Nat Prod Res. 2017 Dec 13:1-6. doi: 10.1080/14786419.2017.1413573. [Epub ahead of print]

Introduction of Adonis aestivalis as a new source of effective cytotoxic cardiac glycoside.

Hosseini M[1], Taherkhani M[2], Ghorbani Nohooji M[3].

Author information

1 a Faculty of Pharmaceutical Chemistry, Department of Phytochemistry and Essential Oils Technology, Pharmaceutical Sciences Branch , Islamic Azad University, (IAUPS) , Tehran , Iran.

2 b Department of Chemistry, College of Science , Takestan Branch , Islamic Azad University , Takestan , Iran.

3 c Medicinal Plants Research Center , Institute of Medicinal Plants, ACECR , Karaj , Iran.

Abstract

Cardiac glycosides are used for treatment of irregular heartbeats, cardiac arrhythmia and congestive heart failures. In this research, digitoxin as a cardiac glycoside was identified and isolated for the first time in the world from Adonis aestivalis and investigated for its cytotoxic activity against cervical cancer cell (HeLa) lines and human lymphocytes by MTT test. Digitoxin extracted from the aerial parts of the plant collected from west of Iran and purified by column and thin layer chromatographic techniques. The structure of isolated cardiac glycoside was identified by IR, ^{1}H NMR and ^{13}C NMR methods and so the presence of digitoxin was established. The half maximal inhibitory concentration values for cervical cancer and lymphocyte cells were obtained to be 5.62 and 412.94 µg/mL. The results of this study introduced the new resource of digitoxin which has considerable cytotoxic effects against HeLa cancer cells but did not damage normal human lymphocyte cells.

KEYWORDS: Adonis aestivalis; Cardiac glycoside; Iran; cytotoxicity; digitoxin

PMID: 29237300 DOI: 10.1080/14786419.2017.1413573

Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Introduction of Adonis aestivalis as a new source of effective cytotoxic cardiac glycoside

Marzieh Hosseini, Mahboubeh Taherkhani & Majid Ghorbani Nohooji

To cite this article: Marzieh Hosseini, Mahboubeh Taherkhani & Majid Ghorbani Nohooji (2017): Introduction of Adonis aestivalis as a new source of effective cytotoxic cardiac glycoside, Natural Product Research, DOI: 10.1080/14786419.2017.1413573

To link to this article: https://doi.org/10.1080/14786419.2017.1413573

NATURAL PRODUCT RESEARCH, 2017
https://doi.org/10.1080/14786419.2017.1413573

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

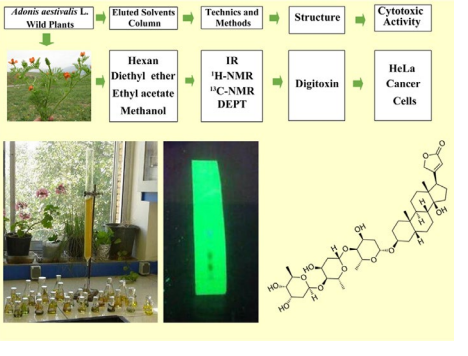
Introduction of *Adonis aestivalis* as a new source of effective cytotoxic cardiac glycoside

Marzieh Hosseini[a], Mahboubeh Taherkhani[b] and Majid Ghorbani Nohooji[c]

[a]Faculty of Pharmaceutical Chemistry, Department of Phytochemistry and Essential Oils Technology, Pharmaceutical Sciences Branch, Islamic Azad University, (IAUPS), Tehran, Iran; [b]Department of Chemistry, College of Science, Takestan Branch, Islamic Azad University, Takestan, Iran; [c]Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

ABSTRACT

Cardiac glycosides are used for treatment of irregular heartbeats, cardiac arrhythmia and congestive heart failures. In this research, digitoxin as a cardiac glycoside was identified and isolated for the first time in the world from *Adonis aestivalis* and investigated for its cytotoxic activity against cervical cancer cell (HeLa) lines and human lymphocytes by MTT test. Digitoxin extracted from the aerial parts of the plant collected from west of Iran and purified by column and thin layer chromatographic techniques. The structure of isolated cardiac glycoside was identified by IR, ^{1}H NMR and ^{13}C NMR methods and so the presence of digitoxin was established. The half maximal inhibitory concentration values for cervical cancer and lymphocyte cells were obtained to be 5.62 and 412.94 µg/mL. The results of this study introduced the new resource of digitoxin which has considerable cytotoxic effects against HeLa cancer cells but did not damage normal human lymphocyte cells.



