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THE EFFECT OF APRICOT SEEDS ON MICROSCOPIC STRUCTURE OF RABBIT LIVER

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ABSTRACT

Natural phytosubstances, such as amygdalin, used in alternative medicine has gained popularity. However, some researchers suspect the protective properties of amygdalin due to a lack of clinical studies. The aim of the present *in vivo* study was to determine the effect of apricot seed administration on microscopic changes in the liver using a rabbit as a biological model. Sixteen male rabbits 45 days old were randomly divided into four groups (control group without any apricot seed administration, and experimental groups fed by crushed apricot seeds at the doses 60, 300 and 420 mg/kg b.w., mixed with commercial feed), which was administered orally a daily during a ten-month period. The liver tissue samples were evaluated by histological analysis. Significant changes were observed in the microscopic structure of rabbit livers after apricot seed ingestion. The morphometric evaluation of rabbit livers after the application of apricot seeds showed an increase of binucleated cells in the *vena centralis* region ($P \leq 0.001$) at the highest dose and in the peripheral zone at all the doses used ($P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$) compared to control. On the other hand, distinct inhibition in the number of binucleated cells in the region *vena centralis* at the doses 300 ($P \leq 0.01$) and 420 mg/kg b.w. ($P \leq 0.05$) and in the peripheral zone at all the doses used ($P \leq 0.001$, $P \leq 0.01$) was observed. No significant differences between the control and experimental groups in *vena centralis* after apricot seed treatment were found ($P \geq 0.05$). In addition, the effect of apricot seeds on the relative volume of liver structures – *vena centralis*, stroma and parenchyma after the application of apricot seeds to rabbit males were assessed. No significant differences between control and experimental groups in the relative volume of *vena centralis* were found ($P \geq 0.05$). On the other hand, the relative volume of the stroma was increased at doses 60 ($P \leq 0.05$) and 300 ($P \leq 0.01$) mg/kg b.w. Interestingly, the relative volume of parenchyma was significantly decreased ($P \leq 0.05$) after the application of apricot seeds in two experimental groups 60 and 300 mg/kg b.w. The current study provides experimental evidence that apricot seeds might affect the liver microscopic structure in rabbits *in vivo* and thus amygdalin present in apricot seeds might present a potential risk for animal health. However, the toxic effect could not be accurately corroborated, as in many cases changes were dose-dependent and not recorded at the highest dose used in the study.

Keywords: amygdalin, apricot seed, liver, rabbit

INTRODUCTION

The field of nutritional research focuses on phytonutrients and their beneficial properties on health. Phytonutrients, which include in particular polyphenols, flavonoids (isoquercitrin, rutin, quercetin, and others) **(Roychoudhury *et al.*, 2018; Kolesarova *et al.*, 2019)**, ellagitannins, especially punicalagin and ellagic acid **(Packova *et al.*, 2015)**, glycosides (amygdalin) **(Halenar *et al.*, 2015, 2017; Kolesar *et al.*, 2018; Kopcekova *et al.*, 2018; Kovacikova *et al.*, 2019)** and others are known to possess potential antioxidant and antitumor activity and the ability to eliminate environmental stress-induced reprotoxicity **(Kolesarova *et al.*, 2012; Halenar *et al.*, 2015)**.
Cancer is a major public health problem worldwide and is the second leading cause of death in the United States **(Siegel *et al.*, 2018)**. Despite advances in radiotherapy and chemotherapy, problems related to these therapies such as side effects and development of drug resistance remained unsolved **(Rogers *et al.*, 2012)**. One of the strategies to solve these problems is to develop novel therapies. In the research for the alternative therapies for cancer treatment, it has been noticed that a natural cyanide-containing substance, amygdalin (AMG), has a gaining reputation as a complementary substance for cancer treatment due to its effectiveness in inhibiting the growth of cancer cells and easy availability **(Chang *et al.*, 2006; Park *et al.*, 2005)**.
Amygdalin is found in the family *Rosaceae*, especially in apricot seeds and almonds which contain vitamins, carbohydrates, organic acids, esters, phenols, terpenoids, except cyanogenic glycoside in the seeds **(Michalcová *et al.*, 2016)**. Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-D-glucoside), a natural substance used in alternative medicine for its many beneficial properties **(Tanwar *et al.*, 2019)**, is a cyanogenic glycoside plant toxin contained in relatively high concentrations in the kernels and seeds of apples, apricots, almonds, cherries, and peaches, and it is abundant in plum seeds **(Lee *et al.*, 2017)**. Seeds contain amygdalin depending on the variety: approximately 20–80 μmol/g AMG may be found in apricot seeds, and its concentration is very high (5.5 g/100 g) in bitter apricot cultivars while it is not detected in the sweet ones **(Kolesar *et al.*, 2018)**. Amygdalin is composed of two molecules of glucose, one molecule of benzaldehyde, and one molecule of hydrocyanide. Amygdalin could be a new therapeutic substance for cancer patients although there has been controversy surrounding the use of AMG as a cancer drug due to concerns of cyanide toxicity (**Chen *et al.*, 2013**).
The present study was designed to reveal the effects of apricot seed administration at the doses of 60, 300 and 420 mg/kg on microscopic changes in the liver of rabbits *in vivo*.

