CO-ADMINISTRATION OF AMYGDALIN AND DEOXYNIVALENOL DISRUPTED REGULATORY PROTEINS LINKED TO PROLIFERATION OF PORCINE OVARIAN CELLS IN VITRO

Marek Halenár, Marína Medveďová, Simona Baldovská,
Katarína Michalcová, Adriana Kolesárová

ABSTRACT

Deoxynivalenol (DON) represents one of the most prevalent trichothecene mycotoxin produced by *Fusarium* species, causing economic and health impacts. On the other hand, amygdalin has been demonstrated to possess both prophylactic and curative properties, thus 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. The aim of this *in vitro* study was to evaluate potential effects of natural product amygdalin combined with mycotoxin deoxynivalenol (DON) on the key regulators of cell proliferation and apoptosis in porcine ovarian granulosa cells. Ovarian granulosa cells were incubated for 24h with amygdalin (1, 10, 100, 1000, 10 000 μg.mL^{-1}) combined with deoxynivalenol (1 μg.mL^{-1}), while the control group remained untreated. The presence of proliferative (cyclin B1, PCNA) and apoptotic markers (caspase-3) in porcine ovarian granulosa cells after amygdalin treatment (1, 10, 100, 1000, 10 000 μg.mL^{-1}) combined with deoxynivalenol (1 μg.mL^{-1}) was detected by immunocytochemistry. The presence of proliferative (cyclin B1, PCNA) and apoptotic markers (caspase-3) in porcine ovarian granulosa cells was detected by immunocytochemistry. Co-administration of amygdalin plus DON significantly ($p < 0.05$) increased the number of granulosa cells containing cyclin B1 and PCNA at all tested concentrations, when compared to control. However, percentage of granulosa cells containing major apoptotic marker caspase-3 did not differ after co-administration of amygdalin and DON. In summary, results form this in vitro study indicate that co-exposure of amygdalin and deoxynivalenol may act to stimulate proliferation-associated peptides in porcine ovarian granulosa cells, and thus alter cell proliferation and normal follicular development.

Keywords: amygdalin; deoxynivalenol; ovarian cell; proliferation; apoptosis

INTRODUCTION

Mycotoxins are a group of toxic secondary metabolites produced by molds and frequently enter the food chain as food contaminants (**Vejdovszky et al., 2016**). Deoxynivalenol (DON) represents one of the most prevalent trichothecene mycotoxin produced by *Fusarium* species responsible of *Fusarium* head blight. Due to a high prevalence of this disease, type B trichothecenes are the most common contaminants of cereal grains in temperate regions of the world (**Alassane-Kpembi et al., 2015**). They are commonly found on cereals grown in the temperate regions of Europe, America and Asia. The extent of infection is dependent on weather conditions, Good Agricultural Practice and storage conditions of cereal crops (**Larsen et al., 2004**). A large scale data survey indicates that DON and its metabolites 15-ADON and 3-ADON are present in 57%, 20% and 8%, respectively of food samples collected in the European Union (**Alassane-Kpembi et al., 2015**). They represent a unique class of mycotoxins that do not only exert toxicity in animal but also are virulence factors in plant disease,

which make them one of the major groups causing significant economic and health impacts (**Desjardins, 2009**). Intoxications following consumption of foodstuffs contaminated with trichothecenes have occurred in both humans and animals with large numbers of people and livestock being affected. Some countries have already established legislative limits in cereals for DON, the most abundant trichothecene, and the European Commission has proposed EU regulatory limits for DON in various raw cereals and their refined products (**Larsen et al. 2004**).

At the molecular level, trichothecenes display multiple inhibitory effects on primary metabolism of eukaryotic cells including inhibition of protein as well as DNA and RNA synthesis, and their activity may eventually produce harmful levels of oxidative stress due to generation of free radicals (**Arunachalam and Doohan 2013**). Thus organs and biological functions involving actively dividing cells appear more sensitive to this class of mycotoxins (**Parent-Massin, 2004; Pestka et al., 2004**). Mycotoxins as contaminants of animal feed can impair growth and/or reproductive effeciency. This is especially prominent in

prepubertal gilts **(Dänicke, 2002)**. The adverse health effects of trichothecenes include emesis, nausea, anorexia, growth retardation, hemorrhagic lesions, neuroendocrine changes and immunosuppression **(Larsen et al., 2004; Pestka et al., 2004; Alassane-Kpembi et al., 2015)**.