ARTICLE HISTORY
Received 16 September 2017
Accepted 2 December 2017

KEYWORDS
Cardiac glycoside; digitoxin; *Adonis aestivalis*; cytotoxicity; Iran

CONTACT Mahboubeh Taherkhani mahtaherkhani@yahoo.com, mah.taherkhani@tiau.ac.ir
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1413573.

1. Introduction

Adonis aestivalis L. is an annual medicinal plant of Buttercup (Ranunculaceae) family distributed in the Middle East and Mediterranean area. Iran is the centre of diversity for the annual species of the genus such as *A. aestivalis* (Ghorbani Nohooji et al. 2008, 2011). Different *Adonis* species are rich sources of secondary metabolites and mostly known for containing of cardiac glycosides (Kopp et al. 1992). The most important usage of *A. aestivalis* is returning to treatment of cardiac arrhythmia and congestive heart failures due to their cardiac glycosides (Al-Snafi 2016). Also some traditional usage of the species was reported from Iran in treatment of Rheumatism and heart diseases (Dolatkhahi et al. 2012).

Cardiac glycosides are a famous group of natural compounds which are used for treatment of some heart maladies. They have various effects on the cancer cells death, proliferation, migration, invasion and blocking effect on growth of tumour cells in both *in vivo* and *in vitro* aspects (Yang et al. 2013; Calderon-Montano et al. 2014). A considerable cytotoxic effect of traditional plants of Egypt (*Urginea maritima*) was reported and it seems that the effect is related with its cardiac glycosides content (El-Seedi et al. 2013).The main mechanism on cancer cells is related on their effects on Na^+/K^+ ATPas activity (Mijatovic et al. 2007; Simpson et al. 2009; Cerella et al. 2013; Mijatovic and Kiss 2013). Also some anti-inflammatory effects are reported by using the glycoside cardenolides. Most famous glycoside cardenolide components which are found in different plants are digoxin, digitoxin, oabalin and oleanderin which are commonly present in *Digitalis*, *Strophanthus* and *Nerium*, respectively. Also one of the natural resources of cardionadis glycosides are the plants of buttercup family especially *Adonis* and *Cimicifuga* genera. (Calderon-Montano et al. 2014). Also noticeable cytotoxic effects of phytochemical compounds in various *Clematis* species (other genus of family) were reported in distinct research projects (Rana et al. 2015; Chang et al. 2017).

Previous investigations have proved the inhibitory effects of sesquiterpene lactones against human carcinoma cells (Fernandes et al. 2008). Chemical researches showed that the cytotoxic activity is due to the reaction of α,β-unsaturated carbonyl structures of the sesquiterpene lactones with thiols (Fernandes et al. 2008). *Digitalis purpurea* and *Digitalis lanata* are the commercial sources of the cardiac glycosides digitoxin and digoxin (Winnicka et al. 2006). Commonly *Digitalis* spp. are using in the cancer therapy due to presence of digitoxin which is the most abundant cardiac glycosides. Although the effect mechanism of digitoxin on the malignant cells is not clearly understood but its effect on the tumour cell lines is well established (Khan et al. 2009). Probably the cardiac glycosides resembling digitoxin are able to reduce c-MYC expression (Yang et al. 2013). The structure of digitoxin is valued for the α,β-unsaturated carbonyl group in γ-lactone ring, it may undergo many nucleophilic reactions. Based on previous researches, Sesquiterpene lactones can be toxic towards cancer cells (Fischedick et al. 2013). Hence, the digitoxin with γ-lactone can be a candidate as anti-cancer agent.

Up to now many reports has proven the presence of flavonoids (Komissarenko et al. 1973), tetraterpenoids, phenolic glycosides, cardenolides and cardiac glycosides (Pauli et al. 1993; Li et al. 2010), in the various species of the genus *Adonis*. In a separate study on the *A. aestivalis* two new cardenolide and strophantidin along with two new glycoside components were reported in the seeds of plant using NMR method. These new components were examined for cytotoxicity in some tumour cell lines and the effects were different in examined lines (Kubo et al. 2012). In other research three compound of *A. amurensis* namely cymarin,

cymarol and cymarinic acid were isolated and identified. These constituents have a diverse cytotoxic effect on some tumour cell lines. But their strongest effect was observed against HUVE (human umbilical venous endothelial) cells (You et al. 2003). Ferric reducing power properties along with total phenolic and flavonoid content of methanol and ethyl acetate extracts of *A. aestivalis* were evaluated in our research by spectrophotometric methods previously. The results showed that the methanol extract of *A. aestivalis* has a high content of phenol, flavonoids and antioxidant activity (Taherkhani and Rustaiyan 2016).

