

ENERGETIC PROFILE OF RABBITS AFTER AMYGDALIN ADMINISTRATION

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ABSTRACT

Amygdalin is a cyanogenic glucoside initially obtained from the seeds of bitter almonds (*Prunus dulcis*). It is a natural product that owns antitumor activity and has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes. It is composed of one molecule of benzaldehyde, two molecules of glucose and one molecule of hydrocyanic acid. Cyanide is not cancerocidal as long as glucose is available. The present *in vivo* study was designed to reveal whether amygdalin is able to cause changes in the energetic profile of rabbit as a biological model. A 12 adult male rabbits were randomly divided into three groups: the control group without any amygdalin administration and two experimental groups receiving daily intramuscular injections (IM) of amygdalin at 0.6 and 3.0 mg/kg b.w. respectively over the period of 28 days. The body weight of each experimental animal was recorded weekly during the whole study. Serum levels of energetic profile (glucose, triglycerides and cholesterol) were determined. Intramuscular application of amygdalin did not affect ($P \geq 0.05$) the serum levels of none of selected biochemical parameters significantly. In this *in vivo* study, no obvious beneficial or negative effects of amygdalin on energetic profile of male rabbits were demonstrated.

Keywords: Amygdalin, rabbits, glucose, triglycerides, cholesterol

INTRODUCTION

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). Amygdalin is a cyanogenic glucoside initially obtained from the seeds of bitter almonds (*Prunus dulcis*) (Chwalek and Plé, 2004). It is a major component of the seeds of prunasin family plants, such as apricots, almonds, peaches, apples, and other rosaceous plants (Fukuda *et al.*, 2003). Amygdalin, when pure, is almost entirely harmless (Sollmann, 1949).

Amygdalin (D-mandelonitrile- β -D-gentiobioside), $C_{20}H_{27}NO_{11}$, is composed of one molecule of benzaldehyde, two molecules of glucose and one molecule of hydrocyanic acid, which is an anti-neoplastic compound (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).

As reported by Levi *et al.* (1965) cyanide is not cancerocidal as long as glucose is available. Therefore it is obvious, the energy profile of patients plays important role in clinical research of amygdalin effect.

The present *in vivo* study was designed to reveal whether amygdalin is able to cause changes in the energetic profile of rabbit as a biological model.

MATERIAL AND METHODS

Chemicals

Amygdalin from apricot kernels ($\geq 99\%$ purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amygdalin was freshly dissolved in sterile saline and 0.5 ml were applied intramuscularly (IM) to *musculus biceps femoris* on a daily basis.

Animals

Meat line P91 Californian rabbit males ($n=12$) from the experimental farm of the Animal Production Research Centre Nitra (Slovak Republic) were used in the experiments. The rabbits were 150 days old, weighing 4.00 ± 0.5 kg, and were housed in individual flat-deck wire cages under a constant photoperiod of 12 h of daylight, temperature 20-24 °C and humidity 55 % \pm 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 were observed throughout the study. The animals were randomly divided into the three groups, leading to 4 male rabbits in each group. The control group received no amygdalin while the two experimental groups P1 and P2 received a daily intramuscular injection of amygdalin at a dose 0.6 and 3.0 mg/kg b.w. respectively during 28 days. The body weight of each experimental animal was recorded weekly during the whole study. Institutional and national guidelines for the care and use of animals were followed appropriately, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3 and Ethic Committee.

Blood Sample Collection.

During the experiment, three blood collections were carried out (at the beginning of the experiment 0; after 14 days and 28 days) to control the health of animals. Venous blood from *vena auricularis* was collected into tubes. Blood serum was separated from whole blood by centrifugation at 3000 rpm for 10 min. at 20 °C. The clear supernatant (serum) was then separated from the pellet and kept frozen until analysis.

Analysis

Quantification of glucose, triglycerides and cholesterol after amygdalin supplementation was performed using photometry. Analyses were provided in biochemical and hematological laboratory at the Department of Animal Physiology of SUA through commercial sets DiaSys (Diagnostic Systems GmbH, Germany) on device Rx Monza (Randox Laboratories Ltd., United Kingdom).