On the other hand, amygdalin as a natural plant compound belongs to the cyanogenic glycosides abundantly present in diverse plants, especially in the rosaceous plant seeds such as bitter almonds, apricots and peaches etc. **(Chang et al., 2006, Lee and Moon 2016)**. This bioactive substance is composed of glucose, benzaldehyde, which induces an analgesic action, and hydrocyanic acid, which is an anti-neoplastic compound **(Fukuda et al., 2003; Chang et al., 2006)**. Amygdalin has been demonstrated to possess both prophylactic and curative anticancer properties, thus 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 **(Moertel et al., 1982; Oyewole and Olayinka 2009; Zhou et al., 2012)**. In addition, the pharmacological activity of amygdalin also include anti-inflammatory, anti-atherogenic and anti-asthmatic effects **(Song and Xu, 2014)**. However, the use of the drug was discouraged when it was demonstrated that amygdalin is metabolized in the body to release significant amount of cyanide thus leading to cyanide poisoning **(Chandler et al., 1984; Bromley et al., 2005)**. β-glucosidase, one of the enzymes that catalyzes the release of cyanide from amygdalin, is present in the human small intestine and is also found in a variety of common foods **(Strugala et al., 1995; Deng et al., 2002)**. Side effects of amygdalin ingestion in humans mirror symptoms of cyanide poisoning which includes nausea, vomiting, headache, dizziness, bluish colouration of the skin, liver damage, hypotension, nerve damage, fever, mental confusion, coma and death **(Howard-Reuben and Miller 1984)**. Considering that amygdalin has been frequently used as alternative therapy, modulatory and chemopreventive potential of amygdalin has not been sufficiently studied yet.

Pig is considered an illustrative case of dual-purpose target and model species that benefits agricultural and biomedical research. In addition, because of its similarity, the pig can be regarded as a good model of extrapolation to human **(Alassane-Kpembi et al., 2015)**.

The aim of this *in vitro* study was to evaluate potential effects of natural product amygdalin combined with mycotoxin deoxynivalenol on the key regulators of cell proliferation and apoptosis in porcine ovarian granulosa cells.

MATERIAL AND METHODOLOGY
Preparation, culture and processing of granulosa cells from ovaries

Ovaries of pre-pubertal gilts (n = 12 per experiment) were obtained after slaughter at a local abattoir. Porcine ovaries were obtained from healthy Slovakian White gilts without visible reproductive abnormalities. The ovaries were transported to the laboratory in containers at 4°C and washed with sterile physiological solution. The follicular fluid was aspirated from 3 – 5 mm follicles. The granulosa cells (GCs) were isolated by centrifugation for 10 min at 200 x g followed by washing in sterile DMEM/F12 1 : 1 medium (BioWhittaker[TM], Verviers, Belgium) and resuspended in the same medium supplemented with 10% fetal calf serum (BioWhittaker[TM], Verviers, Belgium) and 1% antibiotic–antimycotic solution (Sigma, St.Louis, Mo, USA) at a final concentration of 10^6 cells.mL^{-1} (as quantified by a haemocytometer). Portions of the cell suspension were dispensed to Lab-Tek 16 – welled chamber slides (Nunc Inc., International, Naperville, USA, 100 µL per well). The well plates were incubated at 37 °C and 5% CO_2 in humidified air until a 75% confluent monolayer was formed, at this point, the medium was renewed and ovarian GCs were incubated with the same supplements (DMEM/F12 1:1 medium, 10% fetal calf serum, with 1% antibiotic-antimycotic solution), without (control) or with amygdalin (1, 10, 100, 1000, 10 000 µg.mL^{-1}) (≥99% purity, from apricot kernels, Sigma-Aldrich, St. Louis, Mo, USA) in combination with DON (1 µg.mL^{-1}) (Romer Labs Division Holding GmbH, Tulln, Austria) for 24 h. After 24 h of culture the media from wells were removed and the wells from chamber slides were washed in ice-cold phosphate-buffered saline (PBS) (pH 7.5). Cells were fixed for 1h at room temperature in 4% paraformaldehyde.

Immunocytochemistry
Signaling substances within GCs plated on chamber slides were detected using immunocytochemistry method. The ImmunoCruz Staining System and primary mouse monoclonal antibodies against cyclin B1, Proliferating cell nuclear antigen (PCNA) and caspase-3 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were used as directed by the manufacturer. Visualisation of the primary antibody binding sites were achieved with a secondary rabbit polyclonal antibody against mouse IGs, labelled with horseradish peroxidase (Sevac, Prague, Czech Republic; dilution 1 : 1000) and diaminobenzidine (DAB) reagent. Chamber slides stained with peroxidase/DAB reagent were mounted with Glycergel (DAKO, Carpinteria, CA, USA) mounting medium.

