JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

OVARIAN HORMONE PRODUCTION AFFECTED BY AMYGDALIN ADDITION *IN VITRO*

Marek Halenár, Marína Medveďová, Nora Maruniaková, Adriana Kolesárová*

Address(es): Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

*Corresponding author: halenarmarek@gmail.com

doi: 10.15414/jmbfs.2015.4.special2.19-22

ARTICLE INFO

Received 15. 12. 2014
Revised 21. 12. 2014
Accepted 10. 1. 2015
Published 2. 2. 2015

Regular article

OPEN ACCESS

ABSTRACT

Amygdalin, a natural substance, is a cyanogenic glycoside occurring in the seeds of apricots and bitter almonds. It is a controversial anti-tumor compound that has been used as an alternative cancer drug for many years. Amygdalin 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. This *in vitro* study was performed to evaluate the possible impact of amygdalin (1, 10, 100, 1000, 10 000 μg/mL) on the secretory activity of granulosa cells (GCs) from porcine cyclic ovaries. The release of progesterone and estradiol-17*β* by GCs were evaluated by ELISA. In our study, the noticeable changes in estradiol-17*β* release by ovarian GCs were determined after the amygdalin addition. Amygdalin, at the highest dose (10 000 μg/mL), significantly ($P \leq 0.05$) stimulated the release of estradiol-17*β* by GCs, in comparison to the untreated control cells. On the contrary, no significant ($P \geq 0.05$) changes in the progesterone release by GCs caused by amygdalin addition were observed. In conclusion, obtained results showed that the amygdalin application (various doses) to ovarian GCs caused a dose-dependent stimulation of the estradiol-17*β* release, but not progesterone, and its possible modulatory impact on the steroid production in porcine ovaries.

Keywords: Amygdalin, hormone production, ovarian granulosa cells.

INTRODUCTION

Amygdalin is a naturally occurring plant glycoside found mainly in the seeds of apricots and bitter almonds. It is one of the most controversial natural substance that has been used as an anticancer drug for long period. This bioactive compound 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 ($C_{20}H_{27}NO_{11}$, Fig. 1A) is many times confused with **lae**vomandeloni**trile**, which is commonly known as Laetrile ($C_{14}H_{15}NO_7$, Fig. 1B). However, amygdalin and laetrile are different chemical compounds (**Andrew *et al.*, 1980; Du *et al.*, 2005**). Since the early 1950s, a modified form of amygdalin has been developed under the names "laetrile" and "Vitamin B17" to cure cancer, but it is not a vitamin. Studies have found it to be ineffective, dangerously cause cyanide posisoning, and sometimes fatal under realistic conditions (**Zhou *et al.*, 2012**). The decomposition of amygdalin is catalyzed by the action of β-D-glucosidase to yield hydrocyanic acid which stimulates the respiratory center and has antitussive and antiasthmatic effects (**Badr and Tawfik, 2010; Lv *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**).

Amygdalin is one of pharmacological components of crude ingredients of *Keishi-bukuryo-gan*, Japanese herbal medicine (**Yasui *et al.*, 2003**). It has been used for induction of ovulation in women suffering from infertility (**Igarashi, 1988**). *Keishi-bukuryo-gan* and its crude ingredients affected steroidogenesis in pre-ovulatory follicles (**Usuki, 1987, 1990, 1991**) and the *corpus luteum* (**Usuki, 1986, 1988**) in the rat ovary *in vivo* and *in vitro*.

Many studies have reported that amygdalin can be effectively used for prevent and treat various diseases including cancers, migraine, chronic inflammation, relieve fever and pain (**Yan *et al.*, 2006, Fukuda *et al.*, 2003, Zhou *et al.*, 2012**). Besides the mentioned benefits, amygdalin has been used for the treatment of asthma, bronchitis and also diabetes (**Zhou *et al.*, 2012**).

However, amygdalin as a therapeutic agent has not yet received FDA approval for use in the United States owing to insufficient clinical verification of its therapeutic efficacy, and the anticancer effect of amygdalin remains controversial (**Hwang *et al.*, 2008**). 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**). 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**).

Steroid hormones, such as progesterone and estradiol-17*β*, 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 remodelling (**Mahajan, 2008**).

