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POSSIBLE EFECT OF AMYGDALIN IN COMBINATION WITH DEOXYNIVALENOL ON SECRETION ACTIVITY OF PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

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ABSTRACT

Amygdalin is a controversial anti-tumor natural product found in plants of the *Rosaceae* family that has been used as an alternative cancer drug for many years. One of the most widely distributed trichothecene contaminating food and animal feed is deoxynivalenol (DON) produced by *Fusarium* species. Deoxynivalenol has adverse effects on humans, animals, and crops that result in illnesses. The aim of the present *in vitro* study was to investigated the effect of natural compound amygdalin at the selected doses (1, 10, 100, 1000, 10 000 μg/ml) in combination with deoxynivalenol (1000 ng/ml) on secretion of steroid hormones (progesterone and estradiol) by ovarian granulosa cells from non-cyclic gilts. The release of progesterone and estradiol by granulosa cells was significantly (P≤0.05) increased in experimental group with the highest dose of amygdalin (10 000 μg/ml) in combination with deoxynivalenol (1000 ng/ml) compared to control group without administration of the natural compounds. On the other hand no significant differences in secretion of both steroid hormones were observed. Results from our study suggest possible effects of amygdalin in combination with mycotoxin-deoxynivalenol on secretion activity of porcine ovarian granulosa cells and possible involvement in the steroidogenesis.

Keywords: amygdalin, deoxynivalenol, estradiol, progesterone, health granulosa cells

INTRODUCTION

Amygdalin is one of many nitrilosides, which are natural cyanide-containing substances abundant in the seeds of apricots, almond, peaches, apples, and other rosaceous plants (*Chang et al.,* 2006). Its oldest known use was by the ancient Egyptians as a poison for executing capital punishment: "penalty of the peach". Amygdalin (D-mandelonitrile-β-D-gentiobioside, Fig. 1), $C_{20}H_{27}NO_{11}$, is composed of two molecules of glucose, one of benzaldehyde, which induces an analgesic action, and one of hydrocyanic acid, which is an anti-neoplastic compound (*Chang et al.,* 2006).

It has been used as a traditional drug because of its wide range of medicinal benefits, including curing or preventing cancer, relieving fever, suppressing cough, and quenching thirst. In the late 1970s and early 1980s, amygdalin was reported to selectively kill cancer cells at the tumor site without systemic toxicity and to effectively relieve pain in cancer patients (*Zhou et al.,* 2012). However, the Food and Drug Administration (FDA) has not approved amygdalin as a cancer treatment owing to insufficient clinical evidence of its efficacy and potential toxicity. Despite the failure of clinical tests to demonstrate the anticancer effects of amygdalin in the U.S.A. and in Europe, amygdalin continues to be manufactured and administered as an anticancer therapy in northern Europe and Mexico (*Chang et al*.,2006; *Kwon et al*., 2010).

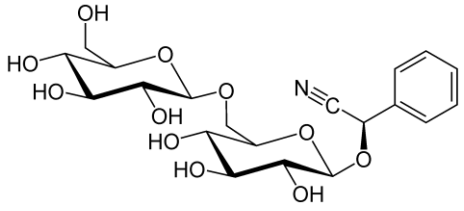

Figure 1

Chemical structure of amygdalin

Deoxynivalenol (DON) is one of the most important and occurring *Fussarium* mycotoxin (*Łazicka and Orzechowski,* 2010; *Klem et al.,* 2007). Occurrence of these mycotoxins is mainly in grains such as wheat, barley and maize (*Creppy,* 2002). DON could be rapidly absorbed after oral administration passively throughout the gastrointestinal tract and actively in the kidneys, liver, muscle, adipose tissue and reproductive tissues. Thus, mycotoxin exposure that alters granulosa cells steroid hormone production may also alter oocyte development, ovulation, reproductive tract function and pregnancy outcome (*Medveďová et al.,* 2011).

Steroid hormones, such as progesterone and estradiol are produced by ovarian cells and both are substantial for normal ovarian cycles (*Hagan et al.,* 2008; *Arnhold et al.,* 2009), contribute to regulation of ovarian follicular development and remodeling (*Mahajan,* 2008). The aim of the present *in vitro* study was to examine the secretion of steroid hormones progesterone and estradiol by health porcine ovarian granulosa cells after addition of natural substances amygdalin and deoxynivalenol.

