EFFECT OF APRICOT SEEDS ON RENAL STRUCTURE OF RABBITS

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ABSTRACT
Amygdalin is the major cyanogenic glycoside present in apricot seeds and is degraded to cyanide by chewing or grinding. The animal data available did not provide a suitable basis for acute human health hazard. The apricot seeds are potentially useful in human nutrition and for treatment of several diseases especially cancer. The present study demonstrates the potential effect of short-term oral application of apricot seeds on renal structure of rabbit as a biological model. Meat line P91 Californian rabbits from the experimental farm of the Animal Production Research Centre Nitra (Slovak Republic) were used in the experiments. The animals were randomly divided into the three groups (C-control, P1, P2 – experimental groups) leading to 8 rabbits in each group. The control group received no apricot seeds while the experimental groups P1 and P2 received a daily dose 60 and 300 mg.kg\(^{-1}\) b.w. of crushed apricot seeds mixed with feed during 28 days, respectively. After 28 days all animals were slaughtered and kidney tissue was processed by standard histopathological techniques. Tissue sections were observed under an optical microscope with camera Olympus CX41 (Olympus, Japan) at a magnification of 10 x 0.40. The basic morphometric criteria of the preparations were quantified using image program MeasurIT (Olympus, Japan). From each sample (n = 24) three histological sections with five different fields of view in each section were analysed and followed parameters were analysed: diameter of renal corpuscles (RC), diameter of glomeruli (G), diameter of tubules (T) and the height of epithelial tubules (E). In our study, we observed a slight increase in the most frequent occurrence parenchyma dystrophy experimental animals. These changes were more pronounced in the experimental group (P2) rabbits received a daily dose of 300 mg.kg\(^{-1}\) of body weight of apricot seeds. Most often, we have found enlarged glomeruli filling the entire space of the capsule, and also glomerular basement membrane thickening. The most frequent alterations of tubular organs manifested by thickening and dilatation of proximal tubules and in the lumen of the occurrence fuchsinophilic mass, grains and hyaline cylinders. The occurrence of the vacuole and parenchymal atrophy was mostly balanced groups. Changes in P2 group are also reflected in morphometric evaluation structures. We have found significant decrease (p <0.001) in the average of all renal structures (diameter of renal corpuscles, diameter of glomeruli, diameter of tubules, and the height of epithelial tubules). Inversely, oral administration a daily dose of 60 mg.kg\(^{-1}\) of body weight of apricot seeds had no significant impact on these parameters. The change displays only the increase of renal tubule diameter. Our data may provide more specific evidence of oral application of apricot seeds on renal structure but further detailed studies are also required.

Keywords: seeds; amygdalin; rabbits; kidney

INTRODUCTION
Apricot (Armeniaca vulgaris L.) is typically ingested as fruits (Hakan et al., 2009). The fruit seeds of apricot trees are classified according to their taste into sweet apricot, semi-bitter apricot, and bitter apricot. Apricot seeds are found as an ingredient in a variety of processed foods, including baked and confectionery products (Lee et al., 2013). Apricot seeds contain a wide variety of bioactive components, and that consumption of apricot kernel has been associated with a reduced risk of chronic diseases (Zhang et al., 2011). Bitter apricot seeds have long been used in Chinese traditional medicine for the treatment of asthma, bronchitis, emphysema, constipation, nausea, leprosy, leucoderma, and pain (Bensky et al., 2004). It is a natural product that owns antitumor activity, less side effects and relatively low priced (Song and Xu, 2014). Alternative cancer therapy represents a variety of treatments used by cancer patients for cancer prevention, treatment or management of symptoms caused by the malignancy or cancer therapies (Balmer, 1998). Natural plant substances like amygdalin are still a major part of traditional medicine. However, its effect on animal and...
human organisms is still not clear (Kováčová, 2016). The use of apricot seeds for human nutrition is limited because of their content of the toxic, cyanogenic glycoside amygdalin, accompanied by minor amounts of prunasin (Gomez et al., 1998). Many edible plants contain cyanogenic glycosides, whose concentrations can vary widely as a result of genetic and environmental factors, location, season, and soil types (JECA, 1993). Amygdalin and prunasin are members of a large class of natural products called CNGs (cyanogenic glycosides) (Yamaguchi et al., 2014). In intact apricot kernels, amygdalin and its catabolic enzymes are stored in separate compartments and are brought into contact by physical processes such as grinding or churning thereby releasing hydrocyanic acid (HCN). Complete degradation of 1 g of amygdalin releases 59 mg HCN (EFSA, 2016). Hydrogen cyanide can be produced by hydrolytic reaction catalysed by one or more enzymes from the plants containing cyanogenic glycosides. In kernels, for example, this reaction is catalysed by the enzyme emulsin (Lasch and El Shawa, 1981) when the seeds are crushed and moistened. Amygdalin (which is also present in cassava, bitter almonds, and peach stones) is converted to glucose, benzaldehyde, and hydrogen cyanide (IPCS, 1992). Liberation of hydrogen cyanide from cyanogenic glycosides occurs usually after ingestion and hydrolysis by the glycosidases of the intestinal microflora and, to a lesser degree, by glucosidases of the liver and other tissues (Padmaja, 1995). Kidney as a central apparatus of urinary system is exposed to high demands for ensuring the homeostasis of the organism (Jelínek et al., 2003). Kidneys have an important role in the body. Any damage to kidney role can damage many organs of the body (Mahjour et al., 2017). The present study demonstrates the potential effect of short-term oral application of apricot seeds on renal structure of rabbit as a biological model.

