Research Article

Detoxification and estimation of Atropine and Hyoscine from Datura seeds by HPLC analysis

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Abstract

Background: *Datura metel* Linn. is an important Ayurvedic medicinal plant used for the treatment of inflammation, respiratory decongestion, skin infection, dental problems, stimulation of central nervous system and alopecia. Since *Datura* is toxic at therapeutic dose, hence its toxicity must be removed before subjecting to medicinal purposes. **Objective:** In the present study seeds of *Datura* detoxified by Ayurvedic process (*Sodhana*). Attempt has been made to identify the chemical constituents responsible for the toxicity by measuring the level of major chemical constituents before and after the detoxification of seeds. **Materials and Methods:** In the present study, seeds of *Datura* were detoxified by two different Ayurvedic process using Cow's milk and Cow's urine. Levels of atropine and hyoscine before and after the purification of seeds were determined by HPLC analysis. **Results:** The quantity of hyoscine and atropine in unpurified sample was found to be 0.109 % & 0.197 % of dried weight of *Datura* seeds. The quantity of hyoscine and atropine in dried seeds of *Datura* was found to be 0.012 % & 0.043 % and 0.004 % & 0.022 % purified with Cow's milk (Method–2) and in both Cow's milk and cow's urine (Method –1) respectively. **Conclusion:** It may be concluded that toxic effect of *Datura* seeds are due to the presence of atropine and hyoscine along with other tropane alkaloids. When *Datura* seeds are subjected *Sodhana* with Cow's milk and/or Cow's urine, the level of tropane alkaloids decreases and therefore toxicity also decreases in the same proportion.

Keywords: Atropine, *Datura metel*, HPLC, Hyoscine, *Sodhana*

Introduction

Ayurveda, a 5000 years old Indian health care system is predominantly based on the illness and opinions of an individual (Dumitrescu, 2015). This traditional system of medicine is now experienced worldwide as complementary and alternative medicine. Over the centuries, several Ayurvedic formulations, as well as surgical procedures have been established for the management of numerous diseases and disorders. Plants are the relevant source of medicines (Saklani and Kutty, 2008). Natural products including medicinal plants play a significant role in drug discovery process. Several lead compounds of medicinal importance have been isolated from medicinal plant. In last few years' global herbal market have scaled down due to toxicity and adverse effects of medicinal plants (Butler, 2004). Though most of the plant drugs are safe, yet few are toxic for human health. These poisonous/toxic plants are categorized as *Visa* (poison) and *Upavisa* (toxic but not lethal for human health) in Ayurvedic texts (Maurya et al., 2015).

The family *Solanaceae* includes about 98 genera and 2,700 species having a great diversity in morphology, habitats and ecology. (Griffin and Lin, 2002) *Datura metel* Linn. is an important Ayurvedic medicinal plant belonging to the family

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Solanaceae. *Datura* comes under well known category of *Upavisa varg* drugs in various text book Ayurveda (Shastri, 2003; Pandey , 2006).

Raja Nighantu, a classical dictionary of *Dravyaguna*, describes five different verities of *Datura viz. Shweta* (white), *Krishna* (black), *Rakta* (red), *Peeta* (yellow) and *Neela* (blue). Out of these five varieties, *Krishna* (black) *Datura* said to have more potent and therapeutically active as compare to other four varieties (Tripathy , 2003). *Datura innoxia* Mill., *Datura metel* Linn., *Datura stramonium* Linn. and *Datura alba* Linn. etc. have identified as *Datura* and among these *Datura metel* Linn. and *Datura innoxia* Mill. are generally used for therapeutic purposes (Vaidya, 2005). Whole plant of *Datura* used for the treatment of inflammation, respiratory decongestion, skin infection, dental problems, stimulation of central nervous system and alopecia. *Datura* has hallucinogenic property which overdose causes serious poisoning. Toxicity of *Datura* is due to its anti–cholinergic properties even in small doses (Das et al., 2012). Apart from this leaves and roots of *Datura* are also used for the treatment of different diseases like diarrhoea, epilepsy, hysteria, rheumatic pains, haemorrhoids, painful menstruation, wounds and burns. *Datura* is considered as bitter, acrid, astringent, anti–phlogistic, anodyne, antiseptic, germicide, narcotic and sedative in Ayurveda (Parrotta, 2001; Anonymous, 2008). Among them seeds are highly toxic which has been reported by various animal models (Boumba 2004; Pereira et al., 1994; Chang et al., 1999; Kovatsis et al., 1993).