So evaluation of *in vitro* anticancer effect of digitoxin extracted from *A. aestivalis* was subjected as the aim of this study using MTT (3-(4,5-di methyl thiazol-2-yl)-2,5-di phenyltetrazolium bromide) assay on HeLa cancer cells and human lymphocytes. This study is the first report in presence of digitoxin in *A. aestivalis* and so this species is a suitable case to being regarded as the new source of digitoxin for medical purposes.

2. Results and discussion

2.1. Separation and identification of digitoxin

Digitoxin was isolated in crystalline form in fraction 8, 9 and 10. The molecular structure of digitoxin was elucidated by FT-IR, ^{1}H NMR and ^{13}C NMR (Figure S1).

2.2. Cytotoxicity test

The cytotoxic effects of digitoxin were determined on HeLa and lymphocyte cells via MTT test (Table S1). At a concentration of 20 µg/mL, digitoxin inactivated HeLa cells by 74.47%, with a 50% cytotoxicity concentration of 5.62 µg/mL ($y = 1.8184x + 39.783$, $r^2 = 0.90$) (Figure S2). Digitoxin was toxic to human carcinoma HeLa cells at low concentrations. At a concentration of 200 µg/mL, digitoxin inactivated lymphocytes by 60% ($y = 0.1792x + 26.001$, $r^2 = 0.92$) (Figure S3). The IC_{50} values for HeLa and lymphocyte cells were obtained to be 5.62 and 412.94 µg/mL, respectively (Table S1). Digitoxin showed a highly significant cytotoxic effect on the human carcinoma HeLa cell line.

The obtained results show that the cytotoxicity of digitoxin on cancer cells is much higher than that seen in human lymphocytes. These results prove low adverse side effects of digitoxin on the human lymphocytes. Some studies have pointed that natural products inhibit tumour growth (Taherkhani and Rustaiyan 2016). Depending on kind and concentration of natural products, they show cytotoxic activities on living cells but are generally non genotoxic (Bakkali et al. 2008). In the present study, digitoxin was found to be cytotoxic to HeLa human carcinoma cells. Based on previous study, cytotoxicity of proscillaridin A, ouabain and digoxin in the breast cancer cells was investigated. Proscillaridin A, ouabain and digoxin induced apoptosis in MDA-MB-231 cells by increasing free calcium concentration and via activating caspase-3 (Winnicka et al. 2007). Li and coworkers showed that asclepin had the strongest cytotoxic activity ($IC_{50} = 0.02$ µM) against the two cancer cell lines and the compound 12beta-hydroxycalotropin had significant cytotoxic activity ($IC_{50} = 0.69$ and 1.46 µM), respectively (Li et al. 2009). Digitoxin and its analogues are able to suppress chemically induced tumours in mice, or the growth of variety of human tumour cells, either in culture, or when xeno grafted into SCID mice (Yang et al. 2013). Upon previous investigation, carcino toxic activities have been observed in mice for chemically induced skin papillomas and

pulmonary tumours with digitoxin (Inada et al. 1993) or oleandrin (Afaq et al. 2004). Cardiac glycosides have been reported to show cytotoxic effect against colorectal cancer cell lines (Felth et al. 2009). The cytotoxic actions of cardiac glycosides such as oleandrin and digitoxin have been investigated in different types of cancer cells. Oleandrin, digitoxin and ouabain have been reported to induce apoptosis in androgen independent PC cells, although the specific mechanism of drug action has not yet been identified (Raghavendra et al. 2007; Fretz et al. 2008; Lefranc and Kiss 2008; Zhang et al. 2008).

3. Conclusion

Based on this study, digitoxin (A Cardiac Glycoside Extracted from *A. aestivalis* L.,) showed a high cytotoxicity against carcinoma HeLa cells but it was not so toxic to lymphocyte.

