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**A REVIEW**

# AMYGDALIN AND ITS EFFECTS ON ANIMAL CELLS

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## ABSTRACT

Amygdalin is a natural compound whose anticancer, anti-inflammatory activity and other medicinal benefits have been known for many years. It has been isolated in 1830 by the French chemists Robiquet and Boutron-Charlard from kernels of the bitter almond (*Prunus amygdalus*). It is a major component of the seeds of prunasin family plants, such as apricots, almonds, peaches, apples, and other rosaceous plants. 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. It has been used as a traditional drug because of its wide range of medicinal benefits. Amygdalin can be used in medicine for preventing and treating migraine, hypertension, chronic inflammation, and other reaction source diseases. This review is focused on the effects of amygdalin on the animal system.

**Keywords:** amygdalin, animal cells cancer, reproduction, proliferation, apoptosis

## INTRODUCTION

### *Characteristic*

Amygdalin is a cyanogenic glucoside initially isolated from the seeds of bitter almonds (*Prunus dulcis*) (**Chwalek and Plé, 2004**). It is a major component of the seeds of

prunasin family plants, such as apricots, almonds, peaches, apples, and other rosaceous plants (**Fukuta *et al.*, 2003**). Its oldest known use was by the ancient Egyptians as a poison for executing capital punishment: "penalty of the peach". It has been isolated in 1830 by the French chemists Robiquet and Boutron-Charlard from kernels of the bitter almond (*Prunus amygdalus*) and has been thoroughly investigated in 1837 by Liebig and Wöhler. Its detailed chemical structure was at last established by the carbohydrate chemists Haworth and Wylam in 1923 (**Rauws *et al.*, 1982**).

Amygdalin (D-mandelonitrile-β-D-gentiobioside, Fig. 1A), $C_{20}H_{27}NO_{11}$, 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**).

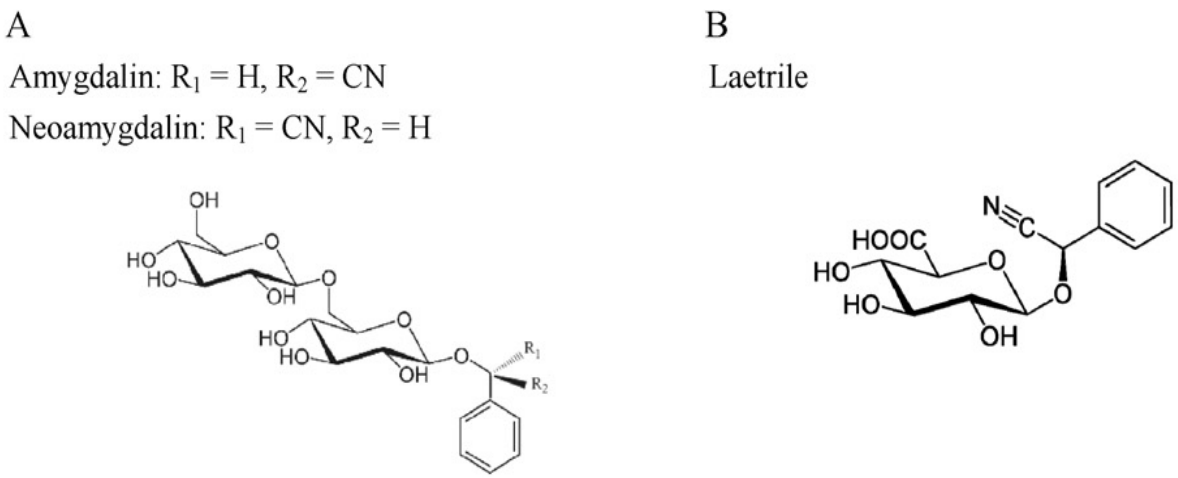


**Figure 1** (A) Molecular structure of amygdalin (D-mandelonitrile-β-D-gentiobioside, $C_{20}H_{27}NO_{11}$). (B) Molecular structure of laetrile (cyanophenylmethyl-β-D-glucopyranosiduronic acid, $C_{14}H_{15}NO_7$) (**Zhou *et al.*, 2012**)

Amygdalin is sometimes confused with laevomandelonitrile (Cyanophenylmethyl-β-D-glucopyranosiduronic acid, Fig. 1B), $C_{14}H_{15}NO_7$, which is commonly known as laetrile. However, amygdalin and laetrile are different chemical compounds (**Andrew *et al.*, 1980; Du *et al.*, 2005**).