Statistical analysis
The proportions of cells containing specific immunoreactivity were calculated from inspection of at least 1000 cells per chamber. The data are presented as means of values obtained from three separate experiments performed on separate days using separate pools of ovaries from 10 – 12 animals. 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 ±SEM. Differences were compared for statistical significance at the *p*-level less than 0.05 ($p <0.05$).

RESULTS
The percentage of porcine ovarian granulosa cells (GCs) containing proliferative and apoptotic markers (cyclin B1, PCNA, caspase-3) after co-administration of amygdalin (1, 10, 100, 1000, 10 000 µg.mL^{-1}) plus deoxynivalenol (1 µg.mL^{-1}) was determined by immunocytochemistry.
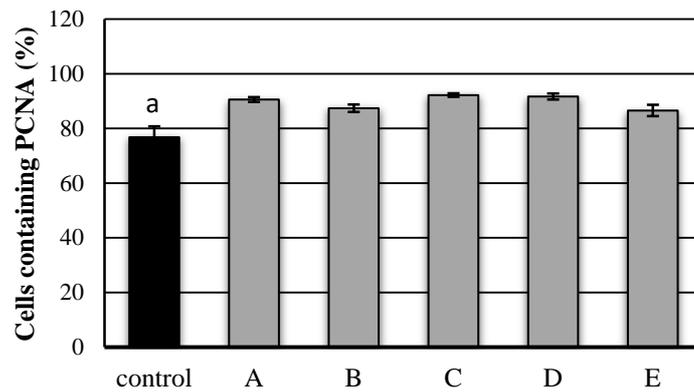
Figure 1 The percentage of granulosa cells containing PCNA (Proliferating cell nuclear antigen) after amygdalin addition combined with deoxynivalenol. The control group represents cells incubated without amygdalin and deoxynivalenol; experimental groups – A: 1 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; B: 10 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; C: 100 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; D: 1000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; E: 10 000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON. Signs *a, b* denote value significantly ($p < 0.05$) different from control group. Significance of differences between the groups was evaluated by One-way ANOVA (Dunnett´s multiple comparison test). The data are expressed as means ±SEM. Immunocytochemistry.
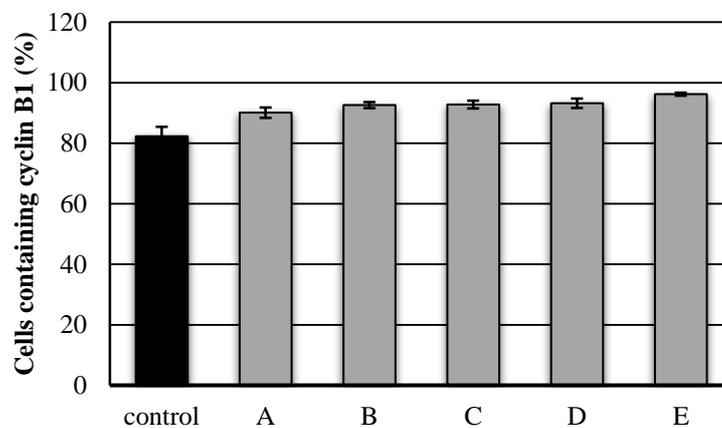

Figure 2 The percentage of granulosa cells containing cyclin B1 after amygdalin addition combined with deoxynivalenol. The control group represents cells incubated without amygdalin and deoxynivalenol; experimental groups – A: 1 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; B: 10 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; C: 100 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; D: 1000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; E: 10 000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON. Signs *a, b* denote value significantly ($p < 0.05$) different from control group. Significance of differences between the groups was evaluated by One-way ANOVA (Dunnett´s multiple comparison test). The data are expressed as means ±SEM. Immunocytochemistry.
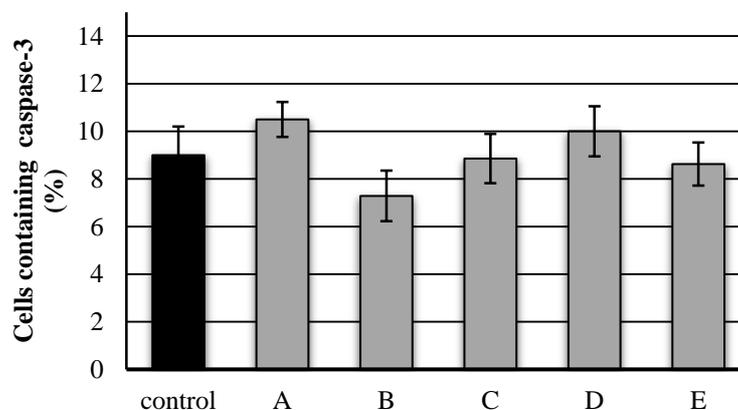