In the present report, release of the steroid hormones (progesterone and estradiol-17*β*) by healthy porcine ovarian granulosa cells after amygdalin treatment (various doses) was observed.

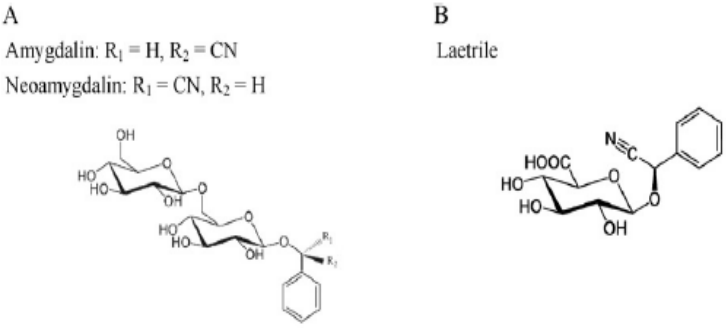

Figure 1 Chemical structure of amygdalin (A) and laetrile (B)

MATERIAL AND METHODS

Preparation, culture and processing of granulosa cells from ovaries

Ovaries from cyclic pigs were obtained from healthy Slovakian White gilts without obvious reproductive abnormalities. The ovaries were transported to the laboratory in containers at 4 ºC and washed in 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 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 a haemocytometer). Portions of the cell suspension were dispensed to 24-welled culture plates (Nunc™, Roskilde, Denmark, 1ml/well; for Enzyme Linked Immuno Sorbent 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 same 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, from apricot kernels, Sigma-Aldrich, St. Louis, Mo, USA) 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-17*β* 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 duplicates. The significance of differences between the control and experimental groups were evaluated by One-Way ANOVA and t-test using the 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≤0.05).

RESULTS

The effect of amygdalin on progesterone and estradiol-17β release by ovarian GCs

The secretory activity of granulosa cells (GCs) from cyclic porcine ovaries after amygdalin addition was observed (Figs. 2, 3). The experimental application of amygdalin (1, 10, 100, 1000, 10 000 µg/mL) to granulosa cells culture did not cause significant (P≥0.05) changes in the progesterone release, compared to the control without addition of the substance (Fig. 2). However, an apparent stimulation of the estradiol-17*β* release by GCs after amygdalin application was detected (Fig. 3). The significant (P≤0.05) increase of the estradiol-17*β*-release by GCs was detected in experimental group with the highest used amygdalin dose (10 000 µg/mL), compared to the control untreated cells. On the other hand, no significant (P≥0.05) differences in release of estradiol-17*β* by ovarian GCs after lower amygdalin doses (1, 10, 100, 1000 µg/mL) were determined. Only slight increase of estradiol-17*β* release by GCs was detected in experimental groups received 10 and 100 µg/mL of amygdalin, compared to the control.

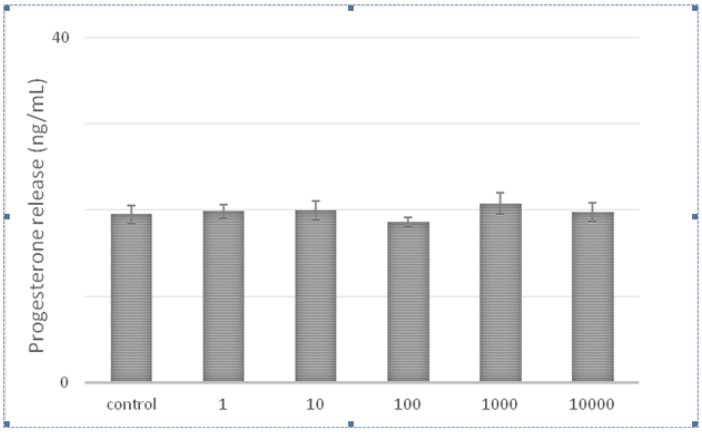

Figure 2 The effect of amygdalin on progesterone release by porcine ovarian granulosa cells. The control represents culture media without amygdalin addition; the experimental groups represent culture media supplemented with amygdalin (1, 10, 100, 1000, 10 000 µg/mL) addition. Differences between the groups were evaluated by One-way ANOVA, t-test. The data are expressed as means ± SEM. ELISA.