Since *Datura* is toxic at therapeutic dose, hence its toxicity must be removed before subjecting to medicinal purposes. *Sodhana* (detoxification) is the Ayurvedic process used for the detoxification from physical or chemical impurity to minimize the side effect and/or to increase the potency/ therapeutic activity of toxic materials used for medicinal purposes. In context of Ayurveda, it is mentioned as by using right procedure of *Sodhana,* toxic materials can be converted as *Amrita* (nector) and by acquisition of inappropriate methods, non–toxic things may become a toxic one (Pal et al., 2014).

Datura, when used without its peculiar *Sodhana* process, it causes dryness of the mouth, cramps, unconsciousness, excessive thirst and giddiness (dizziness). All these symptoms are due to anti–cholinergic property of tropane alkaloids present in it (Reddy, 2007). Common media which are generally used to purify the drugs means for *Sodhana* are cow's milk, cow's urine, ghee, and juice of few plants etc (Joshi and Nagaraju, 1988; Belge and Belge, 2012; Shastri, 2007).

Materials and methods

Chemicals and instrumentation

Standard samples of hyoscine (scopolamine) and atropine were purchased from Sigma–Aldrich Co. LLc., India. All other solvents, chemicals and reagents used were of analytical grade. Distilled water used for purification process. Distilled water wherever used for HPLC was filtered from Millipore filter.

Collection of plant material

Seeds of *Datura* were collected during the month of February and March, 2016 from the herbal garden of Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur (Uttar Pradesh), and duly authenticated by Prof. Anil Kumar Singh, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi. A Boucher specimen (APRL–6) has been submitted at Ayurvedic Pharmacy Research Laboratory, BHU, Barkachha, Mirzapur.

Sodhana of Datura

Two different methods were adopted for Sodhana of Datura seeds.

Method 1

In this method of *Swedana*, Datura seeds (50 g) were kept into a cotton cloth to make *Pottali* and hanged through the *Dola Yantra* in to an iron vessel containing *Godugdha* (Cow's milk, 500 ml) for 3 hours (1 *Prahar*). Cow's milk was collected from Kherigarh breed of cow of age 4-5 years old. A sufficient quantity of fresh milk added to the iron vessel at a regular interval to maintain the volume of milk during *Swedana* process. After completion of *Swedana* process, *Pottali* was removed from the *Dola Yantra* and washed with lukewarm water. It was spread evenly on the outer surface of earthen pot and allowed to dry completely. Testa (seed coat) of seeds was removed by ruining it on abrasive surface (Mishra, 2010; Shastri, 2012).

Method 2

In this method of purification, 50 g *Datura* seeds was soaked in freshly collected 500 ml of *Gomutra* (Cow's urine) and kept it for overnight. After washing with lukewarm water, the seeds taken in the *Pottali* (bundled in cotton cloth). It was hanged through *Dola Yantra* (an instrument used to impregnate material in liquids in such a manner that it dipped in the liquid but not touched to the bottom of the vessel) in an iron vessel containing *Godugdha* (Cow's milk) for 3 hours (1 *Prahar*) (Reddy, 2007; Sastri , 2010). Rest of the process was similar as discussed in method 1.

