no. 4, p. 201-206. https://doi.org/10.1056/NEJM198201283060403 PMid:7033783

Putnam, D. F. 1971. Composition and concentrative properties of human urine (CR-1802) [online]. Washington, DC: NASA. p. 38. [cit.2017-01-28] Available at: https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19710023044.pdf.

Rose, C., Parker, A., Jefferson, B., Cartmell, E. 2015. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Critical Reviews in Environmental Science and Technology*, vol. 45, no. 1, p. 1827-1879. https://doi.org/10.1080/10643389.2014.1000761 PMid:26246784

Santos Pimenta, L. P., Schilthuizen, M., Verpoorte, R., Choi, Y. H. 2013. Quantitative analysis of amygdalin and prunasin in *Prunus serotina Ehrh.* using (1) H-NMR spectroscopy. *Phytochemical analysis : PCA*, vol. 25, no. 2, p. 122-126. PMid:24115144

Seghers, L., Walenbergh-van Veen, M., Salome, J., Hamberg, P. 2013. Cyanide intoxication by apricot kernel ingestion as complimentary cancer therapy. *The Netherlands Journal of Medicine*, vol. 71, no. 9, p. 496-498. PMid:24218429

Seigler, D. S. 1975. Isolation and characterization of naturally occurring cyanogenic compounds. *Phytochemistry*, vol. 14, no. 1, p. 9-29. https://doi.org/10.1016/0031-9422(75)85001-1

Sokolsky, M. L., Wargovich, M. J. 2012. Homeostatic Imbalance and Colon Cancer: The Dynamic Epigenetic Interplay of Inflammation, Environmental Toxins, and Chemopreventive Plant Compounds. *Frontiers in oncology*, vol. 2, no. 57, p. 1-21. https://doi.org/10.3389/fonc.2012.00057

Song, Z., Xu, X. 2014. Advanced research on anti-tumor effects of amygdalin. *Journal of Cancer Resesarch & Therapy*, vol. 1, p. 3-7.

Soxhlet, F. 1879. Die gewichtsanalytische Bestimmung des Milchfettes. *Dinglers Polytechnisches Journal*, vol. 232: p. 461-465.

Tušimová, E., Kováčik, A., Halenár, M., Zbyňovská, K., Chrastinová, Ľ., Ondruška, Ľ., Jurčík, R., Kolesár, E., Kolesárová, A. 2016a. Energetic profile of rabbits after amygdalin administration. *Journal of Microbiology*, Biotechnology and Food Sciences, vol. 5, special no. 1, p. 50-52.

Tušimová, E., Kováčik, A., Halenár, M., Zbyňovská, K., Tomková, M., Tirpák, F., Chrastinová, Ľ., Ondruška, Ľ., Jurčík, R., Kolesár, E., Kolesárová, A. 2016b. Hepatic profile of female rabbits after amygdalin administration. In *12th International Scientific Conference Animal Physiology 2016 : proceedings*. Bořetice : Mendel University in Brno, p. 253-259. ISBN 978-80-7509-416-2.

Vasudevan, D. M., Sreekumari, S., Vaidyanathan, K. 2011. Textbook of Biochemistry for Medical Students, 6th edition. New Delhi : Jaypee Brothers Medical Publishers. p. 332-339. https://doi.org/10.5005/jp/books/11333

Zhou, C., Qian, L., Ma, H., Yu, X., Zhang, Y., Qu, W., Zhang, X., Xia, W. 2012. Enhancement of amygdalin activated with β-D-glucosidase on HepG2 cells proliferation and apoptosis. *Carbohydrate Polymers*, vol. 90, no. 1, p. 516-523. https://doi.org/10.1016/j.carbpol.2012.05.073 PMid:24751072

Acknowledgments:

This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. 1/0039/16, 011SPU-4/2016 KEGA, APVV-0304-12, and European Community under project no. 26220220180: Building Research Centre „AgroBioTech".

Contact address:

Eva Tušimová, Slovak University of Agriculture, Research Centre AgroBioTech, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: tusimova.eva@gmail.com

Anton Kováčik, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: anton.kovacik@yahoo.com

Marek Halenár, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: halenarmarek@gmail.com

Katarína Michalcová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: kmichalcova86@gmail.com

Katarína Zbyňovská, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: zbynovska.katarina@gmail.com

Anna Kolesárova, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Plant Products Storage and Processing, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: kolesarovanna@gmail.com

Jana Kopčeková, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Human Nutrition, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: jana.kopcekova@gmail.com

Jozef Valuch, Health Care Surveillance Authority, Žellova 2, 829 24 Bratislava, Slovakia, E-mail: dodovaluch@gmail.com

Adriana Kolesárová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: adriana.kolesarova@uniag.sk