***Metabolism***

Beta-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**). *In vivo* the enzyme complex emulsin containing the enzymes β-D-glucosidase, benzocyanase, and others, degrades the amygdalin into four components: hydrocyanic acid, benzaldehyde, prunasin, and mandelonitrile, which are absorbed into the lymph and portal circulations (**Chang and Zhang, 2012**).

The enzymatic breakdown of amygdalin occurs most rapidly in alkaline conditions. The β-glucosidase may be deactivated in the acid environment of the stomach but can then be partially reactivated in the alkaline environment of the gut (**JECFA, 1993**). Cyanogenic glycosides can also be hydrolysed by gut flora. Amygdalin is metabolized by the body to produce cyanide, a very rapid poison which impairs cellular respiration leading to a cascade of events culminating in death (**Ballantyne and Marrs, 1987**).

***Effects on the animal organism***

The effects of natural substances on animal organism concentrated on the reproductive system (**Kolesarova *et al*., 2012a; 2012b; 2011**; **Kádasi *et al*., 2012; Tanyildizy and Bozkurt, 2004; Yasui *et al*., 2003; Randel *et al*., 1992;**) were studied in the previous studies. Natural plant origin products like amygdalin are still a major part of traditional medicine (**Nabavizadeh *et al*., 2011**). 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 (**Zhou *et al*., 2012**).

Amygdalin was used as an anticancer agent in Russia as early as 1845, with positive results reported for the first patient treated (**Moss, 1996**). 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**). Various rodent cancers (osteogenic sarcoma, melanoma, carcinosarcoma, lungcarcinoma, and leukemia) were transplanted into rats and mice. The animals were treated with intraperitoneal injections of amygdalin, with or without the enzyme beta-glucosidase. None of the solid tumors or leukemias investigated responded to amygdalin at any dose tested. No statistically significant increase in animal survival was observed in any of the treatment groups (**Wodinsky and Swiniarski, 1975; Laster and Schabel, 1975**).

However, positive results were obtained in other studies. Amygdalin enhanced the antitumor activity of a combination of enzymes and vitamin A in mice bearing spontaneous mammary adenocarcinomas. The amygdalin was given by intramuscular injection, the vitamin A was administered orally through a feeding tube, and the enzymes were injected into and around tumor masses. No anticancer activity was observed when amygdalin was given alone (**Manner *et al.*, 1978**). White blood cells and prostate cancer specimens were used to investigate the potential of amygdalin to stimulate the immune system. Amygdalin caused a statistically significant increase in the ability of a patient's white blood cells to adhere to his own prostate cancer cells, suggesting some immune system boosting potential for amygdalin (**Bhatti *et al.*, 1981**). The ability of amygdalin and beta-glucosidase to indirectly sensitize the hypoxic (oxygen-starved) cells at the center of a tumor to the lethal effects of gamma irradiation was investigated. Cells at the periphery (outer edge) of a tumor are more sensitive to gamma irradiation because they are not oxygen-deprived. Presumably, cyanide uptake by interior tumor cells is less than that of cells located at a tumor's periphery. The investigators found that amygdalin and beta-glucosidase could act as indirect radiation sensitizers of hypoxic tumor cells (**Biaglow and Durand, 1978**). 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**). Amygdalin induced DNA damage, involved in cell cycle on SNU-C4 human colon cancer cells and amygdalin might be used for therapeutic anticancer drug (**Park *et al.*, 2005**). 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**).

Besides the antitumor activity, amygdalin has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes (**Zhou *et al.*, 2012**). It is also decomposed by the action of β-D-glucosidase to yield hydrocyanic acid which stimulates the respiratory center reflexively and produces a kind of antitussive and antiasthmatic effects (**Badr and Tawfik, 2010; Lv *et al.*, 2005**). Amygdalin can be used in medicine for preventing and treating migraine, hypertension, chronic inflammation, and other reaction

source diseases (**Yan *et al*., 2006**). In addition, it can be used as a cerebral function improver that is effective as a therapeutic agent for cerebrovascular lesions such as psychogenic symptoms, nerve symptoms, subjective symptoms, and daily life activity disorder (**Hiromi, 1995**).

