



Datura innoxia anti-angiogenesis properties

B. Khoshkhoo^{1,2}, S. Mohammadi Motamed¹, M. Hamzeloo-Moghadam^{3*}

¹Department of Pharmacognosy, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS).

²Herbal Medicine Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (HMRC).

³Traditional Medicine and Materia Medica Research Center and Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Background and objectives: *Datura innoxia* Miller is one of the two species of *Datura* (Solanaceae) which grow in Iran. There are many reports of the biological activities of *Datura* and in the present study the ability of *Datura innoxia* for inhibiting angiogenesis was evaluated. **Methods:** The methanol extract and petroleum ether, chloroform and methanol fractions of *Datura innoxia* flowers were obtained by maceration method. The extract and fractions were further evaluated for their cytotoxicity and anti-angiogenesis properties in HUV-EC-C cells through MTT and wound healing assays, respectively. **Results:** The methanol extract and the petroleum ether, chloroform and methanol fractions were cytotoxic to HUV-EC-C cells (IC₅₀ 11.25, 63.3, 8.75 and 9.27 µg/mL, respectively). The chloroform fraction demonstrated the most anti-angiogenesis activity in the wound healing assay. **Conclusion:** Evaluating the above activities of the compounds isolated from *Datura innoxia* might be a proper follow up of the present study.

Keywords: angiogenesis, *Datura innoxia*, HUV-EC-C, MTT assay, wound healing assay

Introduction

Datura (Solanaceae) was originally found in Asia. Biological activities such as anti-inflammatory, CNS stimulant and decongestant properties have been reported for the genus. It has also been used for the treatment of skin infections, toothaches and alopecia [1]. *Datura* species are famous for possessing alkaloids, to be more precise, tropane alkaloids. Alkaloids like hyoscyamine, hyoscyne and atropine belong to this group of alkaloids [2]. They are mostly esters of hydroxytropans with a variety of organic

acids, such as tropic, atropic, cinnamic, angelic, tiglic, senecioic, isovaleric, and truxillic acids [3].

Solanaceae family is known to have the most tropane alkaloids though this group of compounds could also be found in Erythroxylaceae, Convolvulaceae, Proteaceae and Rhizophoraceae and in lower amounts in Euphorbiaceae, Brassicaceae and Oleaceae families [4]. Tropane alkaloids demonstrate anticholinergic and antispasmodic properties and can affect the parasympathic nervous system [5].

Two species of *Datura* grow in Iran; *Datura stramonium* L. and *Datura innoxia* Miller [6]. There have been various reports about the biological activities of different organs of the specie and in the present study study, the ability of the methanol extract and fractions of *D. innoxia* flowers to inhibit angiogenesis has been evaluated.

Experimental

Plant material

Datura innoxia flowers were collected from Kerman Province, Iran (2013) and were authenticated by a botanist.

Chemicals and reagents

Dulbecco's modified Eagle medium (DMEM), Fetal Bovine Serum (FBS), Ham-F12 (Gibco, Auckland, New Zealand), penicillin-streptomycin, MTT ([3-(4, 5-dimethylthiazol-2-yl) -2, 4-diphenyl tetrazolium bromide]), phosphate buffer saline, PBS, (Sigma, St. Louis, MO, USA) and DMSO (dimethyl sulfoxide) (Merck, Hohenbrunn, Germany), were used in the cell culture evaluations. The solvents for extraction and fractionation were provided from Mojalali CO. (Iran).

Extraction

Ten mg of the air dried powder of the flowers were macerated with methanol for 24 h. The mixture was filtered afterwards and the residue was macerated with methanol again according to the above mentioned procedure. The extraction process was repeated three times. The combined filtrates were then concentrated by using a rotary evaporator apparatus. The final dry extract was kept in refrigerator for future experiments.

Fractionation

Thirty mg of the dried powered flowers were macerated with petroleum ether with the same method as mentioned for the extraction. On the third day, the procedure was repeated for the residue with chloroform and then methanol; thus the total fractionation process lasted 9 days.