Definition of abbreviations

FTIR Fourier-transform infrared spectroscopy

NMR Nuclear Magnetic Resonance

MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Acknowledgements

The authors wish to thank the research deputy office in Faculty of Pharmaceutical Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS) for financial support and laboratory facilities. We would also like to thank Mrs F. Ebrahimi for help in material collection.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Faculty of Pharmaceutical Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS).

References

Afaq F, Saleem M, Aziz MH, Mukhtar H. 2004. Inhibition of 12O-tetradecanoyl phorbol 13-acetate induced tumor promotion markers in CD-1 mouse skin by oleandrin. Toxicol Appl Pharmacol. 195(3):361–369.

Al-Snafi AE. 2016. *Adonis aestivalis* pharmacological and toxicological activities- a review. Asian J Pharml Sci Technol. 6(2):96–102.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils – a review. Food Chem Toxicol. 46(2):446–475.

Calderon-Montano JM, Burgos-Moron E, Luis Orta M, Maldonado-Navas D, Garcia-Dominguez I, Lopez-Lazaro M. 2014. Evaluating the cancer therapeutic potential of cardiac glycosides. BioMed Res Int. Article ID 794930.

Cerella C, Dicato M, Diederich M. 2013. Assembling the puzzle of anti-cancer mechanisms triggered by cardiac glycosides. Mitochondrion. 13:225–234.

Chang Y, Zhang P, Zhang X, Chen J, Rausch W, Gula A, Bao B. 2017. Cytotoxic activities of flavonoids from a traditional Mongolian medicinal herb *Clematis aethusifolia* Turcz. Nat Prod Res. 31(10):1223–1227.
Dolatkhahi M, Ghorbani Nohooji M, Mehrafarin A, Amini Nejad GR, Dolatkhahi A. 2012. Ethnobotanical study of medicinal plants in Kazeroon, Iran: identification, distribution and traditional usage. J Med Plants. 2(42):163–178.
El-Seedi HR, Burman R, Mansour A, Turki Z, Boulos L, Gullbo J, Göransson U. 2013. The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from *Urginea maritima*. J Ethnopharm. 145(3):746–757.
Felth J, Rickardson L, Rosen J, Wickstrom M, Fryknas M, Lindskog M, Bohlin L, Gullbo J. 2009. Cytotoxic effects of cardiac glycosides in colon cancer cells, alone and in combination with standard chemotherapeutic drugs. J Nat Prod. 72(11):1969–1974.
Fernandes MB, Scotti MT, Ferreira MJP, Emerenciano VP. 2008. Use of self-organizing maps and molecular descriptors to predict the cytotoxic activity of sesquiterpene lactones. Eur J Med Chem. 43(10):2197–2205.
Fischedick JT, Pesic M, Podolski-Renic A, Bankovic J, de Vos RCH, Peric M, Todorovic S, Tanic N. 2013. Cytotoxic activity of sesquiterpene lactones from *Inula britannica* on human cancer cell lines. Phytochem Lett. 6(2):246–252.
Fretz JA, Shevde NK, Singh S, Darnay BG, Pike JW. 2008. Receptor activator of nuclear factor kappaB ligand induced nuclear factor of activated T cells (C1) auto regulates its own expression in osteoclasts and mediates the up-regulation of tartrate-resistant acid phosphatase. Mol Endocrinol. 22(3):737–750.
Ghorbani Nohooji M, Azizian D, Sheidai M, Khatamsaz M. 2008. Pollen morphology of some *Adonis* L. species (Ranunculaceae) from Iran. Iran J Bot. 14(2):65–70.
Ghorbani Nohooji M, Azizian D, Sheidai M, Khatamsaz M. 2011. Taxonomic value of stem anatomical characters in classification of some *Adonis* (Ranunculaceae) species in Iran. Taxon Biosyst. 6:69–78.
Hosseini M, Taherkhani M, Ghorbani Nohooji M. 2016. Evaluation of antioxidant activity, total phenolic and flavonoid content of the extract of *Adonis aestivalis* L. collected from Lorestan province. Sci Res Appl Biol. 6(3):33–41.
Inada A, Nakanishi T, Konoshima T, Kozuka M, Tokuda H, Nishino H, Iwashima A. 1993. Anti-tumor promoting activities of natural products. II Inhibitory effects of digitoxin on two-stage carcinogenesis of mouse skin tumors and mouse pulmonary tumors. Biol Pharm Bull. 16(9):930–931.
Khan MI, Chesney AJ, Laber DA, Miller DM. 2009. *Digitalis*, a targeted therapy for cancer? Am J Med Sci. 337(5):355–359.
Komissarenko NF, Yatsyuk VY, Korzennikova EP. 1973. Flavonoids of *Adonis wolgensis*. Chem Nat Compd. 9(3):417–417.
Kopp B, Krenn L, Kubelka E, Kubelka W. 1992. Cardenolides from *Adonis aestivalis*. Phytochemistry. 31(9):3195–3198.
Kubo S, Kuroda M, Matsuo Y, Masatani D, Sakagami H, Mimaki Y. 2012. New cardenolides from the seeds of *Adonis aestivalis*. Chem Pharm Bull. 60(10):1275–1282.
Lefranc F, Kiss R. 2008. The sodium pump alpha1 subunit as a potential target to combat apoptosis-resistant glioblastomas. Neoplasia. 10(3):198–206.
Li JZ, Qing C, Chen CX, Hao XJ, Liu HY. 2009. Cytotoxicity of cardenolides and cardenolide glycosides from *Asclepias curassavica*. Bioorg Med Chem Lett. 19(7):1956–1959.
Li ZH, Wang Q, Ruan X, Pan CD, Jiang DA. 2010. Phenolics and plant allelopathy. Molecules. 15(12):8933–8952.
Mijatovic T, Kiss R. 2013. Cardiotonic steroids-mediated Na^+/K^+ ATPase targeting could circumvent various chemoresistance pathways. Planta Med. 79(3–4):189–198. doi:10.1055/s-0032-1328243.
Mijatovic T, Van Quaquebeke E, Delest B, Debeir O, Darro F, Kiss R. 2007. Cardiotonic steroids on the road to anti-cancer therapy. Biochim et Biophys Acta. 1776:32–57.
Pauli GF, Junior P, Berger S, Matthiesen U. 1993. Alepposides, cardenolide oligoglycosides from *Adonis aleppica*. J Nat Prod. 5(1):67–75.
Raghavendra PB, Sreenivasan Y, Ramesh GT, Manna SK. 2007. Cardiac glycoside induces cell death via FasL by activating calcineurin and NF AT, but apoptosis initially proceeds through activation of caspases. Apoptosis. 12(2):307–318.