Extraction of purified and unpurified seeds of Datura

Purified and unpurified dried seeds of *Datura* (10 g) was taken separately and converted to coarse powdered form. It was subjected to cold maceration process using methanol

(100 ml) for 24 hours. It was filtered and filtrate obtained was concentrated by rotatory evaporator (Perfit India Pvt. Ltd.) at temperate not more than 60 °C. Semisolid mass (1.12 g) obtained was dried in vacuum desiccator using anhydrous calcium chloride as desiccant material.

HPLC analysis of purified and unpurified seeds of Datura

Purified and unpurified seeds of *Datura* was analysed for the level of hyoscine and atropine by HPLC instrument (Agilent Technologies 1260 infinity Quaternary LC, Agilent Technologies, India). HPLC analysis was performed by reverse phase chromatography using the method developed by Zazhi, 2013. HPLC instrument was equipped with stainless steel column of dimension 25 cm x 4.6 mm and packed with C–18 silica gel (5 µM). A filtered and degassed mixture of acetonitrile and sodium phosphate buffer (adjusted to pH 6.0 with phosphoric acid solution) solution (30:70) was used as mobile phase with flow rate 0.9 mL/min. Samples (20 µL) were injected in the column and temperature was maintained at 25 °C. UV–visible spectrophotometer was used as detector and sample was detected at 207 nm (Zhongguo, 2013).

Determination of physiochemical parameters

Various physicochemical parameters such as total ash value, acid insoluble ash value, loss on drying, alcohol soluble extractive value, and water soluble extractive value were determined in the *Datura* seeds before and after its purification process as per the Ayurvedic Pharmacopoeia of India (Anonymous, 2007).

Results

Results of different physicochemical parameters before and after the purification of seeds of *Datura* are represented in table 1. Remarkable changes observed in the physicochemical parameters before and after the purification of the seeds of *Datura* by both method 1 and 2. Physicochemical such as total ash value, acid insoluble ash value, water soluble ash value, water soluble extractive value and alcohol soluble extractive value decreases after the purification of the seeds while loss on drying increases after purification (Table 1). The quantity of marker

Table 1. Physicochemical parameters of unpurified and purified seeds of Datura

Parameters (%)	Unpurified seeds	Purified seeds	
		Purified in Cow's urine+ Cow's milk	Purified in Cow's milk
Total ash value	2.77	1.56	1.92
Acid insoluble ash value	0.36	0.23	0.11
Water soluble ash value	2.32	1.28	1.74
Loss on drying	4.2	5.6	5.9
Alcohol soluble extractive value	8.16	6.52	7.21
Water soluble extractive value	9.26	5.09	7.52

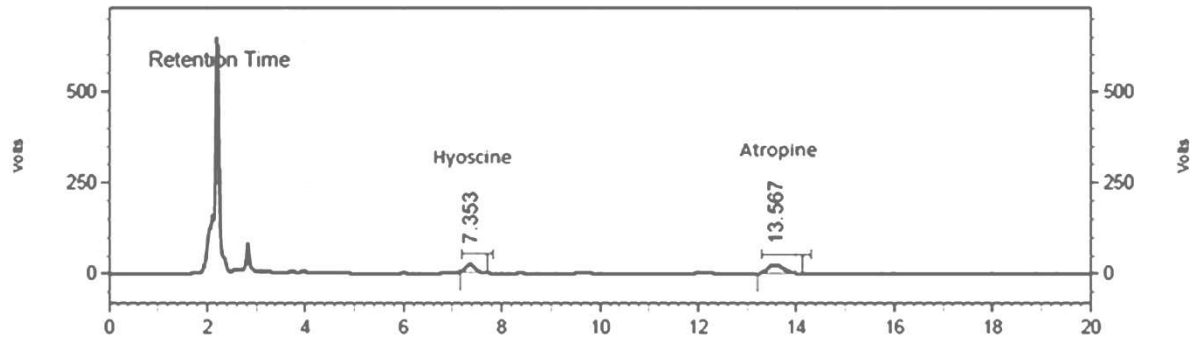

Figure 1. HPLC analysis of unpurified seeds of *Datura* for hyoscine and atropine

