DOES APRICOT SEEDS CONSUMPTION CAUSE CHANGES IN HUMAN URINE?

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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 nitrosides 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 C₁₅H₂₁N₂O₁₃, 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...
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 (Nitriansky 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

<p>| Table 1 Organic content in apricot seeds (%) |</p>
<table>
<thead>
<tr>
<th>Dry matter</th>
<th>Amygdalin</th>
<th>Crude protein</th>
<th>Fat</th>
<th>Crude fibre</th>
<th>Starch</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>95.9</td>
<td>5.8</td>
<td>22.8</td>
<td>39.7</td>
<td>28.5</td>
<td>2.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

<p>| Table 2 Dry matter (%) and mineral content (mg.kg⁻¹) in apricot seeds. |</p>
<table>
<thead>
<tr>
<th>Dry matter</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>95.9</td>
<td>1774</td>
<td>4700</td>
<td>2050</td>
<td>642</td>
<td>5925</td>
</tr>
</tbody>
</table>
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.

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

Note. The level of significance was set at *p < 0.05, **p < 0.01, ***p < 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](image) Na
*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](image) K
*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](image) Cl
*Figure 6* Urine levels of chlorides in men and women during 42 days of experiment (mean ±SEM). The level of significance was set at *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \).
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 21\(^{st}\) day of experiment. In the female group, a slight decrease of P content was observed, though all the values were in the reference range \((20 – 50 \text{ mmol.L}^{-1})\) (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 \((146 \text{ mmol.L}^{-1})\) 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 \((0.6 \text{ and } 3.0 \text{ mg.kg}^{-1})\) 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.

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