Figure 3 The percentage of granulosa cells containing caspase-3 after amygdalin addition combined with deoxynivalenol. The control group represents cells incubated without amygdalin and deoxynivalenol; experimental groups – A: 1 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; B: 10 µg/mL amygdalin +1 µg.mL^{-1} DON; C: 100 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; D: 1000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON; E: 10 000 µg.mL^{-1} amygdalin +1 µg.mL^{-1} DON. Significance of differences between the groups was evaluated by One-way ANOVA (Dunnett´s multiple comparison test). The data are expressed as means ±SEM. Immunocytochemistry.

Significant ($p < 0.05$) differences in the percentage of GCs containing cyclin B1 as well PCNA after amygdalin and deoxynivalenol (DON) treatment were observed, when compared to control untreated cells. Combination of amygdalin and DON induced significant ($p < 0.05$) increase in the number of cyclin B1 (Figure 1) and PCNA-positive (Figure 2) granulosa cells at all used concentrations. Moreover, the percentage of GCs with cyclin B1 peptide was higher along with increasing amygdalin plus DON concentrations. On the other hand, number of granulosa cells containing major apoptotic marker caspase-3 was not influenced by co-administration of amygdalin and DON (Figure 3).

DISCUSSION

Numerous studies demonstrated that granulosa cells play a fundamental role in the process of normal folliculogenesis, oocyte growth and development (**Spicer et al., 2001; Petro et al., 2012; Rawan et al., 2015**). Recently, specific *Fusarium* mycotoxins may directly interfere with cell proliferation and hormone production in porcine ovaries (**Ranzenigo et al., 2008; Caloni et al., 2009; Medvedova et al., 2011; Cortinovis et al., 2014**). Critical cellular kinases involved in signal tranduction related to cell proliferation, differentiation and apoptosis are impaired by deoxynivalenol (**Pestka and Smolinsky 2005**). The ability of these mycotoxins to alter granulosa cell proliferation may compromise normal follicle development and oocyte function because granulosa cells provide essential nutrients to the oocyte (**Petro et al., 2012**).

To assess cell proliferation in porcine ovarian granulosa cells after co-exposure to amygdalin and deoxynivalenol, selected proliferation – associated peptides (cyclin B1 and PCNA) were evaluated in our experiments. Co-exposure of amygdalin and deoxynivalenol at all doses tested significantly increased number of granulosa cells containing both proliferative markers, cyclin B1 and PCNA. Proliferating cell nuclear antigen and cyclin B1 represent the fundamental peptides related to cell cycle, which are involved in the process of ovarian cell proliferation, growth and development (**Naryzhny and Lee 2001; Tomanek and Chronowska 2006; Kolesarova et al., 2008, 2015**).

Our previous study revealed dose-dependent response of porcine ovarian GCs to amygdalin alone or in combination with deoxynivalenol (**Halenar et al., 2015**). The presence of amygdalin alone induced a significant stimulation of 17β-estradiol release, but not progesterone, by porcine GCs at the highest used dose (10 000 µg.mL^{-1}). However, combination of amygdalin with DON increased progesterone and 17β-estradiol release in dose-dependent manner. Therefore, these data suggest that amygdalin is shown to be potential regulator of 17-β-estradiol but not progesterone in porcine ovarian GCs. Stimulatory effect of amygdalin combined with DON on progesterone secretion was clearly due to DON addition, not by amygdalin. Report of **Kolesarova et al. (2012)** has suggested protective actions of the natural substance resveratrol in mycotoxin induced reproductive toxicity in vitro. Their results showed stimulatory effects of resveratrol, DON and their combination on the release of progesterone by ovarian granulosa cells in dose-dependent manner.

Moreover, resveratrol effectively reduced the stimulatory action of DON on steroid hormone production.