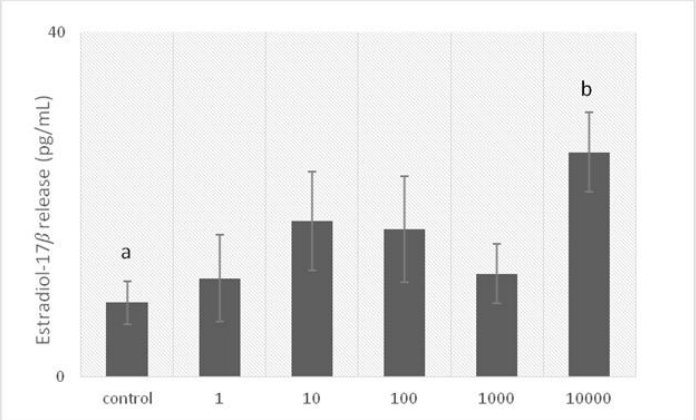

Figure 3 The effect of amygdalin on estradiol-17*β* release by porcine ovarian granulosa cells. The control represents culture medium without amygdalin addition; the experimental groups represent culture media supplemented with amygdalin (1, 10, 100, 1000, 10 000 µg/mL) addition
Signs *a, b* denote value significantly (*P* <0.05) different from control group.
Significance of differences between the groups was evaluated by One ANOVA, t-test. The data are expressed as means ± SEM. ELISA.

DISCUSSION

In the present report, hormone response of the porcine ovarian granulosa cells to amygdalin addition *in vitro* was examined.
Granulosa cells (GCs), isolated from porcine cyclic ovaries, were able to survive, grow in culture and release the steroid hormones after the experimental addition of natural compound amygdalin. Results from this observation suggest possible stimulatory effect of the plant glycoside amygdalin on the release of steroid regulatory molecule (estradiol-17*β*) by GCs, but not progesterone, in a dose-dependent manner.
Amygdalin was one of the most popular, non-conventional, anti-cancer treatments in the 1970s. By 1978, 70,000 US cancer patients had used amygdalin to treat their cancer (**Moss, 2005**). Still, evidence based research on amygdalin is sparse and its benefit controversial. Proponents consider amygdalin a natural cancer cure, whereas opponents warn that amygdalin is ineffective and even toxic. Although it has been argued that amygdalin is unsafe, no serious acute toxicity has been encountered (**Makarević *et al*., 2014**).
Schmidt *et al.* (1978) demonstrated that oral administration of amygdalin in doses equivalent to the recommended human tumoricidial doses along with the sweet almond preparations containing the amygdalin-hydrolyzing enzyme complex emulsion produced high levels of HCN in serum, clinical signs of cyanide toxicity, and death of 6 of 10 experimental animals.
Study focused on the therapeutic effect of amygdalin on various malignant human disease was carried out by **Moertel *et al.* (1982)**. No substantive benefit was observed in terms of cure, improvement, or stabilization of cancer, improvement of symptoms related to cancer, or extension of life span. The hazards of amygdalin therapy were evidenced several patients by symptoms of cyanide toxicity.
On the other hand, unique results were observed in recent study that described inhibitory action of amygdalin on cervical cancer cells. In this study, amygdalin was able to inhibit the growth of human cervical cancer cell line (HeLa cells) both *in vitro* and also *in vivo* through a mechanism of inducing apoptosis. Authors concluded, amygdalin may serve as a potentially effective therapy for cervical cancer (**Chen *et al*., 2013**). Similarly, influence of amygdalin on the tumor growth, proliferation and cell cycle progression of bladder cancer cells was investigated by **Makarevic *et al*. (2014)**. The suppression of cdk2 and cyclin A, key molecules responsible for cell cycle progression and cell division, was suggested as revelant mechanism defining how amygdalin may arrest or diminish tumor growth and proliferation. Otherwise, cultured human bladder cancer cells were treated with amygdalin alone or a combination of amygdalin and an antibody that was coupled (chemically) to beta-glucosidase. The target for this antibody was the glycoprotein (a protein with sugar molecules attached) MUC1. In this study, amygdalin alone was not very effective in killing the bladder cancer cells, but its cell-killing ability was 36 times greater in the presence of the antibody-enzyme complex (**Syrigos *et al*., 1998**).
However, the question whether amygdalin is able to affect the cellular processes in normal tissues, under physiological conditions, is still unanswered. Therefore, uncertain outcomes have led us to evaluate the possible impact of amygdalin on the secretory activity of healthy ovarian granulosa cells *in vitro*.
In our study, the noticeable changes in estradiol-17*β* release by ovarian GCs were determined after the amygdalin addition. Amygdalin, at the highest dose (10 000 µg/mL), stimulated the release of estradiol-17*β* by GCs, in comparison to the untreated control cells. On the contrary, no significant changes in the

