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ISSN: 0975-7538
Research Article

Studies on the process of detoxification and safety evaluation of *shodhit* and *ashodhit Datura metel* seeds for the use in Ayurvedic preparations

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ABSTRACT

Datura metel Linn. is a well known and frequently used herbal drug in Ayurveda. Seeds of *D. metel* are poisonous and according to Ayurvedic literature, they should be used in medicines after appropriate *shodhan* (purification or detoxification procedures). The present study evaluates various physicochemical parameters and HPLC analysis with special reference to hyoscine and atropine content for *D. metel* seeds (DMS), *ashodhit* (before *shodhan*) and *shodhit* (after *shodhan*) samples, as well as for *shodhan* reagent (cow urine). DMS, *ashodhit* as well as *shodhit*, were further evaluated for acute oral toxicity in mice and for cardiotoxicity in the isolated heart preparation of frog. The percentage of water soluble extractives, alcohol soluble extractives, and total alkaloids were found reduced in the *shodhit* DMS. HPLC analysis also showed the depletion of toxic alkaloids like atropine and hyoscine in the *shodhit* DMS as compared to *ashodhit* DMS. *Shodhit* DMS showed no toxicity on isolated heart preparation of frog as compared to *ashodhit* DMS. Thus, the present study reflects the importance of *shodhan* methods for the toxic drugs by means of which reduction of toxic constituents is achieved as advocated by Ayurveda.

Keywords: Atropine; cardiotoxicity; cow urine; *Datura metel* seeds; hyoscine; *shodhan*

INTRODUCTION

Ayurveda, an Indian traditional system of medicine, deals with herbal and herbo-mineral formulations to cure the disease conditions as well as to maintain a good health. *Visha-vaidyak* (Toxicology; administration of poisonous drugs) is one of the eight main branches of Ayurveda (Vagbhatacharya, commentary 1975). Ayurvedic seers believed that 'the poisons are the best and fast acting medicines if they are used in very small doses' (Agnivesha, commentary 1988). Ayurveda has classified such drugs in the *Vishopavisha Varga* (toxic and secondary toxic drugs). They always tried to limit the poisonous effects of these substances while utilizing them in the medicines. To serve this purpose they subjected these substances to the specific processing, collectively known as *shodhan*, which literally means purification. The purpose of *shodhan* is two-fold: to enhance the medicinal or beneficial properties and to limit or nullify the harmful or unwanted properties (Puranik G, 1964). In case of poisonous and secondary poisonous drugs, the later purpose, the detoxification or minimization of toxicity, serves the prime importance. Ayurvedic seers have also cautioned that ignorance or short cuts during *shodhan* processing can lead to fatal outcome.

Datura metel L. is a perennial herbaceous plant belonging to the Solanaceae family and contains tropane alkaloids as major constituents. Ayurveda classifies *D. metel* in *upavisha varga* (secondary toxic drugs). The bark, leaves and seeds of *D. metel* are used in Ayurvedic medicines for the treatment of various ailments. The whole plant is considered as narcotic, anodyne and antispasmodic. According to Ayurveda, seeds are acrid, bitter, tonic, febrifuge, anthelmintic, alexiteric, emetic and useful in leucoderma, skin disorders, ulcers, bronchitis, jaundice, piles and diabetes (Abubakar MG et al, 2010 and Elsa AT et al, 2001). Several pharmacological activities have also been studied including spasmogenic (E. Prabhakar, 1994), antimycotic (Rajesh, 2002), analgesic (Wannang NN, 2009), hallucinogenic (Abubakar MG et al, 2010), and antidiabetic (Krishna MB et al, 2004). According to the Ayurvedic Pharmacopoeia of India, seeds of *D. metel* are a part of many formulations e.g., *Kanakasava, Mahalakshmivilas Rasa, Suvarn Sootashekhar Rasa*, etc. Though it finds various uses in Ayurveda, all parts of *D. metel* plant are poisonous in large doses because of the presence of anticholinergic substances such as hyoscine, hyoscyamine, and atropine (Chopra RN et al, 1965).

In Ayurveda, *shodhan* reagents commonly deployed for *shodhan* of *D. metel* seeds (DMS) are cow milk and cow urine. It is fascinating to note the difference in the

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Received on: 12-08-2012
Revised on: 14-09-2012
Accepted on: 17-09-2012

properties of both the *shodhan* reagents. Cow milk is considered to be sweet in taste, cooling, olient, heavy, strengthening, reducing *vata* and *pitta dosha* (Bhavaprakash, commentary 1999) while cow urine is considered to be bitter, hot, light, penetrating, reducing *kapha* and *vata dosha* (Bhavaprakash, commentary 1999). Here, we are presenting the results obtained while using cow urine as a *shodhan* reagent.