Sample preparation

A stock solution of each sample was prepared in

DMSO (10 mg/mL), serial two fold concentrations were prepared from the above stock solutions (3.125, 6.26, 12.5, 25, 50 and 100 µg/mL); the final DMSO concentration was 1%.

MTT assay

HUV-EC-C cells were obtained from Pasture Institute, Tehran, Iran. The cells were maintained in DMEM medium containing 10% FBS and 25% Ham-F12 and were seeded in 96-well plates. After 24 h, the cells were exposed to different concentrations of extracts/fractions for 72 h. The medium was removed afterwards and MTT (final concentration 0.5 mg/mL) was added to each well. The resulting formazan crystals were then dissolved in DMSO after 4 h exposure of the cells to MTT and the absorbance was recorded at 570 nm using an ELISA reader. Tamoxifen was used as the positive control. Considering the following equation, the viability was measured and the IC₅₀ values were calculated by plotting viability vs. log concentrations (Microsoft Excel).

$$\text{Viability (\%)} = [A]_s/[A]_c \times 100.$$

Where [A]_s is the absorbance of wells with sample and [A]_c is the absorbance of wells in absence of sample [7-9].

Wound healing assay

HUV-EC-C cells were seeded in 6-well plates (50000/well). After 24 h, the center of each well was scratched by a sterile yellow pipette tip imitating a wound. The extracts and fractions were then added to each well (3.125 µg/mL: the concentration in which the viability of the cells was nearly 100%). DMSO 1% was used as the negative control. The changes in the size of the wound were monitored by taking photographs in intervals up to the fourth day after wounding [10].

Results and Discussion

The results of cytotoxic evaluation and wound healing assay of *D. innoxia* have been presented in table 1 and figures 1 and 2, respectively. The results of MTT assay suggested considerable cytotoxic effects for the methanol extract as well as the chloroform and methanol fractions of *D. innoxia* flowers. Other parts of *D. innoxia* have

been investigated before for their cytotoxic or apoptotic activity. In a research about the cytotoxicity and apoptosis potential of *D. innoxia* seeds, it was found that the aqueous extract of the seeds could activate caspase 3 and 9, and restrain VEGF and TNF- α in HeLa cells [1].

Table 1. Results of MTT assay of *Datura innoxia* extract and fractions on HUV-EC-C cells

Samples	ME	PF	CF	MF	Tamoxifen
IC ₅₀ (μ g/mL)	11.2	63.3	8.7	9.2	20.1

ME: methanol extract; PF: Petroleum ether fraction; CF: Chloroform fraction; MF: Methanol fraction

The leaves, stems and fruits of the species have been evaluated in the brine shrimp lethality assay to show cytotoxicity with the fruits being considerably toxic to the larvae ($LC_{50} \leq 100 \mu$ g/mL). The chloroform and *n*-hexane fractions of the fruits were also toxic to human leukemia cells in the same study (IC_{50} 4.52 and 3.49 μ g/mL, respectively) [11]. The methanol extract of the leaves has shown to induce apoptosis and

decrease the expression of Bcl-2 in HCT-15 and Hep-2 cell lines [12] while a lectin isolated from *D. innoxia* seeds has shown anti-proliferative activity against human pancreatic cancer cell line [13].

Datura spp. have been known to possess alkaloids and present their medicinal properties due to the existence of these compounds [14]. El Bazaoui *et al.* have reported the isolation of fifty three alkaloids from different parts of *D. Innoxia* (roots, stems, leaves, flowers seeds) [15]. The alkaloid pattern of the species has previously been reported to be of a significant difference in different organs and even aerial parts [16].

Comparing the IC_{50} of the extract and fractions, it could be suggested that semi-polar to polar constituents might be responsible for the observed cytotoxic results in MTT assay and the alkaloids of the species could have presented the observed effects.