Recent studies demonstrated the effect of various natural substances with protective (**Kolesárova et al., 2012; Halenár et al., 2015**) or toxic (**Ranzenigo et al., 2008; Maruniakova et al., 2014**) potential on the cellular processes in ovarian cells. Confirming previous results (**Medvedova et al., 2011**), porcine GCs exposure to DON had stimulatory effect on proliferative markers depending on concentrations of DON. Higher concentrations of DON increased significantly percentage of cyclin B1 as well PCNA positive cells when compared to control. On the other hand, **Ranzenigo et al. (2008)** demonstrated an inhibitory effect of DON on the proliferation of porcine granulosa cell in vitro. Examination of **Maruniakova et al. (2014)** indicates modulatory impacts of trichothecene mycotoxins, T-2 and HT-2 toxin, combined with insulin-like growth factor I (IGF-I) on the steroid hormone secretion in porcine ovarian granulosa cells in vitro.

Previous studies related to amygdalin have primarily focused on its purification, anti-tumor mechanism, determination in plants, as well as on toxicity caused by the release of cyanide (**Rauws et al., 1982; Yildirim and Askin 2010; Zhou et al., 2012**). An in vitro study of **Makarevic et al. (2014)** demostrated that amygdalin was able to block the bladder cancer cell growth by down-modulating cell cycle regulating proteins cyclin A and cyclin dependent kinase 2. Additionaly, amygdalin acted on the cdk1-cyclin B axis in PC3 cells after 2 weeks but not after 24 h, implying a time dependent mode of action (**Makarevic et al., 2016**). Histological analysis of rabbit femoral bones after intramuscular application of amygdalin was observed by **Kovacova et al. (2016)**. Their results showed a significantly lower value of primary osteons' vascular canals and secondary osteons in bone microstructure of experimental rabbits. However, **Tusimova et al. (2016)** demonstrated no obvious impact of amygdalin application on the energy profile of rabbits in vivo.

As a central substance in death receptor pathway of the apoptosis, caspase-3 plays a key role in mediating nuclear apoptosis and is required for some typical nuclear and other morphological changes (**Porter and Janicke 1999; Denault and Salvesen 2008**). The present study revealed that the presence of caspase-3 in granulosa cells was not influenced by co-administration of amygdalin and deoxynivalenol, compared to untreated control cells. These findings are in accordance with results of **Medvedova et al. (2011)**, who observed no substantial effect of DON on the expression of caspase-3 in porcine ovarian GCs. Conversely, previous report of **Zhu et al. (2012)** demonstrated the increase of caspase-3 accumulation in porcine granulosa cells after addition of other mycotoxin - zeralanenone, and triggered a caspase-3-depended apoptotic process.

Combinatory effects of isoflavone genistein with mycotoxin zearalenone on endometrial adenocarcinoma cell line were studied by **Vejdovszky et al. (2016)**. Authors observed significant increase in alcaline phosphatase activity and cellular protein amounts after addition of both tested substances, suggesting slight proliferative effects and biphasic dose-response.

Taken all together, till obtained results suggest dose and species dependent effects of several natural substances on the cellular processes in ovaries, such as proliferation and programmed cell death - apoptosis.

CONCLUSION

In conclusion, results form this in vitro study indicate that co-exposure of amygdalin and deoxynivalenol may act to stimulate proliferation-associated peptides in porcine ovarian granulosa cells, and thus alter cell proliferation and normal follicular development. Considering the lack of information about the potential effects of natural products on the reproductive function in animals further research should be addressed.

REFERENCES

Alassane-Kpembi, I., Puel, O., Oswald, I. P. 2015. Toxicological interactions between the mycotoxins deoxynivalenol, nivalenol and their acetylated derivatives in intestinal epithelial cells. *Archives of Toxicology*, vol. 89, no. 8, p. 1337-1346. https://doi.org/10.1007/s00204-014-1309-4 PMid:25033990

Arunachalam, C., Doohan, F. M. 2013. Trichothecene toxicity in eukaryotes: cellular and molecular mechanisms in plants and animals. *Toxicology Letters*, vol. 217, no. 2, p. 149-158. https://doi.org/10.1016/j.toxlet.2012.12.003 PMid:23274714

Bromley, J., Hughes, B. G., Leong, D. C., Buckley, N. A. 2005. Life-threatening interaction between complementary medicines: cyanide toxicity following ingestion of amygdalin and vitamin C. *Annals of Pharmacotherapy*, vol. 39, no. 9, p. 1566-1569. https://doi.org/10.1345/aph.1E634 PMid:16014371