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 compound 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*.

### *Mechanism of the effect*

Recent data indicated that amygdalin reduced proliferation potential, decreased mitochondrial activity of cervical cancer cells, accumulated cells in G1 phase and lead to their death (**Jarocha and Majka, 2011**). Amygdalin induces apoptotic cell death by caspase-3 activation through the down-regulation of anti-apoptotic Bcl-2 protein and the up-regulation of pro-apoptotic Bax protein in DU145 and LNCaP prostate cancer cells (**Chang *et al*., 2006**).

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**).

## CONCLUSION

This review suggest possible effects of amygdalin on animal cells. Besides the antitumor activity, amygdalin has also been used for the treatment of asthma, bronchitis, emphysema, leprosy and diabetes. In addition, it can be used as a cerebral function improver that is effective as a therapeutic agent for cerebrovascular lesions. On the other hand there

have been demonstrated its negative effects. There are still few studies that suggest the possible impact of amygdalin on animal reproduction system.

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## REFERENCES

ANDREW, F. – ROSCOE, O.B. – ANDREW, E.G. 1980. A β-glucosidase in feline kidney that hydrolyzes amydalin (Laetrile). In *Archives of Biochemistry and Biophysics*, vol. 201, 1980, p. 363-368.

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

BALLANTYNE, B – MARRS, T.C. 1987. *Clinical and experimental toxicology of cyanides.* Michigan : Wright. 512 p. ISBN 0723608393.

BHATTI, R.A. – ABLIN, R.J. – GUINAN, P.D. 1981. Tumour-associated directed immunity in prostatic cancer: effect of amygdalin. In *IRCS Medical Science research Biochemistry*, vol. 9, 1981, no. 1, p. 19.

BIAGLOW, J.E. – DURAND, R.E. 1978. The enhanced radiation response of an *in vitro* tumour model by cyanide released from hydrolysed amygdalin. In *International Journal of Radiation Biology & Related Studies in Physics, Chemistry & Medicine*, vol. 33, 1978, no. 4, p. 397-401.

DENG, Y. – GUO, Z.G. – ZENG, Z.L. – WANG, Z. 2002. *In Chin. J. Chin. Mater. Med*. Vol. 27, p. 565.

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. In *Journal of Chromatography A,* vol. 1074, 2005, p. 43-46.

FUKUTA, T. – ITO, H. – MUKAINAKA, T. – TOKUDA, H. – NISHINO, H. – YOSHIDA, T. 2003. Anti-tumor promoting effect of glycosides from *Prunus persica* seeds. In *Biological and Pharmaceutical Bulletin,* vol. 26, 2003, p. 271-273.

HIROMI, S. Cerebral function improver. Patent. JP 7,165,589, 1995.

CHANG, J. – ZHANG, Y. 2012. Catalytic degradation of amygdalin by extracellular enzymes from Aspergillus niger. In *Process Biochemistry*, vol. 47, 2012, p. 195-200.

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. In *Biological and Pharmaceutical Bulletin,* vol. 29, 2006, no. 8, p. 1597-1602.

CHWALEK, M. – PLÉ, K. 2004. Convenient syntheses of isomaltose derivatives from amygdalin. In *Tetrahedron Letters*, vol. 45, 2004, p. 4749–4753.

IGARASHI, M. 1988. Kampo medicine in endocrinology. In *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. In *Abstracts of the 2nd Congress of Biochemistry and Cell Biology*. Krakow, 2011, p. 280.

JECFA (1993) Joint Expert Committee on Food Additives. Cyanogenic glycosides. WHO Food Additives Series 30.

KÁDASI, A. – SIROTKN, A.V. – MARUNIAKOVÁ, N. – KOLESÁROVÁ, A. – BULLA, J. – GROSSMANN, R. 2012. The effect of curcumin on secretory activity, proliferation and apoptosis of the porcine ovarian granulosa cells. In *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, 2012, no. 1, p. 349-357.

KOLESAROVA, A. – CAPCAROVA, M. – MARUNIAKOVA, N. – LUKAC, N. – CIERESZKO, RE. – SIROTKIN, AV. 2012a. Resveratrol inhibits reproductive toxicity induced by deoxynivalenol. In *Journal of Environmental Science and Health*, Part A, vol. 47, 2012a, p. 1329-1334.