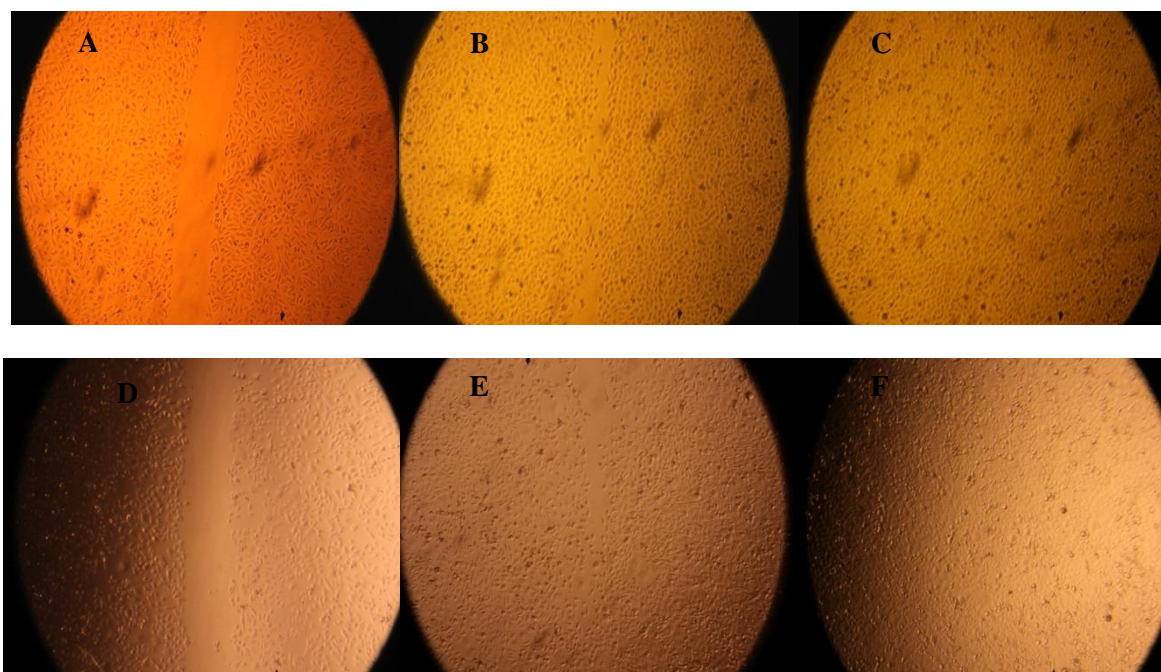


Figure 1. Anti-angiogenesis activity *Datura innoxia*. A, B and C: the effect of the methanol extract on the starting day of the experiment, the 2nd and 3rd day, respectively. D, E and F: the effect of the petroleum ether fraction on the starting day of the experiment, the 2nd and 3rd day, respectively.



Figure 2. Anti-angiogenesis activity *Datura innoxia*. A, B and C: the effect of the chloroform fraction on the starting day of the experiment, the 2nd and 3rd day, respectively. D, E and F: the effect of the methanol fraction on the starting day of the experiment, the 2nd and 3rd day, respectively. G, H and I: the effect of DMSO 1% (negative control) on the starting day of the experiment, the 2nd and 3rd day, respectively.

For evaluating the anti-angiogenesis properties, the ability of the samples to hinder angiogenesis was investigated in a concentration that had not been cytotoxic to the cells and did not decrease viability (3.125 µg/mL).

The chloroform fraction demonstrated more angiogenesis inhibition at the end of day one

compared to other samples; this continued in the second day when closing the wound scratch for the chloroform along with the methanol fractions seemed to be slower compared to other samples. On the third day after wounding the monolayer of the cells, the scratch could not be observed in the petroleum ether fraction or the methanol extract

while it could still be detected in the wells which had received chloroform and methanol fractions. On the fourth day, the scratch had completely closed and could not be observed for any of the samples. Again it was postulated that the semi-polar to polar compounds could have caused the observed anti-angiogenesis activity.

Considering the results of the cytotoxic evaluation and also the anti-angiogenesis experiment, it is proposed that the observed effects could be due to the tropane alkaloids of the flower but to confirm this assumption, the experiment should be carried out with the purified compounds of *D. innoxia* flowers.