MATERIAL AND METHODS

Animals

For the purpose of this study rabbits of meat line P91 (Californian rabbit) from the experimental farm of the Research Institute for Animal Production Nitra (Slovak Republic) were used. The rabbits were housed in breeding cages for

laboratory rabbits in daylight mode 12 h light/12 h dark, at a temperature ranging 20 – 24 °C and humidity 55 ±10 %. All animals had permanent access to water during the experimental ten-month period, which was secured by automatic feeders. Daily amounts of feed (200 g) were administered to the animals, and the rest of the feed was regularly recorded. Conditions of animal care, manipulations, and use corresponded with the instructions of the institutional ethical commission. Care and use of animals and experimental devices met the requirements for the certificate of Authorization to Experiment on Living Animals no. 3398/11-221/3 (certified by State Veterinary and Food Institute of the Slovak Republic). All efforts were made to minimize the suffering of animals.

Application of apricot seeds

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. The chemical composition of the apricot seeds was carried out **(Halenar et al., 2017)**. Animals 45 days old were randomly divided into four groups (C-control, P1, P2, P3-experimental groups) leading to 4 male rabbits in each group. The control group received no apricot seeds while the experimental groups P1, P2, and P3 received a daily dose of 60, 300 and 420 mg/kg b.w. of crushed apricot seeds mixed with feed. Apricot seeds were crushed and processed into a commercially available granulated feed, which was administered orally a day for 10 months. The first experimental group of rabbits was given the lowest dose of 60 mg/kg b.w., the dose of 300 mg/kg b.w. to the second experimental group, and a dose of 420 mg/kg b.w. of apricot seeds to the third experimental group of animals. After 10 months of *in vivo* apricot seed administration, the animals (weighing 4.0±0.5 kg) were sacrificed by electric stunning and subsequent bleeding at the Research Institute for Animal Production Nitra (Slovak Republic).

Histopathological analysis

For histopathological examinations, the liver tissue samples (n=16) were fixed in 10 % neutral buffered formalin (Sigma-Aldrich), dehydrated with ethanol (70 % and 96 % for 2 h and 100 % for 1 h) and embedded in paraffin wax. The samples were cut using rotary microtome AC-820 (American Corporation, USA) and stained with a buffered solution of basic fuchsin with toluidine blue, diluted in a ratio of 3:2 (Bourne and St. John, 1978). Stained sections were mounted in Entelan and examined under an optical microscope fitted with a camera (Olympus AX 70) with installed software for analysis of image M.I.S. Quick Photo at a magnification of 10x40 and 20x40. The basic morphometric criteria of the preparations were quantified using image program MeasureIT (Olympus, Japan). Structural assessment of the liver was carried out by morphometric parameters in a selected area around the *vena centralis* (934.4 µm and 69,594.1 µm2, respectively) and the peripheral zone (1302.4 µm and 104,215.2 µm2, respectively).

Statistical analysis

Statistical analysis was carried out using the Graph Pad Prism program (version 3.02 for Windows; GraphPad Software, La Jolla California USA, www.graphpad.com). Descriptive statistical characteristics (mean, standard error of the mean) were evaluated at first. One-way ANOVA was used for specific statistical evaluation. Dunnett´s test was used as a follow-up test to ANOVA, based on a comparison of every mean to control mean, and computing a confidence interval for the difference between the two means. The levels of significance were set at P≤0.05, P≤0.01, and P≤0.001.