KOLESAROVA, A. – BAKOVA, Z. – CAPCAROVA, M. – GALIK, B. – JURACEK, M. – SIMKO, M. – TOMAN, R. – SIROTKIN, A.V. 2012b. Consumption of bee pollen affects rat ovarian functions. In *Journal of Animal Physiology and Animal Nutrition*, (Berl). 2012b Nov 9. doi: 10.1111/jpn.12013. [Epub ahead of print]

KOLESAROVA, A. – CAPCAROVA, M. – BAKOVA, Z. – GALIK, 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*. In *Journal of Environmental Science and Health*, Part B, vol. 46, 2011, no. 3, p. 207-212.

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. In *Archives of Pharmacal Research*, vol. 33, 2010, p. 81-86.

LASTER, W.R.Jr. – SCHABEL, F.M.Jr. 1975. Experimental studies of the antitumor activity of amygdalin MF (NSC-15780) alone and in combination with beta-glucosidase (NSC-128056). In *Cancer Chemotherapy Reports*, vol. 59, 1975, no. 5, p. 951-965.

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. In *Journal of Chromatographic Science*, vol. 43, 2005, p. 383-387.

MANNER, H.W. – DISANTI, S.J. – MAGGIO, M.I., et al. 1978. Amygdalin, vitamin A and enzyme induced regression of murine mammary adenocarcinomas. In *Journal of Manipulative and Physiological Therapeutics*, vol. 1, 1978, no. 4, p. 246-248.

MOSS, R.W. 1996. *The cancer industry: the classic exposé on the cancer establishment*. Brooklyn, NY: First Equinox Press, 1996, 450 p. ISBN 9781881025092.

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

PARK, H.J. – YOON, S.H. – HAN, L.S. – ZHENG, LT. – JUNG, K.H. – UHM, Y.K. – LEE, J.H. – JEONG, J.S. – JOO,W.S. – YIM, S.V. – CHUNG, J.H. – HONG, S.P. 2005. Amygdalin inhibits genes related to cell cycle in SNU-C4 human colon cancer cells. In *World Journal of Gastroenterology*, vol. 11, 2005, no. 33, p. 5156-5161.

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

RAUWS, A.G. – GRAMBERG, L.G. – OLLING, M. 1982. Determination of amygdalin and its major metabolite prunasin in plasma and urine by high pressure liquid chromatography. In *Pharmacy World & Science*, vol. 6, 1982, p. 172-175.

RAUWS, A.G. – OLLING, M. – TIMMERMAN, A. 1982. The pharmacokinetics of amygdalin. In *Archive of toxicology*, vol. 49, 1982, p. 311-312.

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. In *Human Experimental Toxicology*, vol. 14, 1995, no. 11, p. 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. In *International Journal of Cancer,* vol. 78, 1998, no. 6, p. 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. In *Turkish Journal* of *Veterinary* and *Animal Sciences,* vol. 28, 2004, p. 819-824.

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

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

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

USUKI, S. 1990. Effects of Tokishakuyakusan and Keishibukuryogan on steroidogenesis by rat preovulatory follicles *in vivo*. In *American Journal of Chinese Medicine*, vol. 18, 1990, p. 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*. In *American Journal of Chinese Medicine*, vol. 19, 1991, p. 65-71.

WODINSKY, I. – SWINIARSKI, J.K. 1975. Antitumor activity of amygdalin MF (NSC-15780) as a single agent and with beta-glucosidase (NSC-128056) on a spectrum of transplantable rodent tumors. In *Cancer Chemotherapy Reports*, vol. 59, 1975, no. 5, p. 939-950.

YAN, J. – TONG, S. – LI, J. – LOU, J. 2006. Preparative Isolation and Purification of Amygdalin from *Prunus armeniaca* L. with High Recovery by High-Speed Countercurrent Chromatography. In *Journal of Liquid Chromatography & Related Technologies,* vol. 29, 2006, p. 1271-1279.

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. In *American Journal of Reproductive Immunology*, vol. 50, 2003, p. 90-97.

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. In *Carbohydrate Polymers*, vol. 90, 2012, p. 516-523.