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

Published by Faculty of Biotechnology and Food Sciences

CYANOGENIC GLYCOSIDES AS A POTENTIAL BIOREGULATOR

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doi: 10.15414/jmbfs.2016.6.1.743-746

ARTICLE INFO

Received 3. 3. 2016
Revised 5. 5. 2016
Accepted 8. 6. 2016
Published 1. 8. 2016

Review

OPEN ACCESS

ABSTRACT

Natural substances which are considered to be a food that provides medical and health benefits are called bioregulator. Bioregulators can be used in medicine for preventing and treating migraine, hypertension, chronic inflammation, and other reaction source diseases. Amygdalin is considered for one of the most important bioregulator. It is a controversial nature cyanogenic glycoside abundant in the seeds of *Rosaceae* family. The family includes herbs, shrubs, and trees and most species are deciduous, but some are evergreen. In the past few years, has been a renewed interest about distribution of *Rosaceae* fruits because amygdalin has been used for many years in traditional and alternative medicine. Therefore, it is included in regulatory pathways and processes. Recent data indicate potential regulatory activity of amygdalin in signaling pathways of highly metastatic cells, suggesting that amygdalin might not only be an innovative tool to neutralize metastatic dissemination but also to complement mTOR-inhibitor based regimens.

Keywords: Amygdalin, cyanogenic glycosides, apoptosis, mTOR pathway

INTRODUCTION

In recent years considerable effort has been made to identify the metabolic factors linking nutrition with partial physiological processes **(Monget and Martin, 1997; Kolesárová *et al.*, 2011)**. Numerous data from various mammalian species have shown that natural substances may influence the physiological functions **(Prunier and Quesnel, 2000)** or on the other side tumorigenesis, carcinogenesis, angiogenesis **(Subash *et al.*, 2010; Aggarwal *et al.*, 2009)**. The most widely plant constituents, which are biologically active and provide medical and health benefits are called bioregulators **(Brower, 1998; Zeisel, 1999)**. The function of bioregulators is also important to examine from viewpoint of prevention of many reproductive alterations **(Medveďová *et al.*, 2010).** Neuroendocrine regulation manages reproductive system through axis hypothalamic, pituitary and gonads **(Javorka *et al.*, 2012)**. Sex hormones (female-estrogens, progesterone, male-androgens: testosterone) are steroids (fat soluble compounds) that control sexual maturity and reproduction. The endocrine glands ovaries, testes, or adrenal cortex regulate sexual development of an organism and affects the growth or function of the reproductive organs. 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 **(Chedrese, 2009).** Fruits and vegetables contain many different natural components, some essential nutrients and also contain a variety of bioactive substances, which have other beneficial health effects **(Kris-Etherton *et al.*, 2002)**. In the past few years, there has been a renewed interest in evaluating the bioregulator content and distribution in patterns of fruits and vegetables **(Flood *et al.*,2002; Ruiz *et al.*, 2006)**.

Rosaceae family

Family *Rosaceae*, comprised of over 100 genera and 3000 species, is the third most economically important plant family in temperate regions **(Dirlewanger *et al.*, 2002)**. *Rosaceae* contain edible members such as almonds, apples, plums, peaches, pears, raspberries, sour cherries, sweet cherries, and strawberries. Other non-edible species with almost exclusively ornamental value include rose, hawthorn, potentilla, cotoneaster, and pyracantha. The products of this family are in high demand for their nutritional and esthetic values edible **(Vavilov, 1951)**. *Rosaceae* fruits are also a major human dietary source of phytochemicals, such as flavonoids, cyanogenic glycosides, phytoestrogens **(Mazur *et al.*, 2000)**, and phenols that could potentially yield health and disease - fighting advantages. L-Ascorbic acid, quercetin, kaempferol, myricetin, p-coumaric acid, gallic acid, and ellagic acid are well known antioxidants and/or cancer-inhibiting compounds that have been identified in these fruits **(Macheix *et al.*, 1991, 1998; Selmar, 1999)**. The family *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 **(Fakuta *et al.*, 2003; Chang *et al.*, 2005)**. *In vitro* and *in vivo* studies on animal models provide evidence that fruit and leaf extracts from many *Rosaceae* species inhibit some cancers or have strong antioxidant activities **(Yau *et al.*, 2002)**.
Seeds of *Rosaceae* fruits contain a substantial amount of dietary protein **(Nout et al., 1995)** along with significant amounts of oil and fiber but this part of apricot also depending on the variety, contain the toxic cyanogenic glycoside - amygdalin **(Gomes *et al.*, 1998). Islamiyat *et al*. (2014)** have developed and applied a high performance liquid chromatographic procedure for amygdalin quantification to investigate extraction efficiency and to determine levels in a range of commercially-available foods. Their results showed that seeds from *Rosaceae* species contained relatively high amounts (range 0.1–17.5 mg/g) of amygdalin compared to seeds from *non-Rosaceae* species (range 0.01–0.2 mg/g) **(Yildirim *et al.*, 2010).** The apricot (*Prunus armeniaca L.*) is a member of the *Rosaceae* family. Apricot fruit, being a rich source of vitamins and minerals and is one of the most familiar crops worldwide. Their trees are not ubiquitous since they can only grow in certain regions where the environmental conditions are favourable **(Baytop *et al.*, 1999)**. The fresh apricot fruit contains carbohydrates, vitamins C and K, β-carotene, niacin, and thiamine. Organic acids, phenols, volatile compounds, esters, and terpenoids have also been isolated **(Ruiz *et al.*, 2006; Riu-Aumatell *et al.*, 2005; Safer *et al.*, 2006)**.