Caloni, F., Ranzenigo, G., Cremonesi, F., Spicer, L. J. 2009. Effects of trichothecene, T-2 toxin, on proliferation and steroid production by GCs. *Toxicon*, vol. 54, no. 3, p. 337-344. https://doi.org/10.1016/j.toxicon.2009.05.002 PMid:19463844

Cortinovis, C., Caloni F., Schreiber N. B., Spicer L. J. 2014. Effects of fumonisin B1 alone and combined with deoxynivalenol or zearalenone on porcine granulosa cell proliferation and steroid production. *Theriogenology*, vol. 81, no. 8, p. 1042-1049. https://doi.org/10.1016/j.theriogenology.2014.01.027 PMid:24576714

Dänicke, S. 2002. Fusarium toxins in animal nutrition (Fusarium-toxine in der Tierernährung). *Grosstierpraxis*, vol. 3, p. 5-18.

Denault, J. B., Salvesen, G. S. 2008. Apoptotic caspase activation and activity. *Methods in Molecular Biology*, vol. 414, p. 191-220. https://doi.org/10.1007/978-1-59745-339-4_15

Deng, Y., Guo, Z. G., Zeng, Z. L., Wang, Z. 2002. Studies on the pharmacological effects of saffron (*Crocus sativus* L.). *Zhongguo Zhong Yao Za Zhi*, vol. 27, no. 8, p. 565-568. PMid:12776492

Desjardins, A. E. 2009. From yellow rain to green wheat: 25 years of trichothecene biosynthesis research. *Journal of Agricultural and Food Chemistry*, vol. 57, no. 11, p. 4478-4484. https://doi.org/10.1021/jf9003847 PMid:19385595

Fukuda, T., Ito, H., Mukainaka, T., Tokuda, H., Nishino, H., Yoshida, T. 2003. Anti-tumor promoting effect of glycosides from *Prunus persica* seeds. *Biological and Pharmaceutical Bulletin*, vol. 26, no. 2, p. 271-273.

https://doi.org/10.1248/bpb.26.271 PMid:12576693

Halenár, M., Medveďová, M., Maruniaková, N., Kolesárová, A. 2015. Assessment of a potential preventive ability of amygdalin in mycotoxin-induced ovarian toxicity. *Journal of Environmental Science and Health B*, vol. 50, no. 6, p. 411-416. https://doi.org/10.1080/03601234.2015.1011956 PMid:25844862

Howard-Ruben, J., Miller, N. J. 1984. Unproven methods of cancer management. Part II: Current trends and implications for patient care. *Oncology Nursing Forum*, vol. 11, no.1, p. 67-73. PMid:6318190

Chandler, R. F., Anderson, L. A., Phillipson, J. D. 1984. Laetrile in perspective. *Canadian Pharmacists Journal*, vol. 117, p. 517-520.

Chang, H. K., Shin, M. S., Yang, H. Y., Lee, J. W., Kim, Y. S., Lee, M. H., Kim, J., Kim, K. H., Kim, C. J. 2006. Amygdalin Induces Apoptosis through Regulation of Bax and Bcl-2 Expressions in Human DU145 and LNCaP Prostate Cancer Cells. *Biological and Pharmaceutical Bulletin*, vol. 29, no. 8, p. 1597-1602. https://doi.org/10.1248/bpb.29.1597 PMid:16880611

Kolesárová, A., Sirotkin, A. V., Kováčik, J. 2008. Endocrine and intracellular mechanisms of sexual gestation of gilts (Endokrinné a vnútrobunkové mechanizmy pohlavného dospievania prasničiek). 1st ed. Nitra, Slovakia : Slovak University of Agriculture, p. 131. ISBN 978-80-552-0109-2.

Kolesarova, A., Capcarova, M., Maruniakova, N., Lukac, N., Ciereszko, R. E., Sirotkin, A. V. 2012. Resveratrol Inhibits Reproductive Toxicity Induced by Deoxynivalenol. *Journal of Environmental Science and Health Part B*, vol. 47, no. 9, p. 1329-1334. https://doi.org/10.1080/10934529.2012.672144 PMid:22540658

Kolesarova, A., Sirotkin, A. V., Mellen, M., Roychoudhury, S. 2015. Possible intracellular regulators of female sexual maturation. *Physiological research*, vol. 64, no. 3, p. 379-386. PMid:25536325

