Influence of Apricot Kernels on Blood Plasma Levels of Selected Anterior Pituitary Hormones in Male and Female Rabbits \textit{in vivo}

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Abstract
Amygdalin is represented in the family \textit{Rosacea} more precisely in apricot kernels and almonds. There are a lot of components such as trace elements, vitamins, carbohydrates, organic acids, esters, phenols, terpenoids, except cyanogenic glycoside in the seeds. It is known that bioregulators can modulate the activity of specific enzymes and hormones very exactly at low levels and in a short time. The aim of our study was examine the effects of selected doses (0, 60, 300, 420 mg/kg b.w.) of apricot kernels in feed on the plasma levels of anterior pituitary hormones in young male and female rabbits \textit{in vivo}. A sensitive, biochemical method, ELISA was used to determine the hormones prolactin (PRL), luteinizing hormone (LH) and follicle stimulating hormone (FSH). 28-day application of apricot kernels did not affect the concentration of PRL, LH, FSH in blood plasma of males. No significant (P $\geq$ 0.05) differences in case of PRL and LH levels in the blood plasma of females were found. On the other hand a significant (P $\leq$ 0.05) inhibition of FSH release induced by kernels at the doses 300, 420 mg/kg was found. Our results indicate that apricot kernels could affect secretion of anterior pituitary hormone FSH in female rabbits.

Keywords: cyanogen glycoside, prolactin, luteinizing hormone, follicle stimulating hormone

1. Introduction

The most widely plant constituents, which are biologically active and provide medical and health benefits are called bioregulators [1, 2]. The family \textit{Rosaceae} has found for own rich generic representation of an application in the prevention and treatment of many pathological conditions. In the past few years, has been a renewed interest about distribution of these fruits [3,4]. The apricots have been used in folk medicine as a remedy, because a fresh apricot fruit contains carbohydrates, vitamins C and K, $\beta$-carotene, niacin, and thiamine. Organic acids, phenols, volatile compounds, esters, and terpenoids have been isolated [5-8]. Apricot kernels contain the toxic cyanogenic glycoside - amygdalin depending on variety [9]. It has been proved that amygdalin is a major component of the seeds of prunasin family plants, such as apricots, almonds, peaches, apples, and other \textit{Rosaceous} [3] plants but most abundant is in the apricots and almonds. There are differences between various amounts of amygdalin depending on cultivars. The amygdalin contents of bitter cultivars were found to be higher

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than those of sweet cultivars [5]. Yildirim et al. [10] have found that the amygdalin contents of seeds of sweet almond genotypes ranged from 1.53 to 11.25 mg/g. Amygdalin is known natural substance has used in alternative and traditional medicine for its powerful effects [11-12]. It is composed of two molecules of glucose, one of benzaldehyde, which induces an analgesic action, and one of hydrocyanic acid, which is an antineoplastic compound [4]. It is also decomposed by the action of β-D-glucosidase to yield hydrocyanic acid [13, 14]. The function of bioregulators is important to examine from viewpoint of prevention of many reproductive alterations [15]. The endocrine and extracellular signalling systems provide a means of communication between distant organs via the circulatory system, specific cell populations, neighbouring cell populations, and the external and internal environments [16]. The main centre hypothalamus secretes gonadolibersins releasing hormone (GnRH), which cause a corresponding release of gonadotropins - for example follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones control the production of gametes and sex hormones in the gonads. And prolactin (PRL) which is a polypeptide regulates the differentiation of mammary gland [17].

The aim of our study was to examine the effects of selected doses (0, 60, 300, 420 mg/kg b.w.) of apricot kernels in feed on the plasma levels of anterior pituitary hormones in the blood plasma of young male and female rabbits in vivo.

2. Materials and methods

2.1 The animals included in the experiment

Experimental animals, meat line P91 Californian rabbits, from the experimental farm of the Animal Production Research Centre Nitra (Slovak Republic) were used for our in vivo study. The rabbits were 40 days old and housed in individual flat-deck wire cages under a constant photoperiod of 12 h of daylight, temperature 20-24 °C and humidity 55 % ± 10 %.

2.2 Application of apricot kernels

Overall 48 animals were randomly divided into the four groups, leading to 8 female and 4 male rabbits in each group. The control group received no apricot kernels while the three experimental groups P1, P2 and P3 received daily doses 60, 300, 420 mg/kg b.w. in feed respectively during 28 days.

2.3. Analysis of blood plasma

During the experiment, blood collections were carried out to control the health of animals. Venous blood from vena auricularis was collected into EDTA-treated tubes. Blood plasma was separated from whole blood by centrifugation at 3000 rpm for 10 min. at 20 °C. The clear supernatant (plasma) was then separated from the pellet and kept frozen until analysis.