RESULTS AND DISCUSSION

Amygdalin is one of the main biologically active substances present in apricot seeds, hot almonds, flax seeds, and others **(Tanwar *et al*., 2019).** The effect of AMG present in apricot seeds has been partially described in our previous studies using the porcine model **(Halenar et al., 2015)**, rabbits (**Halenar *et al*., 2017; Kolesar *et al*., 2018; Kovacikova *et al*., 2019)** and humans **(Kopčeková *et al*., 2018; Kovacikova *et al*., 2017**). The objective of this study was to evaluate the effects of apricot seeds on microscopic characteristics of rabbit liver *in vivo*.

The effect of apricot seeds on the microscopic structure of rabbit liver after oral application of apricot seeds at the doses of 60, 300 and 420 mg/kg b.w. for ten months was examined. Binucleated cells in the *vena centralis* region and the peripheral zone are shown in Figs. 1-5. The relative representation of structures of the liver – *vena centralis*, stroma and parenchyma are given in (Figs. 6-8).

The effect of apricot seeds on the morphometric evaluation of rabbit liver after application of apricot seeds on young rabbit males was assessed in this *in vivo* study (Figs. 1-5). A distinct increase (P≤0.001) was observed in the number of binucleated cells in the *vena centralis* region and the peripheral zone at the highest dose compared to control (Fig. 1,4). Likewise, a slight increase was observed at the two lowest doses of apricot seeds compared to control (P≤0.01, P≤0.05; Fig.4). On the contrary, there was a distinct inhibition in the number of binucleated cells in the region of *vena centralis* and the peripheral zone (Fig. 3, 5). A significant decrease was shown at doses 300 (P≤0.01) and 420 mg/kg b.w. (P≤0.05; Fig. 3), and also at the doses 60 (P≤0.01), 300 (P≤0.001), and 420 (P≤0.01) mg/kg b.w. compared to the control group (Fig. 5). No significant differences between the control and experimental groups were noted in *vena centralis* after apricot seed treatment. There was moderate stimulation in all experimental groups, but the results were not conclusive (Fig. 2).

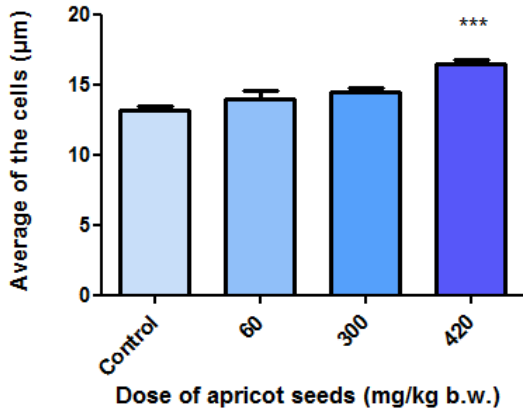

Figure 1 Binucleated cells in the *vena centralis* region after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Significant differences (***P<0.001) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

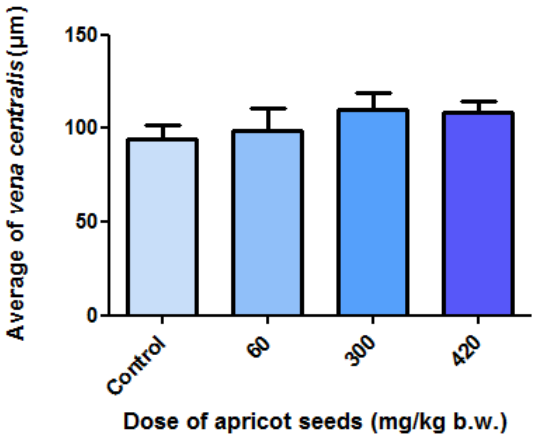

Figure 2 *Vena centralis* diameter after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Differences between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

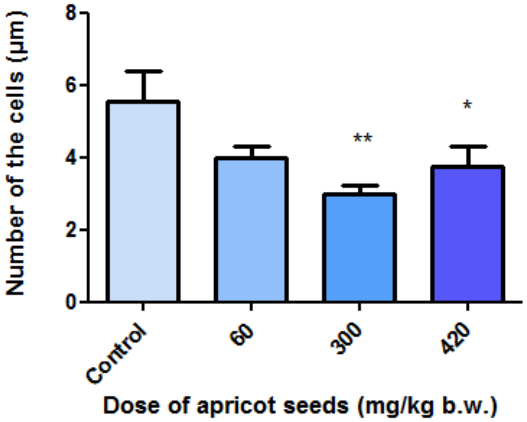

Figure 3 Binucleated cells in the *vena centralis* region after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Significant differences (**P<0.01, * P<0.05) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