Bioactive substance - Amygdalin

Natural plant origin products like amygdalin are still a major part of traditional medicine **(Hwang *et al.*, 2008)**. Amygdalin (vitamin B17; previously called laetrile) is one of many nitrilosides, which are natural cyanide-containing substances abundant in the seeds of prunasin family and other *Rosaceous* plants **(Chang *et al.*, 2005; Pak *et al.*, 1999)**. The distribution of the cyanogenic glycosides in the plant kingdom is relatively wide and they are present mainly in more than 2650 plant species **(Franciscu and Pinotti, 2000; Haque and Bradbury, 2002**). There are approximately 25 cyanogenic glycosides which have found in the edible parts of plants being: amygdalin (almonds); dhurrin (sorghum); linamarin (cassava, lima beans); lotaustralin (cassava, lima beans); prunasin (stone fruit); and taxiphyllin (bamboo shoots) **(Gonzales and Sabatini,**

1989). Cyanide is a toxic substance, mainly due to its affinity for the terminal cytochrome oxidase in the mitochondrial respiratory pathway **(Brattsten *et al*., 1983)**. The lethal dose of cyanide for vertebrates lies in the range of 35–150 μmol/kg, if applied in a single dose. Much higher amounts of HCN can be tolerated if consumed or administered over a longer period **(Davis and Nahrstedt, 1985).** Biosynthesis and degradation of cyanogenic glycosides (CNGs) are well documented in many plants **(Jones *et al*., 2000; Lechtenberg and Nahrstedt, 1999).**

But the genetic control of cyanogenesis has no unique mechanism, the plants show variation in the amount of the produced HCN. The production of HCN depends on both the biosynthesis of CNGs and on the existence (or absence) of its degrading enzymes. The biosynthetic precursors of the CNGs are different L-amino acids, these are hydroxylated then the *N*-hydroxylamino acids are converted to aldoximes, these are turned into nitriles. The last ones are hydroxylated to α-hydroxynitriles and then they are glycosilated to CNGs. The generation of HCN from CNGs is a two steps process involving a deglycosilation and a cleavage of the molecule (regulated by β-glucosidase and α-hydroxynitrilase). The tissue level compartmentalisation of CNGs and their hydrolysing enzymes prevents large-scale hydrolysis in intact plant tissue. The actual level of CNGs is determined by various factors both developmental and ecological ones, which are reviewed too **(Vetter, 2000)**.

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 **(Zhou *et al*., 2012**).

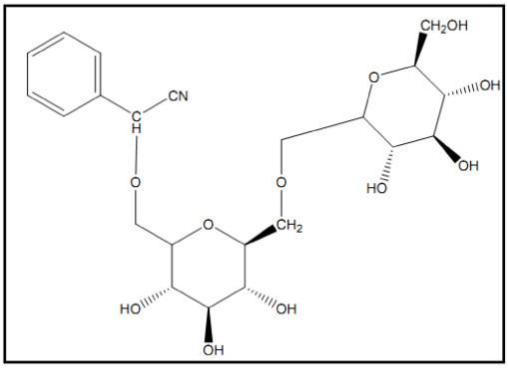

Figure 1 Chemical structure of amygdalin **(Abdel-Rahman, 2011)**.