progesterone release by GCs caused by amygdalin addition were observed in this study.

These results are in accordance with our previous investigation, in which has described the amygdalin influence on the release of progesterone by GCs from cyclic and also non-cyclic porcine ovaries *in vitro*. No significant differences in the progesterone release by GCs from cyclic and non-cyclic ovaries after amygdalin treatment (1, 10, 100, 1000, 10 000 µg/mL) were detected (**Halenár *et al*., 2013a**). Recently, **Kádasi *et al.*** (**2012**) reported also stimulatory effect of curcumin, a natural plant molecule, on the release of progesterone and testosterone by porcine ovarian GCs. Furthermore, authors suggested a direct impact of curcumin on the steroidogenesis, proliferation as well as apoptosis of ovarian granulosa cells *in vitro*. Similarly, **Kolesarova *et al.*** (**2012**) demostrated stimulatory effect of resveratrol, a natural polyphenol, on the progesterone release by porcine ovarian GCs at the doses 50 µg/mL but not at 30 and 10 µg/mL.

Previous studies examined the effects of natural compounds on different parts of animal reproductive system (**Kolesárová *et al*., 2012a,b; 2011; Tanyildizi and Bozkurt, 2004; Halenár et al., 2013b; Yasui *et al*., 2003; Randel *et al*., 1992**).

Likewise, cultured HTB-35 cells line, as a model of cervical carcinoma, was used for the evaluation anticancer properties of amygdalin. Results from this study indicate that amygdalin reduced proliferation potential, decreased mitochondrial activity, accumulated cells in the G1 phase and lead to their death (**Jarocha and Majka, 2011**). Recent observation, carried out by **Nabavizadeh *et al*.** (**2011**), has also suggested the preventive and therapeutic effects of amygdalin on absolute alcohol-induced gastric ulcer in rats. The results of this study showed that amygdalin protected gastric mucosa from alcohol-induced gastric ulcer, and the protective action was mediated via gastric mucosal nitric oxide production and TNF-α suppression.

There are many studies which have described the effects of different natural substances on the secretory activity of porcine (**Medveďová *et al*., 2011, Maruniaková *et al*., 2013, Ranzenigo *et al*., 2008**) and rats ovarian cells (**Kolesárová *et al*., 2011**). The adverse impacts of various naturally cyanide-containing substances on the motility and morphological abnormality of bull sperm, were observed previously (**Tanyildizi and Bozkurt, 2004**).

Steroid hormones, such as progesterone and estradiol, are produced by ovarian cells and both play irreplaceable role in ovarian cycles (**Hagan *et al*., 2009; Arnhold *et al*., 2009**), contribute to regulation of ovarian follicular development and remodeling (**Mahajan, 2008**). Exposure to toxic concentrations of deoxynivalenol, resveratrol and their combination on the release of progesterone by porcine ovarian granulosa cells was studied by **Kolesárová *et al*.** (**2012a**). Results from this *in vitro* study suggested that reproductive toxicity of animals induced by a mycotoxin – deoxynivalenol can be inhibited by a protective natural substance - resveratrol.

Amygdalin, as a therapeutic agent, has not yet received FDA approval for use in the United States owing to insufficient clinical verification of its therapeutic efficacy, and the anticancer effect of amygdalin remains controversial (**Hwang *et al*., 2008**).

Possible modulatory impact of amygdalin (only high doses) on the steroid production of porcine ovaries is presented here.