Acknowledgments

This study was based on a Pharm. D. Thesis (Bent-ol- Hoda Khoshkhoo). The authors wish to thank the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran for the financial support.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Pandey M, Saraswati S, Agrawal SS. Antiproliferative effects of *Datura innoxia* extract in cervical HeLa cell line. *J Pharm Res.* 2011; 4(4): 1124-1126.

[2] Manske RHF. *The alkaloids chemistry and physiology.* New York: Academic Press Inc. Publishers, 1995.

[3] Fattorusso E, Tagliatella-Scafati O. Modern alkaloids structure, isolation, synthesis and biology. Weinheim: WILEY-VCH, 2008.

[4] Christen P. *Tropane alkaloids: old drugs used in modern medicine.* In: *Studies in natural products chemistry.* Atta-ur-Rahman, Ed. Amsterdam: Elsevier, 2000.

[5] Kim N, Estrada O, Chavez B, Stewart C, Auria JCD. Tropane and granatane alkaloid

biosynthesis: a systematic analysis. *Molecules.* 2016; Article ID 21111510.

[6] Mozaffarian V. *Dictionary of Iranian plant names.* Tehran: Farhang-e-Moaaser, 2007.

[7] Hamzeloo-Moghadam M, Firouzi J, Saeidnia S, Hajimehdipoor H, Jamili S, Rustaiyan A, Gohari AR. Cytotoxic hydroperoxy sterol from the brown alga, *Nizamuddiniazanardinii.* *Daru J Pharm Sci.* 2013; 21:1-4.

[8] Hamzeloo-Moghadam M, Naghibi F, Atoofi A, Asgharian-Rezaie M, Irani M, Mosaddegh M. Cytotoxic activity and apoptosis induction by gaillardin. *Z. Naturforsch C.* 2013; 68(3-4): 108-112.

[9] Esmaili S, Irani M, Moazzeni Zehan H, Keramatian B, Tavakoli Harandi Z, Hamzeloo-Moghadam M. Cytotoxic activity of some ethnic medicinal plants from southwest of Iran. *Res J Pharmacogn.* 2016; 3(1): 43-47.

[10] Mosaddegh M, Esmaili S, Eslami-Tehrani B, Keramatian B, Mohebbi S, Hamzeloo-Moghadam M. Anti-angiogenesis properties of *Crocus pallasii* subsp. *haussknechtii*, a popular ethnic food. *Res J Pharmacogn.* 2015; 2(3): 37-42.

[11] Fatima H, Khan K, Zia M, Ur-Rehman T, Mirza B, Haq I. Extraction optimization of medicinally important metabolites from *Datura innoxia* Mill.: an *in vitro* biological and phytochemical investigation. *BMC Complement Altern Med.* 2015; 15: 1-18.

[12] Arulvasu C, Babu G, Manikandan R, Srinivasan P, Sellamuthu S, Prabhu D, Dinesh D. Anti-cancer effect of *Datura innoxia* P.mill. leaf extract *in vitro* through induction of apoptosis in human colon adenocarcinoma and larynx cancer cell lines. *J Pharm Res.* 2010; 3(7): 1485-1488.

[13] Singh R, Nawale L, Sarkar D, Suresh CG. Two chitotriose-specific lectins show anti-angiogenesis, induces caspase-9-mediated apoptosis and early arrest of pancreatic tumor

- cell cycle. *Plos One*. 2016; Article ID 0146110.
- [14] Maheshwari NO, Khan A, Chopade BA. Rediscovering the medicinal properties of *Datura* sp.: a review. *J Med Plants Res*. 2013; 7(39): 2885-2897.
- [15] El Bazaoui A, Bellimam MA, Soulaymani A. Tropane alkaloids of *Datura innoxia* from Morocco. *Z Naturforsch C*. 2012; 67(1-2): 8-14.
- [16] Witte L, Müller K, Arfmann HA. Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass spectrometry. *Planta Med*. 1987; 53(2): 192-197.