MATERIAL AND METHODS

Preparation, culture and processing of granulosa cells from ovaries

Ovaries from non-cyclic gilts were obtained from healthy Slovakian White gilts without obvious reproductive abnormalities. Isolated ovaries were transported to the laboratory in containers at 4ºC and washed in sterile physiological solution. Follicular fluid was aspirated from 3-5 mm follicles. Granulosa cells were isolated by centrifugation for 10 min at 200xg followed by washing in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and resuspended in the same medium supplemented with 10% fetal calf serum (BioWhittaker™, Verviers, Belgium) and 1% antibiotic-antimycotic solution (Sigma, St. Louis, Mo, USA) at the final concentration of 10^6 cells/ml (as detected by haemocytometer). Portions of the cell suspension were dispensed to 24-welled culture plates (Nunc™, Roskilde, Denmark, 1ml/well; for Enzyme Linked ImmunoSorbent Assay,ELISA). The well plates were incubated at 37 °C and 5% CO_2 in humidified air until a 75% confluent monolayer was formed (4-5 days), at this point, the medium was renewed and ovarian granulosa cells were incubated with the similar supplements (DMEM/F12 1:1 medium, 10% fetal calf serum, without 1% antibiotic-antimycotic solution) and without (control) or with amygdalin (1, 10, 100, 1000, 10 000 μg/ml) (99 % purity, Sigma-Aldrich, St. Louis, Mo, USA) combined with deoxynivalenol (1000 ng/ml) (Romer Labs Division Holding GmbH, Tulln, Austria) for 24h. After 24h of incubation the culture media from well plates were aspirated and kept at –80°C for subsequent assay. The concentrations of steroid hormones progesterone and estradiol were assayed using ELISA (Dialab, Wiener Neudorf, Austria) according to the manufacturer´s instructions.

Statistical Analysis

Each experimental group was represented by four culture wells of granulosa cells. Assay of hormone level in the incubation media was performed in duplicate. Significance of differences between the control and experimental groups were evaluated by one-way ANOVA and t-test using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at the P – level less than 0.05 ($P \leq 0.05$).

RESULTS

Release of progesterone and estradiol by porcine ovarian granulosa cells

The release of progesterone and estradiol by porcine ovarian granulosa cells after addition of amygdalin (1, 10, 100, 1000, 10 000 μg/ml) combined with DON (1000 ng/ml) was detected (Figs. 2, 3). Amygdalin at the highest dose (10 000 μg/ml) in combination with DON (1000 ng/ml) significantly ($P \leq 0.05$) stimulated the release of progesterone (Fig. 2) and estradiol (Fig. 3) by granulosa cells

compared to control group without addition of natural substances. No significant (P≥0.05) differences in secretion of these steroid hormones by granulosa cells were found between control group without addition of both natural compounds and experimental groups with 1, 10, 100, 1000 µg/ml of amygdalin treatment combined with 1000 ng/ml of DON.

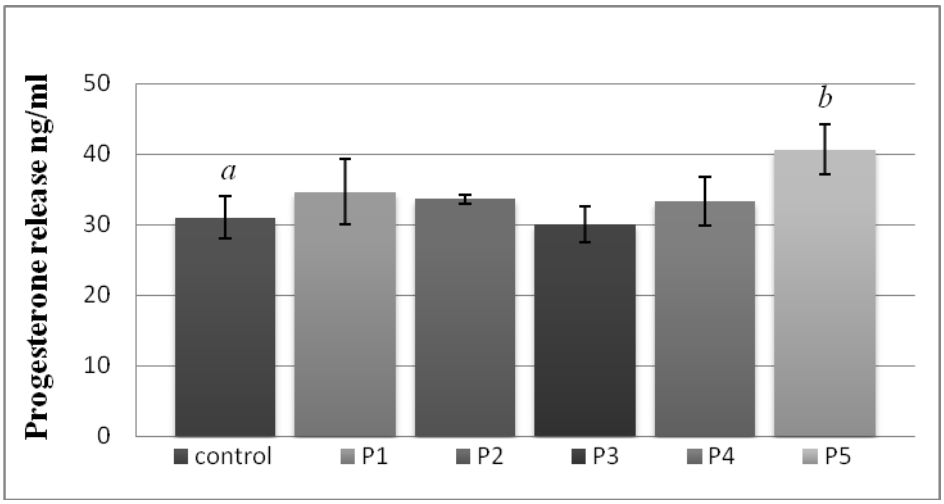

Figure 2

The effect of amygdalin in combination with deoxynivalenol on progesterone release by porcine ovarian granulosa cells.

Control represents culture medium without amygdalin and deoxynivalenol addition. Experimental group P1 represents culture medium with amygdalin (1 µg/ml) plus deoxynivalenol (1000 µg/ml), other groups represent culture medium with amygdalin P2 (10 µg/ml), P3 (100 µg/ml), P4 (1000 µg/ml) and P5 (10 000 µg/ml) plus in each group deoxynivalenol (1000 ng/ml). Signs a,b denote values significantly different from control group (P≤0.05) evaluated by one-way ANOVA and t-test. ELISA.

DISCUSION

Dose-response of amygdalin combined with DON addition to ovarian granulosa cells was examined in this study. Isolated ovarian granulosa cells were able to survive in culture and release hormonal substances progesterone and estradiol after experimental addition of both natural compounds. Findings from our observation suggest that the release of steroid hormones progesterone and estradiol was affected by amygdalin addition in combination with trichothecene deoxynivalenol.

Natural plant origin products like amygdalin are still a major part of traditional medicine (*Nabavizadeh et al*., 2011). In the late 1970s and early 1980s, amygdalin was reported to selectively kill cancer cells at the tumor site without systemic toxicity and to effectively relieve pain in cancer patients (*Zhou et al*., 2012).