CONCLUSION

Predictable impact of reputed anticancer compound amygdalin on the release of steroid hormones by GCs from porcine cyclic ovaries was demonstrated in this report. The amygdalin application (various doses) to ovarian GCs caused a dose-dependent stimulation of estradiol-17*β* release. On the contrary, no differences in the progesterone release by ovarian GCs were obtained after amygdalin addition, compared to untreated control cells. In conclusion, results obtained from this *in vitro* study, together with our ongoing animal study, could significantly contribute to evaluate the possible effects of amygdalin on healthy animal system.

Acknowledgments: This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. 1/0022/13, APVV-0304-12, and European Community under project no 26220220180: Building Research Centre „AgroBioTech".

REFERENCES

ANDREW, F., ROSCOE, O.B., ANDREW, E.G. 1980. A β-glucosidase in feline kidney that hydrolyzes amydalin (Laetrile). *Archives of Biochemistry and Biophysics*, 201, 363-368. **http://dx.doi.org/10.1016/0003-9861(80)90523-8**

ARNHOLD, I.J., LOFRANO-PORTO, A., LATRONICO, A.C. 2009. Inactivating mutations of luteinizing hormone beta-subunit or luteinizing hormone receptor cause oligo-amenorrhea and infertility in women. *Hormone Research*, 71(2), 75-82. **http://dx.doi.org/10.1159/000183895**

BADR, J.M., TAWFIK, M.K. 2010. Analytical and pharmacological investigation of amygdalin in *Prunus armeniaca* L. kernels. *Journal of Current Pharmaceutical Research*, 3, 2134-2137.

DENG, Y., GUO, Z.G., ZENG, Z.L., WANG, Z. 2002. Studies on the pharmacological effects of saffron (Crocus sativus L.). *Chin. J. Chin. Mater. Med*. 27(8), 565-568.

DU, Q., JERZ, G., HE, Y., LI, L., XU, Y., ZHANG, Q., ZHENG, Q., WINTERHALTER, P., ITO, Y. 2005. Semi-industrial isolation of salicin and amygdalin from plant extracts using slow rotary counter-current chromatography. *Journal of Chromatography A*, 1074, 43-46. **http://dx.doi.org/10.1016/j.chroma.2005.03.064**

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*, 26, 271-273. http://dx.doi.org/10.1248/bpb.26.271

HAGAN, C.R., FAIVRE, E.J., LANGE, C.A. 2009. Scaffolding actions of membrane-associated progesterone receptors. *Steroids*, 74, 568-572. **http://dx.doi.org/10.1016/j.steroids.2008.12.004**

HALENÁR, M., MARUNIAKOVÁ, N., MEDVEĎOVÁ, M., KOLESÁROVÁ, A. 2013a. The effect of amygdalin on porcine ovarian granulosa cells *in vitro*. *Journal of Microbiology, Biotechnology and Food Sciences*, 2, 14.

HALENÁR, M., MEDVEĎOVÁ, M., MARUNIAKOVÁ, M., KOLESÁROVÁ, A. 2013b. Possible effect of amygdalin in combination with deoxynivalenol on secretion activity of porcine ovarian granulosa cells in vitro. *Animal welfare, ethology and housing system*, 9(3), 471-476.

HOWARD-RUBEN, J., MILLER, N.J. 1984. Unproven methods for cancer management, Part II: Current trends and implicaitons for patient care. *Oncology Nursing Forum*, 11(1), 67-73.

HWANG, H.J., LEE, H.J., KIM, CH. J., SHIM, I., HAHM, D.H. 2008. Inhibitory effect of amygdalin on lipopolyccharide-inducible TNF-α and IL-1β mRNA expression and carrageenan-induced rats arthritis. *Journal of Microbiology and Biotechnology*, 18(10), 1641-1647.

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*, 29(8), 1597-1602. **http://dx.doi.org/10.1248/bpb.29.1597**

CHEN, Y., MA, J., WANG, F., HU, J., CUI, A., WEI, C., YANG, Q., LI, F. 2013. Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells. *Immunopharmacology and Immunotoxicology*, 35(1), 43-51. **http://dx.doi.org/10.3109/08923973.2012.738688**

IGARASHI, M. 1988. Kampo medicine in endocrinology. *Recent Advancesin the Pharmacology of Kampo (Japanese herbal) medicines*, E. Hosoya, Y. Yamamura (eds). Tokyo, Excepta Medica, 1988, p. 157-160.