Rana S, Rawat K, Mahendru M, Padwad Y, Pakade Y, Lal B, Bhushan S. 2015. Screening of bioconstituents and *in vitro* cytotoxicity of *Clematis gouriana* leaves. Nat Product Res. 29(23):2242–2246.

Simpson CD, Mawji IM, Anyiwe K, Williams MA, Wang X, Venugopal AL, Gronda M, Hurren R, Cheng S, Serra S, et al. 2009. Inhibition of the sodium potassium adenosine triphosphatase pump sensitizes cancer cells to Anoikis and Prevents Distant Tumor Formation. Cancer Res. 69(7):2739–2747.

Taherkhani M, Rustaiyan A. 2016. Investigation of *in vitro* cytotoxic, mutagenic and anti-mutagenic effects of Shirazolide extracted from *Jurinea leptoloba*. Nat Product Res. 17:1–4.

Winnicka K, Bielawski K, Bielawska A. 2006. Cardiac glycosides in cancer research and cancer therapy. Acta Pol Pharm. 63:109–115.

Winnicka K, Bielawski K, Bielawski A, Miltyk W. 2007. Apoptosis mediated cytotoxicity of ouabain, digoxin and proscillaridin A in the estrogen independent MDA-MB-231 breast cancer cells. Arch Pharm Res. 30(10):1216–1224.

Yang QF, Dalgard CL, Eidelman O, Jozwik C, Pollard BS, Srivastava M, Pollard HB. 2013. Digitoxin induces apoptosis in cancer cells by inhibiting nuclear factor of activated T cells driven c-MYC expression. J Carcinog. 12(8):1–24.

You YJ, Kim Y, Nam NH, Ahn BZ. 2003. Inhibitory effect of components on tube-like formation of *Adonis amurensis* human umbilical venous cells. Phytother Res. 17:568–570.

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, et al. 2008. Digoxin and other cardiac glycosides inhibit HIF 1 alpha synthesis and block tumor growth. Proc Natl Acad Sci U S A. 105(50):19579–19586.