The effects of natural substances on animal organism focus on the reproductive system were studied in the previous studies (*Kolesárová et al*., 2012a; 2012b; 2011; *Tanyildizy and Bozkurt*, 2004; *Yasui et al*., 2003; *Randel et al*., 1992). Amygdalin (at 10 000 but not at 1, 10, 100, 1000 µg/ml) combined with DON (1000 ng/ml) significantly (P≤0.05) stimulated the release of steroid hormones

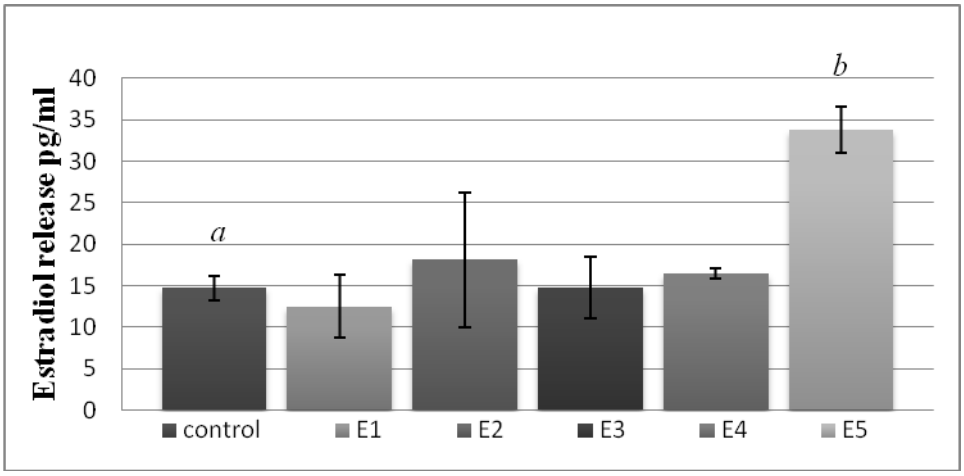

Figure 3

The effect of amygdalin in combination with deoxynivalenol on estradiol release by porcine ovarian granulosa cells.

Control represents culture medium without amygdalin and deoxynivalenol addition. Experimental group E1 represents culture medium with amygdalin (1 µg/ml) plus deoxynivalenol (1000 ng/ml), other groups represent culture medium with amygdalin E2 (10 µg/ml), E3 (100 µg/ml), E4 (1000 µg/ml) and E5 (10 000 µg/ml) plus in each group deoxynivalenol (1000 ng/ml). Signs a,b denote values significantly different from control group (P≤0.05) evaluated by one-way ANOVA and t-test. ELISA.

progesterone and estradiol by porcine ovarian granulosa cells. On the other hand, our recent *in vitro* investigation showed that the release of steroid hormone progesterone by health granulosa cells from cyclic and non-cyclic porcine ovaries was not affected by the amygdalin addition at the doses 1, 10, 100, 1000 and 10 000 µg/ml (*Halenár et al.,* 2013). Previous studies on amygdalin have focused on its purification, toxicity related to the release of cyanide, anti-tumor mechanism, and identification of its metabolites in plasma or herbs, and its pharmacological effect on cancers (*Rauws et al.,* 1982). Possible effect of amygdalin on the male reproductive system was observed in previous study (*Tanyildizi and Bozkurt,* 2004). There are many studies which suggest dose-dependent impact of different mycotoxins on the secretion activity of porcine (*Medveďová et al.,* 2011, *Maruniaková et al.,* 2013, *Ranzenigo et al*., 2008) and rat ovarian cells (*Kolesárová et al.,* 2011). The effect of deoxynivalenol on the steroid secretion by porcine ovarian granulosa cells was examined by *Medveďová et al.* (2011). Progesterone release by porcine ovarian granulosa cells was stimulated by DON treatment at the dose 1000 ng/ml but not at 10 and 100 ng/ml. Previous data also indicated dose-depended effects of DON on ovarian granulosa cells (*Ranzenigo et al.,* 2008). On the other hand, the release of progesterone by porcine ovarian granulosa cells was significantly (P<0.05) inhibited after administration of trichothecene T-2 toxin at the doses 10, 100 and 1000 ng/ml (*Maruniaková et al.,* 2013). The release of progesterone by porcine ovarian granulosa cells after exposure to toxic concentrations of DON, resveratrol, and their combination was studied by *Kolesárová et al.* (2012). These *in vitro* results suggest that reproductive toxicity of animals induced by a mycotoxin – deoxynivalenol can be inhibited by a protective natural substance - resveratrol.

The results of our *in vitro* experiments indicate that amygdalin combined with DON could modify secretion of steroid hormones by granulosa cells and potentially regulate process of steroidogenesis in porcine ovaries.

CONCLUSION

This *in vitro* study was focused on assessment the interaction between natural anti-cancer substance amygdalin and mycotoxin deoxynivalenol. In conclusion, there are still a few studies that suggest possible positive or negative effects of natural substances depending on their toxicity. The

results suggest possible effects of amygdalin in combination with mycotoxin-deoxynivalenol on secretion activity of porcine ovarian granulosa cells and possible involvement in the steroidogenesis.

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