The typical features of *D. metel* toxicity includes, dryness of mouth, intense thirst, increase in heart rate, and blurring of vision with prominent mydriasis; followed by hallucinations, delirium, and loss of motor coordination which may further lead to coma and ultimately to death by respiratory failure. DMS are reported to be highly toxic which is evidenced by the various animal studies (Monira KM, 2012).

Thus, the main aim of the study was to assess the impact of *shodhan* method on DMS as compared to DMS before *shodhan*, that is, in their crude stage. Further, the samples were also evaluated for acute oral toxicity in mice and cardiotoxicity in the isolated heart of frog.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Dried DMS were obtained from three different stockists; two from Mumbai vendors (Samples: DM01 and DM02) and one from Jabalpur vendor (Sample: DM03) and were authenticated at Agharkar Research Institute (ARI), Pune. Voucher specimens (Numbers: S-127, S-128, and S-129) were deposited in the herbarium of the same department.

Physicochemical Parameters

The dried DMS were subjected for determination of physicochemical parameters; namely, foreign organic matters, loss on drying, ash content, acid insoluble ash, water soluble extractives, alcohol soluble extractives, and % total alkaloids. The tests were carried out according to standard methods as mentioned in Ayurvedic Pharmacopoeia of India (1989). Microbial profile of the samples was studied as per World Health Organisation (WHO) norms. The best suitable raw material drug sample that complied with the API limits was selected for further *shodhan* methods.

TLC of Different Raw Materials - DMS Powder

Different raw materials of DMS powders were spotted on TLC system using pre-coated silica G plate as stationary phase, toluene: ethyl acetate: diethylamine (7:2:1 v/v/v) as mobile phase and detection was done after derivatization with dragendorffs reagent to detect the presence of atropine.

Shodhan Procedure of DMS

Shodhan Method 1 (for DS01)

This was performed according to the traditional Ayurvedic texts, detailed in the '*Rasa Tarangini* (Sadan- and S, 1975). Briefly, 0.5 kg DMS were cleaned with dry and dust free cloth. The cleaned seeds were placed on the muslin cloth and the four edges of the cloth were brought together to form a pouch. Further, 5 L of fresh cow urine was taken in a vessel. The above vessel was placed on a heating mantle. The pouch containing the seeds was carefully dipped into the cow's urine such that the pouch was completely immersed in the liquid and still did not touch the bottom of the vessel. The whole assembly is named as *Dolayantra*. This assembly was then heated on a medium flame for 3 hr. After 3 hr, the pouch was taken out and opened. The urine was collected separately and the seeds were washed with lukewarm water. The seeds were spread to dry and stored in a labelled container till further use.

Shodhan Method 2 (for DS02)

This was performed according to the traditional Ayurvedic texts, detailed in the '*Bharat Bhaishajya Ratnakar*' (Shah NC, 1986). Seeds of *D. metel* are immersed in cow urine for a period of 12 hours. The seeds were then removed and the covering of the seeds were separated. After removal of the covering the seeds were washed with lukewarm water and then dried in shade and stored in a labelled container till further use. Sample of cow urine, after removal of the seeds from it, was also preserved in a clean labelled container.

Quantitative Estimation of Hyoscine and Atropine in *Ashodhit* and *Shodhit* DMS and Post-*Shodhan* reagent (cow urine), by HPLC Method

Preparation of Sample

Atropine and hyoscine content was determined using HPLC method for the *ashodhit* (DM03), *shodhit* samples of DMS (DS01 and DS02), and post-*shodhan* reagent samples (cow urine [SR01 and SR02]). Each of these materials separately alkalinized with ammonia and extracted with 80 ml methanol using soxhlet apparatus. It was then cooled and transferred in 100 ml volumetric flask and diluted up to the mark with methanol. Two ml was pipetted out in a 25 ml volumetric flask and diluted up to the mark with methanol. This solution was used for HPLC analysis. Standard solution of atropine and hyoscine was prepared in the methanol.

HPLC analysis was performed with a JASCO system consisting of a manual sample injection valve (Rheodyne 7725i) equipped with a 20 µL loop, and a UV - visible detector (UV - 1575) using column Microsorb - MV (100 - 5, C18, 250 X 4.6 mm). Mobile phase consisted of MeOH: 0.02M Sodium acetate buffer (80:20) containing 0.02% triethanolamine with 1 ml/min flow rate and pH was adjusted to 6.0 with glacial acetic acid.