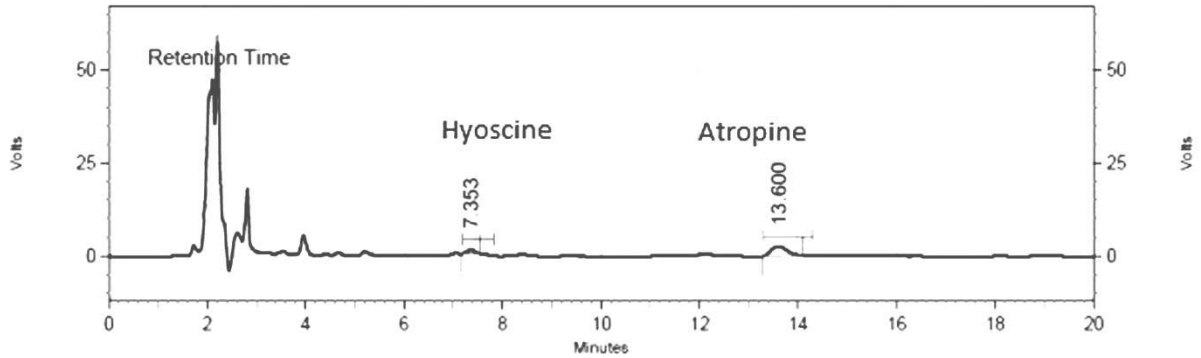

Figure 2. HPLC analysis of Datura seeds purified with both Cow's milk and Cow's urine for hyoscine and atropine

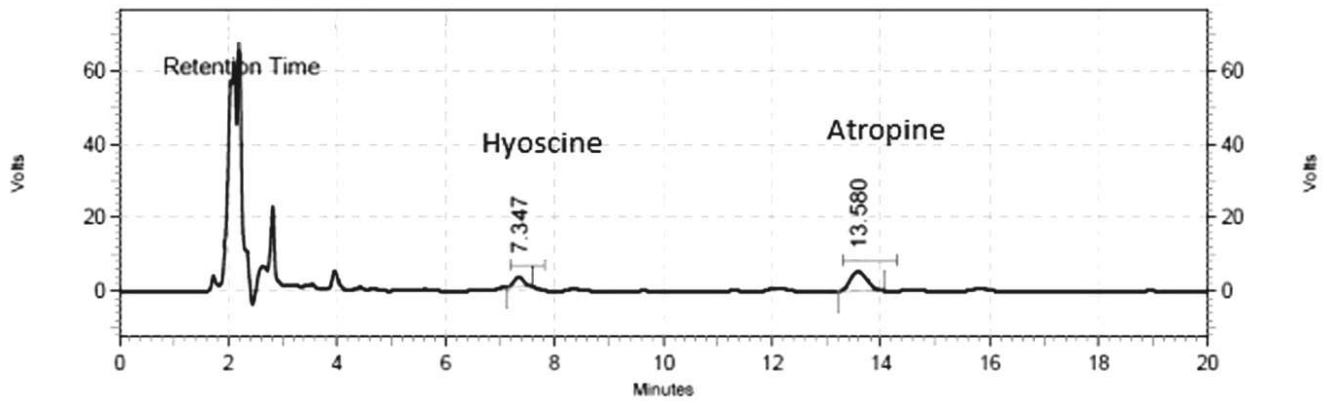

Figure 3. HPLC analysis of Datura seeds purified with Cow's milk for hyoscine and atropine

compound hyoscine and atropine in unpurified sample was found to be 0.109% & 0.197% of dried weight of *Datura* seeds (Figure 1). Remarkable decrease in the quantity of marker compounds observed after purification of the seeds of *Datura*. The quantity of hyoscine and atropine in dried seeds of *Datura* was found to be 0.012% & 0.043% and 0.004% & 0.022% of the dried weight of *Datura* seeds purified with Cow's milk (Figure 3) (Method 1) and in both Cow's milk and Cow's urine (Figure 2) (Method 2) respectively.