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 (5.2%) 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 uL of solution was applied onto TLC plates Kieselgel UV 254 20x20 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 densitometry at 205 nm. An external standard was used (1% amygdalin solution in methanol).

Animals

Twenty four healthy rabbits females of meat line P91 (Californian rabbit) from the experimental farm of the Animal Production Research Centre Nitra (Slovak Republic) were used for the purpose of this study. The rabbits were 150 days old, weighing 4.00 ±0.5 kg, and were housed in individual wire cages and kept in 12-h dark/light cycle, at a temperature between 20 – 24 °C and humidity 55% ±10%. The rabbits were fed a standard commercially available feed based on a pelleted concentrate. Animals had free access to feed and water during the study period and no toxic or side effects or death was observed throughout the study. The animals were randomly divided into the three groups (C-control, P1, P2 – experimental groups) leading to 8 rabbits in each group. The control group received no apricot seeds while the experimental groups P1 and P2 received a daily dose 60 and 300 mg.kg⁻¹ b.w. of crushed apricot seeds mixed with feed during 28 days, respectively. The body weight of each experimental animal was recorded weekly during the whole study. Conditions of animals care, manipulations and use corresponded with the instruction of ethical commission. Care and use of animals and experimental devices met the requirement of the certificate of Authorization to Experiment on Living Animals, no. 3398/11-221/3 (certified by State Veterinary and Food Institute of Slovak Republic). All efforts were made to minimize suffering.

Collection of tissue samples

The animals were killed by technology used for the Animal Production Research Centre Nitra – electrocution and then bled. Using surgical scissors were taken from the abdominal organs. The kidneys were evaluated macroscopically (visual) and processed for histological analysis. Kidney samples were washed in physiological saline solution and then individually weighed. For histopathological and histochemical examinations, small pieces of the liver and kidney were collected and rinsed in 10% buffered neutral forminal solution.

Histopathological analysis

For histopathological examinations, the kidney tissue samples (n = 24) were fixed in 10% neutral buffered formalin (Sigma-Aldrich), dehydrated with (of) ethanol (70% and 96% 2 hours 100% 1 hour) and embedded in paraffin wax. The samples were cut rotary microtome AC-820 (American Corporation, USA) cut rotary microtome AC-820 (American Corporation, USA) and stained with hematoxyline eosin (H & E). Stained sections were mounted in Entelan and examined with an Olympus CX41 optical microscope with camera (Olympus Optical Co., Osaka, Japan) at a magnification of 10x40.

The basic morphometric criteria of the preparations were quantified using image program MeasurIT (Olympus, Japan). From each sample three histological sections with five different fields of view in each section were analysed and followed parameters were analysed: diameter of renal corpuscles (RC), diameter of glomeruli (G), diameter of tubules (T) and the height of epithelial tubules (E).

Statistical analysis

Statistical analyses were performed using the program STATISTICA Cz version 10 belonging to the available statistical programs. All values were expressed as mean ±standard deviation (SD). Differences between control and experimental groups were assessed by Tukey HSD test (one-way ANOVA). Differences from controls (p <0.05, p <0.01, p <0.001) were considered as significant.
RESULTS AND DISCUSSION

Kidney is a parenchyma apparatus with microscopic structure looks like tubular gland. Kidney’s parenchyma consists of nephron and intrarenal efferent urinary tract. The nephron consists of capillary tuft (glomerulum) coated by two-bladed Bowman’s capsule (capsula glomerula) and known as renal corpuscle (corpusculum renis) (Lukáč et al., 2006). After the pathological-anatomical autopsy we assess kidney macroscopically. We found out russet color, fabaceous shape of rabbit kidney with a smooth surface, cover by a gentle fibrosis casing. Kidney parenchyma on section showed no pathological damage. On the histological preparations were clearly formed glomeruli encapsulated two-bladed Bowmanov coated casing, which produced typically renal corpuscle. As the excisions came from cortex area of kidney, marked representation was created by proximal convoluted tubules.