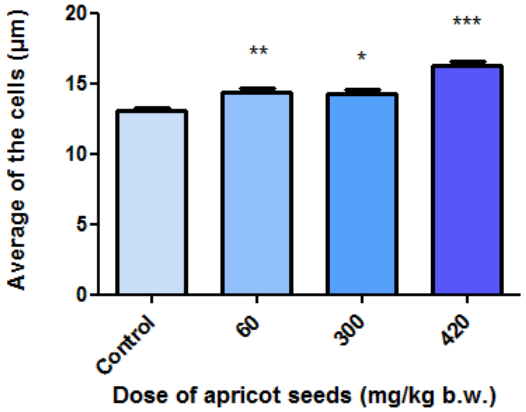

Figure 4 Binucleated cells in the peripheral zone after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Significant differences (***P<0.001, **P<0.01, *P<0.05) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

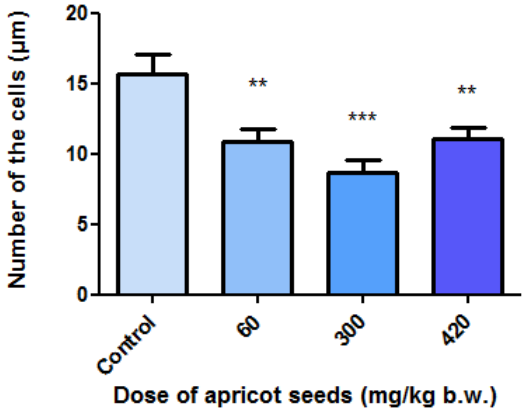

Figure 5 Binucleated cells in the peripheral zone after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significant differences (***P<0.001, **P<0.01) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

The effect of apricot seeds administration on the relative volume of structures of rabbit liver – *vena centralis*, stroma and parenchyma were assessed in this *in vivo* study (Figs. 6-8). No significant (P≥0.05) difference was found between control and experimental groups in the relative volume of *vena centralis*, although a moderate inhibition in two experimental groups of 300 and 420 mg/kg b.w. was observed as compared to the control group without the addition of natural substance (Fig. 6). Significant differences were found in the relative volume of stroma between control and experimental groups 60 (P≤0.05) and 300 (P≤0.01) mg/kg b.w. (Fig. 7). On the other hand, a significant (P≤0.05) inhibition of relative volume of parenchyma was noted after the application of apricot seeds in two experimental groups 60 and 300 mg/kg b.w. in comparison to control (Fig. 8).

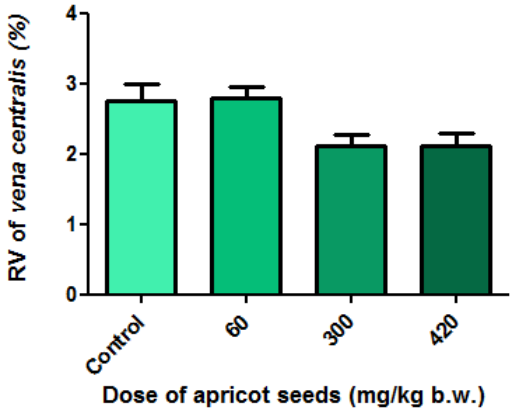

Figure 6 The relative volume of *vena centralis* after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Differences between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

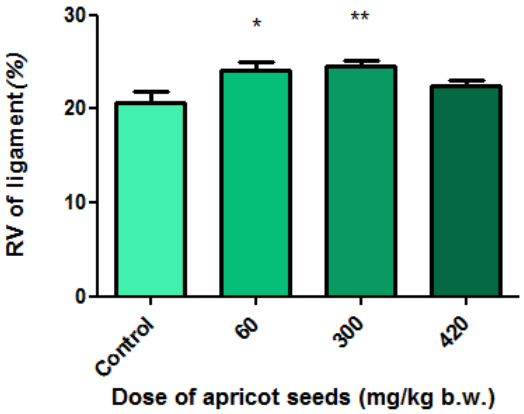

Figure 7 The relative volume of stroma after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Significant differences (**P<0.01, *P<0.05) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