Kovacova, V., Omelka, R., Sarocka, A., Sranko, P., Adamkovicova, M., Toman, R., Halenar, M., Kolesarova, A., Martiniakova, M. 2016. *Potravinarstvo,* vol. 10, no. 1, p. 393-399. https://doi.org/10.5219/625

Larsen, J. C., Hunt, J., Perrin, I., Ruckenbauer, P. 2004. Workshop on trichothecenes with a focus on DON: summary report. *Toxicology Letters*, vol. 153, no. 1, p. 1-22. https://doi.org/10.1016/j.toxlet.2004.04.020 PMid:15342076

Lee, H. M., Moon, A. 2016. Amygdalin regulates apoptosis and adhesion in Hs578T Triple-Negative Breast Cancer Cells. *Biomolecules & Therapeutics (Seoul)*, vol. 24, no. 1, p. 62-66. https://doi.org/10.4062/biomolther.2015.172 PMid:26759703

Makarević, J., Rutz, J., Juengel, E., Kaulfuss, S., Reiter, M., Tsaur, I., Bartsch, G., Haferkamp, A., Blaheta, R. A. 2014. Amygdalin blocks bladder cancer cell growth *in vitro* by diminishing cyklin A and cdk2. *PLoS ONE*, vol. 9, no. 8, p. 1-9. https://doi.org/10.1371/journal.pone.0105590 PMid:25136960

Makarević, J., Juengel, E., Tsaur, I., Borgmann, H., Nelson, K., Thomas, C., Bartsch, G., Haferkamp, A., Blaheta, R. A. 2016. Amygdalin delays cell cycle progression and blocks growth of prostate cancer cells in vitro. *Life Sciences*, vol. 147, p. 137-142. https://doi.org/10.1016/j.lfs.2016.01.039 PMid:26827990

Maruniakova, N., Kadasi, A., Sirotkin, A. V., Bulla, J., Kolesarova, A. 2014. T-2 toxin and its metabolite HT-2 toxin combined with insuin-like growth factor-I modify progesterone secretion by porcine ovarian granulosa cells. *Journal of Environmental Science and Health Part A*, vol. 49, no. 4, p. 404-409. https://doi.org/10.1080/10934529.2014.854650 PMid:24345238

Medvedova, M., Kolesarova, A., Capcarova, M., Labuda, R., Sirotkin, A. V., Kovacik, J., Bulla, J. 2011. The effect of deoxynivalenol on the secretion activity, proliferation and apoptosis of porcine ovarian granulosa cells in vitro. *Journal of Environmental Science and Health*, vol. 46, no.3, p. 213-219. https://doi.org/10.1080/03601234.2011.540205 PMid:21442537

Moertel, C. G., Fleming, T. R., Rubin, J. A. 1982. Clinical trial of amygdalin in the treatment of human cancer. *The New England Journal of Medicine*, vol. 306, no. 4, p. 201-206. https://doi.org/10.1056/NEJM198201283060403 PMid:7033783

Naryzhny, S. N., Lee, H. 2001. Protein profiles of the Chinese hamster ovary cells in the resting and proliferating stages. *Electrophoresis*, vol. 22, no. 9, p. 1764-1775. https://doi.org/10.1002/1522-2683(200105)22:9<1764::AID-ELPS1764>3.0.CO;2-V

Oyewole, O. I., Olayinka, E. T. 2009. Hydroxocobalamin (vitb12a) effectively reduced extent of cyanide poisoning arising from oral amygdalin ingestion in rats. *Journal of Toxicology and Environmental Health Sciences*, vol. 1, no. 1, p. 8-11.

Parent-Massin, D. 2004. Haematotoxicity of trichothecenes. *Toxicology Letters*, vol. 153, no. 1, p. 75-81. https://doi.org/10.1016/j.toxlet.2004.04.024 PMid:15342083

Pestka, J. J., Zhou, H. R., Moon, Y., Chung, Y. J. 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. *Toxicology Letters*, vol. 153, no. 1, p. 61-73. https://doi.org/10.1016/j.toxlet.2004.04.023 PMid:15342082

Pestka, J. J., Smolinski, A.T. 2005. Deoxynivalenol: toxicology and potential effects on humans. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, vol. 8, no. 1, p. 39-69. https://doi.org/10.1080/10937400590889458 PMid:15762554

Porter, A. G., Jänicke, R. U. 1996. Emerging roles of caspase-3 in apoptosis. *Cell Death and Differentiation*, vol. 6, no. 2, p. 99-104. https://doi.org/10.1038/sj.cdd.4400476 PMid:10200555