Figure 1 Representative photomicrographs of kidney sections from control groups: normal renal architecture – renal corporcles (RC), glomeruli (G), tubules (T).

Figure 2 Representative photomicrographs of kidney sections from experimental P1 groups: part of atrophic renal parenchyma with renal corpuscle and left dark atrophic glomerulus.

Figure 3 Representative photomicrographs of kidney sections from experimental P2 groups: in the tubules are visible dark hyaline cylinders.
The results of histopathological analysis of the control (C) and experimental (P1, P2) rabbit kidney samples presented in the summary table (Table 1), prepared from data obtained by examination of histological slides of all (n = 24) and the section (n = 72). Control rabbits’ kidney sections showed normal architecture of the characteristic renal parenchyma with normal renal tubular epithelium (Figure 1). Most often, we found enlarged glomeruli filling the entire space of the capsule, and also glomerular basement membrane thickening. The proximal tubule is uniquely susceptible to a variety of metabolic and hemodynamic factors (Thomas et al., 2005). The most frequent alterations of tubular organs manifested by thickening and dilatation of proximal tubules and in the lumen of the occurrence fuchsinophilic mass, grains and hyaline cylinders. The occurrence of the vacuole and parenchymal atrophy was mostly balanced groups (Figure 2 and Figure 3).

Table 1

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>Glomeruli expansion</th>
<th>thickening of bm</th>
<th>Dystrophic changes of tubules</th>
<th>vacuoles</th>
<th>atrophy p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+ fm</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P1</td>
<td>8</td>
<td>++</td>
<td>++</td>
<td>++ fm, hc, dt</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P2</td>
<td>8</td>
<td>++</td>
<td>++</td>
<td>++ fm, hc, dt, fg</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: intensity of change: + low, ++ medium, +++ high, bm- basement membrane; pk- proximal convoluted tubule; atrophy p- parenchymal atrophy; pf- periglomerular fibrosis; fm- fuchsinophilic mass; fg- fuchsinophil grains; h- hyaline cylinder; dt- tubular dilatation.

Table 2

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>Diameter of renal corpuscles (µm) (µm)</th>
<th>Diameter of glomeruli (µm)</th>
<th>The height of epithelial (µm)</th>
<th>Diameter of tubules (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>255</td>
<td>129.44 ±23.67</td>
<td>111.86 ±20.36</td>
<td>15.20 ±2.78</td>
<td>55.88 ±7.73</td>
</tr>
<tr>
<td>P1</td>
<td>175</td>
<td>130.08 ±22.14</td>
<td>110.43 ±17.83</td>
<td>15.55 ±2.85</td>
<td>58.88 ±7.73</td>
</tr>
<tr>
<td>P2</td>
<td>266</td>
<td>118.01 ±26.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.36 ±23.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.79 ±3.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.88 ±9.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: data are expressed as the mean ±SD, p-values resulted from ANOVA, a – statistically significant differences compared to control group, p <0.001, C- control; P1, P2 – experiment.

The proximal tubule is uniquely susceptible to a variety of metabolic and hemodynamic factors (Thomas et al., 2005). The most frequent alterations of tubular organs manifested by thickening and dilatation of proximal tubules and in the lumen of the occurrence fuchsinophilic mass, grains and hyaline cylinders. The occurrence of the vacuole and parenchymal atrophy was mostly balanced groups (Figure 2 and Figure 3).

Glomerular filtration rate has a central role in the pathophysiology of chronic kidney disease complications. Chronic kidney disease occurs when the impaired kidney function persists for three months or more. In this disorder, there is a decrease kidney function based on the presence of kidney damage (Levey et al., 2012).

In our study morphometric evaluation of each kidney structures (Table 2) showed a decrease diameters of renal corpuscles and diameter of glomeruli in P2 group when compared to the control groups. These changes were statistically significant (p <0.001). Diameters of renal corpuscles decreased about 11.43 µm and a diameter of glomeruli decreased about 9.5 µm. The differences between C and P1 group were no significant in these structures. The authors (Tulsawani et al., 2005) found no significant changes in body weight and organ-body index in the treatment group Female Wistar rats dosed by gavage to 7 mg KCN/kg<b>1</b> b.w. once daily for 14 days. Glomerular congestion, tubular lesions displaying vacuolar degeneration at proximal tubular epithelial cells and scattered tubule disorganisation due to damaged renal parenchyma were also observed. Other authors have found that marked changes take place in tubular epithelial cells in experimental postischemic (ischemia-reperfusion) rat kidney (Aunapuu et al., 2005).