2.4 ELISA (Enzyme linked immunosorbent assay)

Quantification of hormones after apricot kernels supplementation was performed using ELISA. ELISA assays (Dialab, Wiener Neudorf, Austria) were performed according to the manufacturer’s instructions and the color intensity was inversely proportional to the concentration of hormones in the sample.

2.5 Statistical Analysis

Analysis of substances was performed in duplicate. The significance of differences between the control and experimental groups was evaluated by One-Way ANOVA (Dunnett’s multiple comparison test) using the 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).

3. Results and discussion

The effect of short-term application of apricot kernels on the plasma levels of anterior pituitary hormones: PRL, LH, FSH in males.

Plasma levels of PRL, LH, FSH after short-term application of apricot kernels to young male were assessed in this in vivo study (Figs. 1-3). No significant (P≥0.05) differences between control
and experimental groups were found. Nevertheless, the lowest dose of apricot kernels showed a slight increase of PRL concentrations in the blood plasma compared to the control (Fig. 1). The effect of apricot kernels on plasma levels of LH did not differ statistically significant (P≥0.05) between the control and experimental groups (Fig. 2). Slight increase was observed at the two lowest doses compared to control (Fig. 3).

**Figure 1** The plasma levels of PRL after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). The data are expressed as means ± SD. ELISA.

**Figure 2.** The plasma levels of LH after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). The data are expressed as means ± SD. ELISA.

The effect of short-term application of apricot kernels on the plasma levels of anterior pituitary hormones: PRL, LH, FSH in females

Plasma levels of PRL, LH and FSH after short-term application of apricot kernels to young female were assessed in this in vivo study (Figs. 4-6). No significant (P≥0.05) differences between control and experimental groups were found (Figs. 4, 5). On the other hand significant (P≤0.05) inhibition of FSH plasma level was detected in two experimental groups 300 and 420 mg/kg b.w. compared to control group without addition of natural substance. (Fig. 6)

**Figure 3.** The plasma levels of FSH after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). The data are expressed as means ± SD. ELISA.

**Figure 4.** The plasma levels of PRL after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). The data are expressed as means ± SD. ELISA.
Figure 5. The plasma levels of LH after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). The data are expressed as means ± SD. ELISA.

Figure 6. The plasma levels of prolactin after oral treatment of apricot kernels. The control group (control) represents a group of rabbits without application of apricot kernels. Experimental groups with apricot kernels at doses 60, 300 and 420 mg/kg b.w. Significance of differences between the groups was evaluated by One way ANOVA (Dunnet’s multiple comparison test). a,b – represent significant (P≤0.05) differences between control and experimental groups. The data are expressed as means ± SD. ELISA.

Previous works evaluated the influence of selected natural substances on the reproductive system [17, 20-24]. Kolesár et al. [21] describe amygdalin effects on female and male reproductive system. The possible impact of different naturally cyanide-containing substances on the male reproductive system, focused on spermatozoa motility and morphological abnormalities in bull spermatozoa, was observed previously [25]. This in vivo study was designed to reveal whether apricot seeds were able to cause changes in the endocrine profile, as well and thus alter the reproductive and physiological functions, using rabbits as a biological model. We focused on selected hormones of anterior pituitary such as PRL, LH, FSH. In case of males were demonstrated that the plasma levels of anterior pituitary hormones did not statistically (P≥0.05) differ after oral application of apricot kernels, compared to the hormone levels of animals in the control group. On the other hand, the presence of apricot kernels at selected doses (60, 300, 420 mg/kg) did not affect the levels of PRL, LH in females. The highest dose of kernels (300, 420 mg/kg) caused a significant (P≤0.05) decrease of FSH levels in female plasma. The modulating potential of bitter leaf extract in rats was demonstrated by Saalu et al. [26]. Their results showed a decrease in the blood level of FSH, an increased serum testosterone and luteinizing hormone after a 30-day treatment period. Halenár et al. [27,28] investigated the combination of natural substances amygdalin the mycotoxin deoxynivalenol (DON) on the secretion of steroid hormones by porcine ovarian granulosa cells in vitro. Their results indicated significant stimulation in the release of the steroid hormones progesterone, and 17-β estradiol, depending on the concentrations of both substances (amygdalin at a concentration 10 000 μg/ml, DON at a concentration 1000 ng/ml).

4. Conclusions

Application of apricot kernels did not affect the concentration of PRL, LH, FSH in blood plasma of males. No significant (P≥0.05) differences in case of PRL and LH levels in the blood plasma of female were found. On the other hand a significant (P≤0.05) inhibition of FSH release induced by kernels at the doses 300, 420 mg/kg was found. Our results indicate that apricot kernels could affect secretion of anterior pituitary hormone FSH in female rabbits.

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