Action of endogenous plant enzymes can release hydrogen cyanide causing potential toxicity issues for animals and humans, including cell death by blocking cytochrome oxidase and the arrest of the ATP production **(Bolarinwa *et al*., 2014)**. Amygdalin has been used to treat cancers and relieve pain **(Ellison *et al*., 1978; Shim *et al*., 2000)**. 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 acute toxicity experiments of amygdalin have proved that the toxicity of oral administration route is far greater than the intravenous route **(Adewusi and Oke, 1985; Park *et al*., 2013)**. The maximum tolerance dose of intravenous and intramuscular injection of amygdalin in mice, rabbits, dogs are 3g/kg, 0.075 g/kg orally respectively **(Zhang and Jin, 1986; Rauws *et al*., 1982)** and human intravenous injection are 5g (approximately 0.07 g/kg). 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, Song and Xu., 2014)**. Recent studies examined the effects of natural compound amygdalin on female reproductive system concentrated on secretory activity of porcine ovarian granulosa cells (GC) *in vitro* **(Halenár *et al*., 2013a)**. **Halenár *et al.* (2015)** have the investigated release of steroid hormone progesterone by GC from cyclic and non-cyclic porcine ovaries. The progesterone release was not significantly *(P>0.05)* affected by the amygdalin treatment at all experimental doses (1, 10, 100, 1000 and 10000 mg/ml) compared to the control group without addition. However, a significant stimulation *(P<0.05)* of the 17-β-estradiol release after amygdalin addition at the highest dose (10000 mg/ml) was observed. Other experimental doses of amygdalin (1, 10, 100 and 1000 mg/ml) did not cause differences in the 17β-estradiol secretion. **Kolesár *et al*., (2015)** in their review have described the characteristic, metabolism and possible effects of amygdalin on reproductive processes. Previous studies described the effects of natural compound amygdalin on female and male reproductive systems focused on process of steroidogenesis **(Halenár *et al*., 2013a, 2015)**, spermatozoa motility and morphological abnormalities of bull spermatozoa **(Tanyildizy and Bozkurt, 2004)**. Amygdalin significantly inhibited sperm hyaluronidase activity. The inhibition of hyaluronidase activity can cause a drop in the fertilization ability of bull spermatozoa due to the prevention of acrosomal reaction. However, amygdalin did not produce any morphological abnormality in bull spermatozoa. The inhibition of sperm hyaluronidase activity and spermatozoa motility showed that these compounds have deleterious effects on bull sperm in vitro **(Tanyildizy and Bozkurt, 2004)**.

Amygdalin is one of main 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*.

The characterization and role of mTOR

Mammalian target of rapamycin (mTOR) is a *serine/threonine kinase*, which belongs to phosphatidylinositol-3 kinase (PI3K) related kinases (PIKKs) family. It regulates cellular metabolism, growth, proliferation, and therefore is a target for the development of a number of mTOR inhibitors **(Pópulo *et al*., 2012)**. Akt functions as a component of the PI3K cell survival pathway. In cancer, Akt activity is frequently elevated due to multiple mechanisms, including loss of function of the PTEN tumor-suppressor gene and mutations of the PIK3CA gene. Akt functions as a component of the PI3K cell survival pathway **(Manning et al., 2005)**. Akt acts as a survival kinase in many cancers **(Cheng *et al*., 2005)**. The PI3K pathway is implicated in cell survival and cell growth, and can be activated by growth factors binding to cell-surface receptors. It is an intricate signaling cascade that is among the most frequently activated pathways in different types of cancer. PI3K is the subject of extensive research **(Knight *et al*., 2007)**.

Qian *et al.* (2015) submitted a study where Western blot results showed that amygdalin had no significant impact on Akt and Rictor expression. Rictor as a subunit of mTOR plays an important role in the Akt-mTOR signaling pathway; its phosphorylation level is positively regulated by Akt **(Chen *et al*., 2010)**. But the determination of Akt and rictor phosphorylation level showed that amygdalin significantly reduced the phosphorylation level of these two proteins in highly metastatic cells, suggesting that amygdalin was able to regulate the activity of Akt and rictor signaling pathways **(Qian *et al*., 2015)**. The PI3K pathway is a key signal transduction system that links oncogenes and multiple receptor classes to many essential cellular functions, and is perhaps the most commonly activated signalling pathway in human cancer. This pathway therefore presents both an opportunity and a challenge for cancer therapy. Even as inhibitors that target PI3K isoforms and other major nodes in the pathway, including Akt and mTOR, reach clinical trials, major issues remain. **Liu *et al*., (2009)** describe the progress made in understanding of the PI3K pathway and discuss the potential of and challenges for the development of natural therapeutic agents that target this pathway in cancer (**Liu *et al.*, 2009)**. Thus, amygdalin might not only be an innovative tool to neutralize metastatic dissemination but also to complement mTOR-inhibitor based regimens **(Gupta *et al*., 2013)**.

SUMMARY, CONCLUSIONS, AND FUTURE PERSPECTIVES

This review described possible effects of natural bioregulators on various types of animal cells. In recent years, increasing attention has been paid to natural substances and their impact on specific pathways in the cell. Amygdalin, as natural product shows lot of evidences. This natural compound is known for its anticancer, anti-inflammatory activity and other medicinal benefits, but on the other side represents one of the most controversial substance. Although amygdalin itself is non-toxic but its production HCN splitted by some endogenous plant enzymes is toxic substance for animals including humans. These agents that inhibit the downstream protein kinase mTOR as well as agents that inhibit multiple kinases, including components of the PI3K-Akt pathway are under clinical evaluation. There are still only a few studies which could suggest the possible involvement of amygdalin in mTOR pathway and thus influence animal reproductive system. Therefore other in vitro and in vivo experiments are necessary.

Acknowledgments: This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. 1/0039/16, KEGA 011SPU-4/2016, Slovak Research and Development Agency, APVV-0304-12, and European Community under project no 26220220180: Building Research Centre „AgroBioTech".

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