JAROCHA, D., MAJKA, M. 2011. Influence of amygdalin on biology of cervical carcinoma cells. *Abstracts of the 2nd Congress of Biochemistry and Cell Biology*, Krakow, 280.

KÁDASI, A., SIROTKIN, A.V., MARUNIAKOVÁ, N., KOLESÁROVÁ, A., BULLA, J., GROSSMANN, R. 2013. The effect of curcumin on secretory activity, proliferation and apoptosis of the porcine ovarian granulosa cells. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(1), 349-357.

KOLESÁROVÁ, A., CAPCAROVÁ, M., BAKOVÁ, Z., GÁLIK, B., JURACEK, M., SIMKO, M., SIROTKIN, A.V. 2011. The effect of bee pollen on secretion activity, markers of proliferation and apoptosis of porcine ovarian granulosa cells *in vitro*. *Journal of Environmental Science and Health*, Part B, 46(3), 207-212. **http://dx.doi.org/10.1080/03601234.2011.540202**

KOLESÁROVÁ, A., CAPCAROVÁ, M., MARUNIAKOVÁ, N., LUKÁČ, N., CIERESZKO, R.E., SIROTKIN, A.V. 2012a. Resveratrol inhibits reproductive toxicity induced by deoxynivalenol. *Journal of Environmental Science and Health*, Part A, 47, 1329-1334. **http://dx.doi.org/10.1080/10934529.2012.672144**

KOLESÁROVÁ, A., BAKOVÁ, Z., CAPCAROVÁ, M., GÁLIK, B., JURACEK, M., SIMKO, M., TOMAN, R., SIROTKIN, A.V. 2012b. Consumption of bee pollen affects rat ovarian functions. *Journal of Animal Physiology and Animal Nutrition*, 97, 1059-1065. **http://dx.doi.org/10.1111/jpn.12013**

KWON, H.J., LEE, J.H., HONG, S.P. 2010. Improvement of the extraction efficiency of D-amygdalin from Armeniacae Semen powder through inactivating emulsion and suppressing the epimerization of D-amygdalin. *Archives of Pharmacal Research*, 33, 81-86. **http://dx.doi.org/10.1007/s12272-010-2229-3**

LV, W.F., YU, D., ZHENG, R. 2005. Isolation and quantitation of amygdalin in apricot kernel and *Prunus Tomentosa* Thunb. by HPLC with solid phase extraction. *Journal of Chromatographic Science*, 43, 383-387. **http://dx.doi.org/10.1093/chromsci/43.7.383**

MAHAJAN, D.K. 2008. Pig model to study Dynamics of steroid during ovarian follicular growth and maturation. *Sourcebook of Models for Biomedical Research*, Totowa, New Jersey: Humana Press, 778. **http://dx.doi.org/10.1007/978-1-59745-285-4_45**

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*, 9(8), 1–9. **http://dx.doi.org/10.1371/journal.pone.0105590**

MARUNIAKOVÁ, N., KOLESÁROVÁ, A., KÁDASI, A., MEDVEĎOVÁ, M., HALENÁR, M., SIROTKIN, A.V., GROSSMANN, R., BULLA, J. 2013.Release of progesterone and testosterone by ovarian granulosa cells after addition of T-2 toxin and its combination withgrowth factor IGF-I. *Journal of Microbiology, Biotechnology and Food Sciences,* 2, 1864-1874.

MEDVEĎOVÁ, M., KOLESÁROVÁ, A., CAPCAROVÁ, M., LABUDA, R., SIROTKIN, A.V., KOVÁČIK, J., BULLA, J. 2011. The effect of deoxynivalenol on the secretory activity, proliferation and apoptosis of porcine ovarian granulosa cells *in vitro*. *Journal of Environmental Science and Health Part B*, 46(3), 213-219. **http://dx.doi.org/10.1080/03601234.2011.540205**

MOERTEL, C.G., R., FLEMING, T.R., RUBIN, J., KVOLS, L.K., SARNA, G., KOCH, R., CURRIE, V.E., YOUNG, C.W., JONES, S.E., DAVIGNON, J.P. 1982. A clinical trial of amygdalin (laetrile) in the treatment of human cancer. *The New England Journal of Medicine*, 306(4), 201-206. **http://dx.doi.org/10.1056/NEJM198201283060403**

MOSS, R.W. 2005. Patient perspectives: Tijuana cancer clinics in the post-NAFTA era. *Integrative Cancer Therapies*, 4, 65–86. **http://dx.doi.org/10.1177/1534735404273918**

NABAVIZADEH, F., ALIZADEH, A.M., SADROLESLAMI, Z., ADELI, S. 2011. Gastroprotective effects of amygdalin on experimental gastric ulcer: Role of NO and TNF-α. *Journal of Medical Plants Research*, 5(14), 3122-3127.