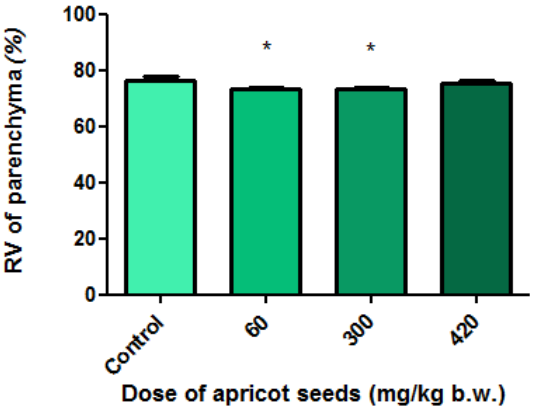

Figure 8 The relative volume of parenchyma after feeding the male rabbits with apricot seeds for 10 months. The control group represents rabbits without the application of apricot seeds. Experimental groups with apricot seeds at doses 60, 300 and 420 mg/kg b.w. Significant differences (*P<0.05) between control and experimental groups were evaluated by One-way ANOVA (Dunnett´s test). Data are expressed as means ± SEM. Histology.

In the current study, the effect of apricot seeds on the microscopic structure of the rabbit liver was investigated. Apricot seeds containing the active ingredient AMG have found widespread use in the treatment of many diseases. There have been several hypotheses that it kills tumour cells selectively at the site of their proliferation without systemic toxicity and can effectively relieve pain in cancer patients **(Michalcová *et al.*, 2016)**. They investigated the effect of AMG present in apricot seeds on the concentration of blood plasma hormones of male and female rabbits *in vivo* at doses 60, 300 and 420 mg/kg b.w. of apricot seeds, which were administered to rabbits orally. The lowest dose of apricot seeds showed a slight increase of prolactin (PRL) concentrations in the blood plasma (P≥0.05) of males. No significant (P≥0.05) difference was observed in the case of PRL and LH levels in the blood plasma of females. On the other hand, a significant (P≤0.05) inhibition of FSH release was induced by seeds at the doses of 300, 420 mg/kg b.w.

Another study examined the inhibitory effects of AMG in itself and activated with β-d-glucosidase on the proliferation of the investigated HepG2 cells. Compared with AMG, the activated ingredients showed a stronger inhibitory effect on the proliferation rate among the cells tested. The findings indicate that AMG had no strong anti-HepG2 activity, however, the ingredients of AMG activated with β-D-glucosidase had a higher and efficient anti-HepG2 activity. It was, therefore, suggested that this combination strategy may be applicable for treating tumours with a higher activity **(Zhou *et al.*, 2012)**.

Chang *et al.* (2006) stated that despite the significant effects of AMG, the Food and Drug Administration (FDA) has not approved it in cancer treatment owing to insufficient clinical evidence of efficacy and potential toxicity of AMG. Despite the failure of clinical tests to demonstrate the anticancer effects of AMG in the USA and in Europe, it continues to be manufactured and administered as an

anticancer therapy in northern Europe and Mexico **(Chang *et al*., 2006; Kwon *et al*., 2010)**. The effect of AMG is generally known in the treatment of cancer. According to **Arshi *et al*. (2019),** AMG an anti-cancer effect on human cancer cell lines, where AMG significantly inhibits the expression level of two anti-apoptotic genes (Survivin, XIAP), showing potential as a natural therapeutic anticancer drug. Some of the studies showed the inhibition of tumour cell growth by AMG **(Juengel *et al*., 2016; Makarević *et al*., 2014; 2016)**.
Other studies have also described AMG toxicity. It is known that the harmful effects of apricot seeds are associated with the cyanide toxicity that is released upon hydrolysis of AMG. According to **Adewusi and Oke (1985)**, AMG toxicity due to cyanide release requires the activity of intestinal microflora. The current review highlights apoptosis-inducing attributes of AMG towards different cancers and its potential application as an anti-cancer agent in cancer therapy. However, well-planned clinical trials are still needed to prove the effectiveness of this substance *in vivo* and for its approval for human use **(Saleem *et al*., 2018)**.

CONCLUSION

Based on the findings, it may be concluded that apricot seeds administered orally to experimental animals had a modulating effect on the microscopic structure of rabbit liver *in vivo*. However, the toxic effect can not be accurately corroborated as in many cases the changes were not recorded at the highest dose used in the study. It is necessary to evaluate the blood parameters particularly hematological and biochemical indicators, including liver enzymes, which might help in either confirming or refuting the toxicity of apricot seeds. Our results suggest that 10 months of consumption of apricot seeds affected the microscopic structure of the liver cells of male rabbits, but as the liver is an organ with a high regenerative capacity, we assume that these dose-dependent changes may also be are reversible.

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