Intra-assay, inter-assay coefficients and sensitiveness for selected parameters are shown in Table 1.

Table 1 Intra-assay, inter-assay coefficients and sensitivity for selected parameters

Parameter	Intra-assay coefficient (%)	Inter-assay coefficient (%)	Sensitivity
Glucose	≤1.05	≤3.8	0.22 mmol.l ⁻¹
Triglycerides	≤1.6	≤1.23	0.01 mmol.l ⁻¹
Cholesterol	≤0.95	≤1.09	0.08 mmol.l ⁻¹

Statistical analysis

The significance of differences between the control and experimental groups was evaluated by one-way analysis of variance (ANOVA), with the Dunnett’s multiple comparison test using statistical software GraphPad Prism 3.01 (GraphPad Software Inc., San Diego, CA, USA). The data are expressed as means ± SD. Differences were compared for statistical significance at the *p*-level less than 0.05 (*P*<0.05).

RESULTS AND DISCUSSION

The effect of short-term amygdalin administration on the serum levels of glucose

Serum levels of glucose after short-term IM injection of amygdalin to adult male rabbits were assessed in this *in vivo* study (Figure 1). During 28 days treatment period, no significant (*P*≥0.05) differences in serum levels of glucose were observed when compared to the untreated control group.

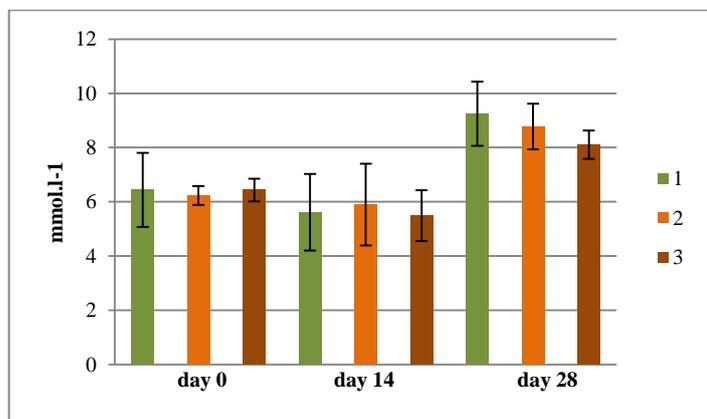


Figure 1 Serum levels of glucose during 28 days. 1 - control group (without amygdalin administration); 2 - group P1 (0.6 mg/kg); 3- group P2 (3.0 mg/kg)

All the measured values of serum glucose were compared to reference range 4.1 – 8.5 mmol.l⁻¹ (Merck Sharp & Dohme Corp., 2012). Two groups did not comply this range – control group (9.25 mmol.l⁻¹) and P1 group (8.78 mmol.l⁻¹), both after 28 days of experiment. On the other hand, Özkan et al. (2012) indicate there are some differences in reference ranges between male (3.83-10.77 mmol.l⁻¹) and female (4.94-8.32 mmol.l⁻¹) rabbits. It has been reported that increased glucose levels in rabbits are generally due to various stress factors (Melillo, 2007; Jenkins, 2008).

The effect of short-term amygdalin administration on the plasma levels of triglycerides

Changes of triglycerides levels in response to amygdalin application were determined in this study as well. The effect of IM amygdalin application on rabbit serum levels of triglycerides is shown in Figure 2. The analysis has shown that the serum triglycerides levels of animals administered with 0.6 and 3.0 mg/b.w. amygdalin did not statistically (*P*≥0.05) differ, when compared to the control group.