Discussion

Datura is a well known plant in Ayurvedic system of medicine, used for the treatment of inflammation, respiratory decongestion, skin infection, dental problems, stimulation of central nervous system and alopecia. *Datura* plant has hallucinogenic as well as anti–cholinergic properties which cause serious poisoning (dryness of the mouth, cramps, unconsciousness, excessive thirst and dizziness) even in small dose (Das et al., 2012). Therefore in Ayurvedic system of medicine it is used after its *Sodhana* with Cow's milk and/or Cow's urine. Major phytochemical constituents of *Datura* responsible for its anti–cholinergic property are hyoscine and atropine (Reddy, 2007).

In the present study an attempt has been made to purify the seeds of *Datura* by Ayurvedic process known as *Sodhana* by using Cow's milk (Method 1) or both Cow's milk and Cow's urine (Method 2). Different physicochemical parameters were determined before and after the purification of the seeds of *Datura*. Levels of marker compounds (atropine and hyoscine) were also determined by HPLC before and after the purification of the seeds. Remarkable changes have been observed in different physicochemical parameters before and after the purification in the seeds of *Datura* (Table 1).

Results obtained from HPLC analysis revealed that remarkable decrease in the level of hyoscine and atropine were observed when seeds of *Datura* treated by Method 1 and Method 2 as compared to unpurified seeds (Figure 1-3). Though the level of hyoscine and atropine were found to be more in seeds of *Datura* purified by Method 1 as compared to seeds of *Datura* purified by Method 2. Atropine and hyoscine are slightly soluble in water while freely soluble in organic solvents like chloroform, diethyl ether and alcohol (Alexander et al., 2008). Milk is a natural emulsion which has both water and oil phase (Pal et al., 2014). So it may be speculated that when seeds of *Datura* treated with Cow's milk, oil (butter) in the milk removes a portion of tropane alkaloids from the seeds of *Datura* and therefore decreases the level of marker compounds atropine and hyoscine after its purification.

Levels of atropine and hyoscine again decreases when seeds of *Datura* treated by both Cow's milk and Cow's urine (Method 2). Range of pH of Cow's urine is from 7.27 to 8.71 (Kume et al., 2011). Atropine and hyoscine are weak base and its solubility decreases with increase in pH (Block , 1991). At lower basic pH atropine and hyoscine are soluble and therefore when seeds of *Datura* soaked overnight in Cow's urine and then after treated with Cow's milk, level of atropine and hyoscine decreases in the seed of *Datura* as compared to the seeds of *Datura* treated with only Cow's milk.

Hyoscine and atropine along with other alkaloids present in the seeds of *Datura* are responsible for its toxicity (excessive anti–cholinergic activity) (Boumba 2004; Pereira et al., 1994; Chang et al., 1999; Kovatsis et al., 1993). Since treatment of seeds of *Datura* with Cow's milk and/or Cow's urine decreases the level of alkaloids, therefore it may be speculated that toxicity of seeds decreases after its purification due to decrease in the level of hyoscine and atropine along with other alkaloids.

Conclusion

Literature survey revealed that toxic effect of *Datura* seeds are due to the presence of atropine and hyoscine along with other tropane alkaloids present in it. When *Datura* seeds are

subjected to Ayurvedic process of purification (*Sodhana*) with Cow's milk and/or Cow's urine, the level of tropane alkaloids decreases and therefore toxicity also decreases in the same proportion. After purification, *Datura* seeds may use in therapeutic dose for the treatment of various disorders *viz*. inflammation, respiratory decongestion, skin infection, dental problems, stimulation of central nervous system and alopecia.

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Conflict of interests

Authors declare no any conflict of interests.

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