RANDEL, R.D., CHASE, C.C.JR., WYSE, S.J. 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *Journal of Animal Science*, 70, 1620- 1638.

RANZENIGO, G., CALONI, F., CREMONESI, F., AAD, P.Y., SPICER, L.J. 2008. Effects of *Fusarium* mycotoxins on steroid production by porcine granulosa cells. *Animal Reproduction Science*, 107(1-2), 115-130. **http://dx.doi.org/10.1016/j.anireprosci.2007.06.023**

SCHMIDT, E.S., NEWTON, G.W., SANDERS, S.M., LEWIS, J.P., CONN, E.E. 1978. Laetrile toxicity studies in dogs. *Journal of the American Medical Association*, 239, 943-947. **http://dx.doi.org/10.1001/jama.1978.03280370039021**

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*, 14(11), 895-901.

SYRIGOS, K.N., ROWLINSON-BUSZA, G., EPENETOS, A.A. 1998. *In vitro* cytotoxicity following specific activation of amygdalin by beta-glucosidase conjugated to a bladder cancer-associated monoclonal antibody. *International Journal of Cancer*, 78(6), 1998, 712-719.

TANYILDIZI, S., BOZKURT, T. 2004. *In Vitro* Effects of Linamarin, Amygdalin and Gossypol Acetic Acid on Hyaluronidase Activity, Sperm Motility and Morphological Abnormality in Bull Sperm. *Turkish Journal* of *Veterinary* and *Animal Sciences*, 28, 819-824.

USUKI, S. 1986. Effects of Chinese herbal medicines on progesterone secretion by *corpus luteum*. *Japanese Journal of Fertility and Sterility*, 31, 482-486.

USUKI, S. 1987. Effects of Hachimijiogan, Tokishakuyakusan and Keishibukuryogan on estrogen and progesterone secretion in ovarian follicles. *Japanese Journal of Fertility* and *Sterility*, 32, 276-283.

USUKI, S. 1988. Effects of Hachimijiogan, Tokishakuyakusan and Keishibukuryogan on progesterone and 17α-hydroxyprogesterone secretion by rat corpora lutea *in vivo*. *Japanese Journal of Fertility* and *Sterility*, 33, 60-66.

USUKI, S. 1990. Effects of Tokishakuyakusan and Keishibukuryogan on steroidogenesis by rat preovulatory follicles *in vivo*. *American Journal of Chinese Medicine*, 18, 1990, 149-156.

USUKI, S. 1991. Effects of Hachimijiogan, Tokishakuyakusan and Keishibukuryogan, Ninjinto and Unkeito on estrogen and progesterone secretion in preovulatory follicles incubated *in vitro*. *American Journal of Chinese Medicine*, 19, 65-71.

YAN, J., TONG, S., LI, J., LOU, J. 2006. Preparative Isolation and Purification of Amygdalin from *Prunusarmeniaca* L. with High Recovery by High-Speed Countercurrent Chromatography. *Journal of Liquid Chromatography & Related Technologies*, 29, 1271-1279. **http://dx.doi.org/10.1080/10826070600598985**

YASUI, T., MATSUZAKI, T., USHIGOE, K., KUWAHARA, A., MAEGAWA, M., FURUMOTO, H., AONO, T., IRAHARA, M. 2003. Stimulatory effect of the herbal medicine *Keishi-bukuryo-gan* on a cytokine-induced neutrophil chemoattractant, in rat ovarian cell culture. *American Journal of Reproductive Immunology*, 50, 90-97. **http://dx.doi.org/10.1034/j.1600-0897.2003.00055.x**

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*, 90, 516-523. **http://dx.doi.org/10.1016/j.carbpol.2012.05.073**