Animals

Swiss albino mice were used for acute oral toxicity study. Frogs of *Rana tigrina* species were used for the cardiotoxicity studies. The experimental protocols

Table 1: Physicochemical Analysis of Raw Material of DMS - DM01, DM02, and DM03

Parameters	Units	API Limits	DM01	DM02	DM03
Foreign Matter	%/wt	NMT 2 %w/w	4.5	3.5	2
Loss On Drying@105°C, 1 hr	%/wt	-	3.682	4.229	4.267
Ash Content	%w/w	NMT 15 %w/w	< 1	< 1	< 1
Ash Insoluble in dil. HCl	%w/w	NMT 2 %w/w	< 1	< 1	< 1
Water Soluble Extractives	%w/w	NLT 10 %w/wt	6.75	5.926	15.409
Alcohol Soluble Extractives	%w/w	NLT 6 %w/w	2.067	3.131	12.006
% Total Alkaloids in Terms of Atropine Sulfate	-	-	0.453	0.421	0.416
Microbial Load:		WHO Limits			
Total Bacterial Count@37°C	cfu/g	NMT 105 cfu/g	200	200	250
Total Fungal Count	cfu/g	NMT 103 cfu/g	40	nil	60

Table 2: Yields Following Shodhan Methods for D. metel Seeds

Parameters	Material	*Shodhan* Reagent	Material	*Shodhan* Reagent
Specification	DS01	SR01	DS02	SR02
Shodhan Method	*Dolayatra*		Immersion	
Quantity Before *Shodhan*	500 gm	5 L	500 gm	5 L
Quantity After *Shodhan*	640 gm	4.15 L	670 gm	4.8 L

Table 3: Comparison of Physicochemical Properties of DM03 and DS01 and DS02

Parameters	Units	Methods	DM03 (*Ashodhit* DMS)	DS01 (*Shodhan* in Cow Urine: *Dolayantra*)	DS02 (*Shodhan* in Cow Urine: Immersion)
Colour	-	-	Brown	Light brown	Light brown
Odour	-	-	Characteristic	Characteristic (Strong, ammonic)	Characteristic (Strong, ammonic)
Appearance	-	-	Auriform	Auriform, Swollen	Auriform, Swollen
Foreign matter	%/wt	IS:1797:2001	2	2	1
Loss on drying @105oC, 1hr	%/wt	Gravimetric	4.267	3.1	4.3
Ash content	%/wt	IS:1797:2001	<1	2.49	2.12
Acid insoluble ash	%/wt	IS:1797:1985	<1	0.2	0.26
Water soluble extractives	%/wt	IS:1797:2001	15.409	4.12	4.31
Alcohol soluble extractives	%/wt	IS:1797:2001	12.006	4.46	4.62
Total Alkaloids	%/wt	-	0.416	0.40	0.25
Microbiological					
Total bacterial count @ 37oC	cfu/gm	IP:1996	250	186	210
Total fungal count	cfu/gm	IP:1996	60	26	30

were approved by Institutional Animal Ethics Committee (UICT/PH/IAEC/0807/35) of Institute of Chemical Technology (ICT), Mumbai. The animals were maintained in standard laboratory conditions with food and water *ad libitum*, under a 12 hr light/12 hr dark cycle.

Acute Oral Toxicity

Acute Oral Toxicity study was performed according to OECD 425 (OECD, 2008). Swiss albino mice were administered with *ashodhit* and *shodhit* DMS powder and observed for mortality during 14 days study period toxicity. Based on short-term profile of drug, the lethal and safe dose of the extract for animals were determined as per as OECD guideline. The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental Protection Agency, USA.

Frog Heart *in situ* Preparation

Frog was pithed and the heart exposed. The inferior vena cava was cannulated and the frog's Ringer Solution was used to perfuse the heart. (The composition of the frog Ringer solution in millimoles: NaCl-110; KCl-1.9; $CaCl_2$-1.1; $NaHCO_3$-2.4; NaH_2PO_4-0.06; Glucose-11.1). After perfusion, the basal cardiac contraction was recorded on a student's physiograph. The *ashodhit* and *shodhit* DMS extracts were administered through the cannula. The effects obtained with the extracts were transposed to the respective percentage of the basal values. The frog's heart was washed with

Table 4: Results of physicochemical characterization of DMS using cow urine

Parameters	Units	Methods	SR01 (Cow Urine) Pre- *Shodhan*	SR01 (Cow Urine: Dolayantra) Post- *Shodhan*	SR02 (Cow Urine) Pre- *Shodhan*	SR02 (Cow Urine: Immersion) Post- *Shodhan*
Colour	-	-	Yellow	Dark brown	Yellow	Dark brown
Odour	-	-	Characteristic (Strong, ammonic)	Characteristic (Strong, ammonic)	Characteristic (Strong, ammonic)	Characteristic (Strong, ammonic)
Appearance	-	-	Yellow Liquid	Dark Brown Liquid	Yellow Liquid	Dark Brown Liquid
pH	-	IS:3025 (Part-11-2): 2002	8.5	8.6	8.5	8.4
Total Solids	%/wt	IS:3025 (Part-16):2002	3.32	6.2	3.32	4.45
Total Alkaloids	-	-	Nil	0.24	Nil	0.34