Most of the substances presented in the glomerular filtrate were in a greater or lower extent absorbed in the tubules (Trojan, 1992). In the assessment the average of the tubules diameter, we found a statistically significant increase (p <0.001) in the groups P1 (about 3.0 µm) and significant decrease (about 6.53 µm) in the group P2 in comparison with the control group. Epithelium height was statistically significant decrease (p <0.001) only in the C/P2 group (about 1.41 µm). The differences between C and P1 group were remained insignificant.

Sousa et al. (2002) found moderate to severe congestion and cytoplasmic vacuolisation of the epithelial cells of the kidney proximal tubules at fifteen-day study male adult Wistar rats treated with KCN in drinking water adjusting KCN concentration to body weight and water consumption in order to administer 3.0 or 9.0 mg.Kg⁻¹ per day.

Hydrogen cyanide after oral administration is ready absorbed. After absorption, cyanide is rapidly distributed in the body through the blood (EPA, 1990). Cyanogenic glycosides are hydrolyzed by β-glucosidase produced by intestinal bacteria to glucose, HCN and benzaldehyde or acetone (Oke, 1979). Beta-glucosidase was demonstrated in cat, rat and rabbit kidney tissue that catalyzed the hydrolytic cleavage of terminal glucose residue of amygdalin (Freeze et al., 1980).

In a 13-week study, male Sprague-Dawley rats were administered potassium cyanide in drinking-water at a dose level of 40, 80, or 160/140 mg.Kg⁻¹ body weight per day. Histopathological investigation of the brain, heart, liver, testes, thyroid, and kidneys did not reveal adverse effects (Leuschner et al., 1989). Acute oral median lethal dose (LD50) values for cyanide in laboratory animals range from 2.13 to 6 mg.Kg⁻¹ body weight. Lethal levels of cyanide lead to dyspnoea, irregular and gasping breathing, ataxia, tremor, spasms, loss of consciousness, convulsions and eventually asphyxiation. Short-term dietary exposure...
to cyanide leads to histopathological changes and alterations in organ weights (EFSA, 2016).

In none of the reported short-term studies was mortality observed at doses up to 40 mg CN.kg⁻¹ bw per day, even though some of the doses were equal to or higher than the oral LD50 for cyanide. Since in the short-term studies analysed, cyanide was administered through the diet or drinking water, absence of mortality is possibly due to a slower absorption rate following dietary exposure, thus not exhausting the detoxification capacity of the enzyme rhodanese, which occurs after bolus administration in LD50 tests (Hayes, 1967; US EPA, 2010).

The dystrophy of parenchyma, which we most frequently observed is the lowest level of regressive damage cells and extracellular tissue. It is a reversible process caused by changes in cell metabolism, manifested by morphological changes. The changes were more pronounced in the experimental group of rabbits which received a daily dose 300 mg.kg⁻¹ b.w. of crushed apricot seeds mixed with feed during 28 days, respectively.

The pathologic findings of renal fibrosis are often described as glomerulosclerosis, tubulo-interstitial fibrosis, inflammatory infiltration, and loss of renal parenchyma characterized by tubular atrophy, capillary loss, and podocyte depletion (Liu, 2006). Cyanide causes a decrease in the utilization of oxygen in the tissues, producing a state of histotoxic anoxia (Solomonson, 1981). Parenchymal kidney cells contained a small grains or larger or smaller vacuoles of watery fluid. Slightly increased incidence of parenchymal organs dystrophy experimental rabbits could cause damage in the circulatory system and insufficient supply of oxygen-hypoxia or nutrient substances to the cells, but also an excessive load on the kidney function in pregnancy.

CONCLUSION

In our short-term study, we most often observed a slight increase in the incidence of renal parenchyma dystrophy of experimental animals. The changes were more pronounced in the experimental group of rabbits which received a daily dose 300 mg.kg⁻¹ b.w. of apricot seeds. Changes in this group is also reflected in morphometric evaluation changes in this group is also reflected in morphometric evaluation structures and a significant decrease in the average of all renal structures (diameter of renal corpuscles, diameter of glomeruli, diameter of tubules, and the height of epithelial tubules). Inversely, oral administration a daily dose of 60 mg.kg⁻¹ of body weight of apricot seeds had no significant impact on these parameters. The change displays only the increase of renal tubules diameter. These changes inform about possible nephrotic dysfunction and thereby disrupt the homeostasis of urinal excretion, but further detailed studies are also required.

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Potravinarstvo Slovak Journal of Food Sciences


Acknowledgments:

This work was financially supported by the research projects VEGA, 1/0039/16, KEGA 011SPU–4/2016, APVV-0304-12, and European Community under project no 26220220180: Building Research Centre „AgroBioTech“.

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