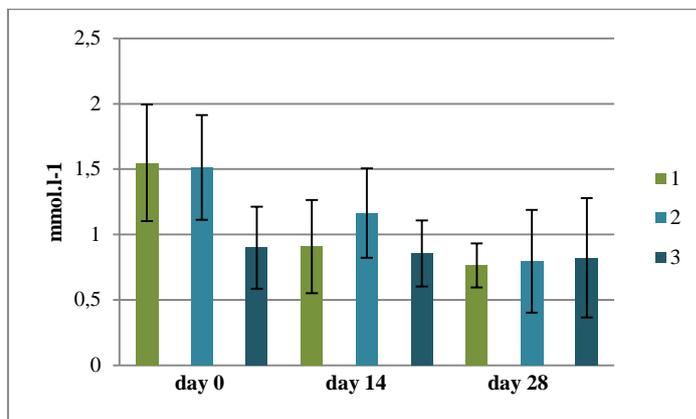


Figure 2 Serum levels of triglycerides during 28 days. 1 - control group (without amygdalin administration); 2 - group P1 (0.6 mg/kg); 3- group P2 (3.0 mg/kg)

However, a non-significant decrease of triglycerides levels in all groups during the experiment was observed. It could be connected to slightly increased concentration of glucose, since the secretion of triglycerides serves as an additional potential energy source in the form of circulating lipids (Tuvdendorj et al., 2015).

The effect of short-term amygdalin administration on the plasma levels of cholesterol

Serum levels of cholesterol after short-term IM injection of amygdalin to adult male rabbits were assessed and are shown in Figure 3. During 28 days of experiment, no significant (*P*≥0.05) differences in serum levels of cholesterol were observed when compared to the untreated control group.

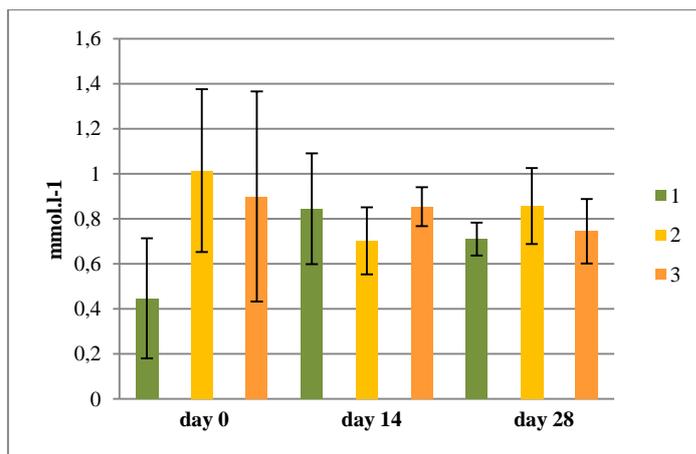


Figure 3 Serum levels of cholesterol during 28 days.1 - control group (without amygdalin administration); 2 - group P1 (0.6 mg/kg); 3- group P2 (3.0 mg/kg)

Cholesterol molecule plays an important role in organism, e. g. in incorporation to cell membranes, or as precursor of steroid hormones (Vasudevan et al., 2011). According to veterinary manual of Merck Sharp & Dohme Corp. (2012) levels of serum cholesterol of all groups were not outside the reference range (0.3-2.1 mmol.l⁻¹) and no significant differences between the groups were noticed during our experiment. These results could partly explain possible modulatory impact of amygdalin on the steroid production in porcine ovaries *in vitro*, as Halenár et al. (2015a, 2015b) published in their study. Keishi-bukuryo-gan (a traditional Chinese herbal remedy containing seeds of *Prunus persica* or *P. persiba*) and its crude ingredients affected steroidogenesis in pre-ovulatory follicles (Usuki, 1990, 1991) in the rat ovary *in vivo* and *in vitro*.

There are just a few studies of blood chemistry changes after amygdalin administration *in vivo*. Similarly to our results, Miller et al. (1981) did not observe changes in blood chemistry of rats fed a diet containing 10% ground apricot kernels.

Our results showed some non-significant differences within the same groups between each blood collections. It is hypothesised that this difference may be due to the variations in some factors such as stress, blood collection methods and housing conditions (Özkan et al., 2012).

CONCLUSION

It can be summarized, that amygdalin had no significant effect (negative or beneficial) on energetic profile of male rabbits in this *in vivo* study. Our results

showed some non-significant differences within the same groups between each blood collections, which may occur due to the variations in some factors such as stress, blood collection methods and housing conditions.

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