Petro, E. M., Leroy, J. L., Van Cruchten, S. J., Covaci, A., Jorssen, E. P., Bols, P. E. 2012. Endocrine disruptors and female fertility: focus on (bovine) ovarian follicular physiology. *Theriogenology*, vol. 78, no. 9, p. 1887-1900. https://doi.org/10.1016/j.theriogenology.2012.06.011 PMid:22925646

Ranzenigo, G., Caloni, F., Cremonesi, F., Aad, P. Y., Spicer, L. J. 2008. Effects of Fusarium mycotoxins on steroid production by porcine GCs. *Animal Reproduction Science*, vol. 107, no. 1-2, p. 115-130. https://doi.org/10.1016/j.anireprosci.2007.06.023 PMid:17656051

Rauws, A. G., Olling, M., Timmerman, A. 1982. The pharmacokinetics of prunasin, a metabolite of amygdalin. *Journal of Toxicology. Clinical Toxicology*, vol. 19, no. 8, p. 851-856. https://doi.org/10.3109/15563658208992518 PMid:7182513

Rawan, A. F., Yoshioka, S., Abe, H., Acosta, T. J. 2015. Insulin-like growth factor-1 regulates the expression of luteinizing hormone receptor and steroid production in bovine granulosa cells. *Reproduction in Domestic Animals*, vol. 50, no. 2, p. 283-91. https://doi.org/10.1111/rda.12486 PMid:25626338

Song, Z., Xu, X. 2014. Advanced research on anti-tumor effects of amygdalin. *Journal of Cancer Resesarch & Therapy*, vol. 1, p. 3-7.

Spicer, L. J., Alonso, J., Chamberlain, C. S. 2001. Effects of Thyroid Hormones on Bovine Granulosa and Thecal Cell Function *In Vitro*: Dependence on Insulin and Gonadotropins. *Journal of Dairy Science.* vol. 84, no. 5, p. 1069-1076. https://doi.org/10.3168/jds.S0022-0302(01)74567-5

Strugala, G. J., Stahl, R., Elsenhans, B., Rauws, A. G., Forth, W. 1995. Small-intestinal transfer mechanism of prunasin, the primary metabolite of the cyanogenic glycoside amygdalin. *Human Experimental Toxicology,* vol. 14, no. 11, p. 895-901. https://doi.org/10.1177/096032719501401107 PMid:8588951

Tomanek, M., Chronowska, E. 2006. Immunohistochemical localization of proliferating cell nuclear antigen (PCNA) in the pig ovary. *Folia Histochemica et Cytobiologica*, vol. 44, no. 4, p. 269-274. PMid:17219721

Vejdovszky, K., Schmidt, V., Warth, B., Marko, D. 2016. Combinatory estrogenic effects between the isoflavone genistein and the mycotoxins zearalenone and alternariol in vitro. *Molecular Nutrition & Food Research*, vol. 61, no. 3, p. 1-12.

Yidirim, F. A., Askin, M. A. 2010. Variability of amygdalin content in seeds of sweet and bitter apricot cultivars in Turkey. *African Journal of Biotechnology*, vol.9, no. 39, p. 6522-6524.

Zhou, C., Qian, L., Ma, H., Yu, X., Zhang, Y., Qu, W., Zhang, X., Xia, W. 2012. Enhancement of amygdalin activated with β-D-glucosidase on HepG2 cells proliferation and apoptosis. *Carbohydrate Polymers*, vol. 90, no. 1, p. 516-523. https://doi.org/10.1016/j.carbpol.2012.05.073 PMid:24751072

Zhu, L., Yuan, H., Guo, Ch., Lu, Y., Deng, S., Yang,Y., Wei,Q., Wen, L., He, Z. 2012. Zearalenone induces apoptosis and necrosis in porcine granulosa cells via a caspase-3- and caspase-9-dependent mitochondrial signaling pathway. *Journal of Cellular Physiology*, vol. 227, no. 5, p. 1814-1820. https://doi.org/10.1002/jcp.22906 PMid:21732350

Contact address:
Marek Halenár, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: halenarmarek@gmail.com
Marína Medveďova, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department

of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: mata.medvedova@yahoo.com

Simona Baldovská, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: sbaldovska@gmail.com

Katarína Michalcová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department

of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: kmichalcova86@gmail.com

Adriana Kolesárová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: adriana.kolesarova@uniag.sk