Table 5: Hyoscine and Atropine Content in DM03, DS01 and DS02; and SR01 and SR02

DM03		DS01		DS02		SR01		SR02	
Hyoscine	Atropine	Hyoscine	Atropine	Hyoscine	Atropine	Hyoscine	Atropine	Hyoscine	Atropine
1.72	0.13	1.32	0.07	0.70	0.08	0.36	0.02	0.39	0.04

the Ringer solution after every administration of different doses of *ashodhit* and *shodhit* DMS extracts till it regained the normal rhythm (Shah RK et al, 2010).

RESULT AND DISCUSSION

The results of the physicochemical parameters obtained from different locations of DMS as raw materials (DM01, DM02 and DM03) are depicted in Table 1. Foreign organic matter, water extractive and alcohol extractive values for DM03 raw material lied within the limits as compared to Ayurvedic Pharmacopeia of India. This signified that DM03 sample has better quality and purity of raw material as compared to DM01 and DM02. DM03 was selected as the best suitable raw material sample and were used further to evaluate the effect of *shodhan* methods on DMS in crude stage and after the *shodhan* process.

Identification of Active Constituents by Chromatography

Figure 1 demonstrated the TLC of methanolic extract of *ashodhit* DMS, wherein T_2, T_3, T_4 lanes represented the pre-*shodhan* sample and T_1 lane represented standard atropine. Methanolic extract of *D. metel* was found to contain principle constituents like atropine having R_f values of 0.36 (Figure 2).

Shodhan was performed according to traditional literature on DMS. After *shodhan,* the weight of DMS (DS01) increased and quantity of the *shodhan* reagent (cow urine [SR01 and SR02]) decreased (Table 2).

Physicochemical parameters like water extractive and alcohol extractive values for DS01 and DS02 were reduced as compared to DM03 (Table 3).

The hyoscine and atropine content of DM03, DS01 and DS02; SR01 and SR02 were determined by HPLC method. In DM03 (RM sample), hyoscine and atropine content was found to be 1.729% and 0.132% respectively. In post-*shodhan* samples, hyoscine content was higher in DS01 (1.32%) compared to DS02 (0.70%), while atropine content was higher in DS02 (0.08%) compared to DS01 (0.07%). From the result it can be observed that the content of atropine and hyoscine decreased in DS01 and DS02 as compared to DM03 (Table 5; Graphs 1-5). It is also evident from the results that the toxic substance atropine was extracted into SR01 and SR02 (Table 4). Thus, it was observed from the results that the process of *shodhan* resulted into the reduction, loss, or modification of few components like atropine and hyoscine from the original seed powder (Banerjee AA et al, 2011). This may be due to their extraction into the *shodhan* medium or any structural change in these compounds. The removal of these compounds is likely to cause the reduction in their toxic effects. (Kamble R et al, 2008)

Acute Oral Toxicity in Mice

According to AOT 425 guideline LD_{50} found to be greater than 2000 mg/kg for DM03- and DS01 and DS02- treated groups as all the five animals survived. It was found that the DM03-treated group revealed tachycardia and isolated jerks at 2000 mg/kg dose of the sample which was absent in the DS-1 and DS02-treated groups. Thus, DM03, DS01, and DS02 were found to be non-toxic at 2000 mg/kg in mice except for little isolated jerks and tachycardia as mentioned above at 60 to 90 min after dosing on the same day.

Table 6: Effect of DM03, DS01 and DS02 on Isolated Frog Heart

Sr.No.	Extract Code	Concentration	Result
1	DM03	0.1 ml	Produced Cardiac diastolic arrest for seven seconds
		0.2ml, 0.4 ml and 0.8ml	Produced cardiac arrest for the increasing time period of 31 sec, 64 sec, and more than 2 min respectively. After that heart revived back to normal after 2 min
2	DS01	0.1 ml, 0.2ml, 0.4ml and 0.8ml	Extract did not produce any cardiotoxicity at increasing doses on the perfused heart of frog.
3	DS02	0.1 ml, 0.2ml, 0.4ml and 0.8ml	Extract did not produce any cardiotoxicity at increasing doses on the perfused heart of frog.

Figure 1: TLC Results for Raw Materials (DM01, DM02 and DM03)

T_1: Atropine sulphate (standard); T_2: Methanolic extract of *D. metel* DM01; T_3: Methanolic extract of *D. metel* DM02; T_4: Methanolic extract of *D. metel* DM03

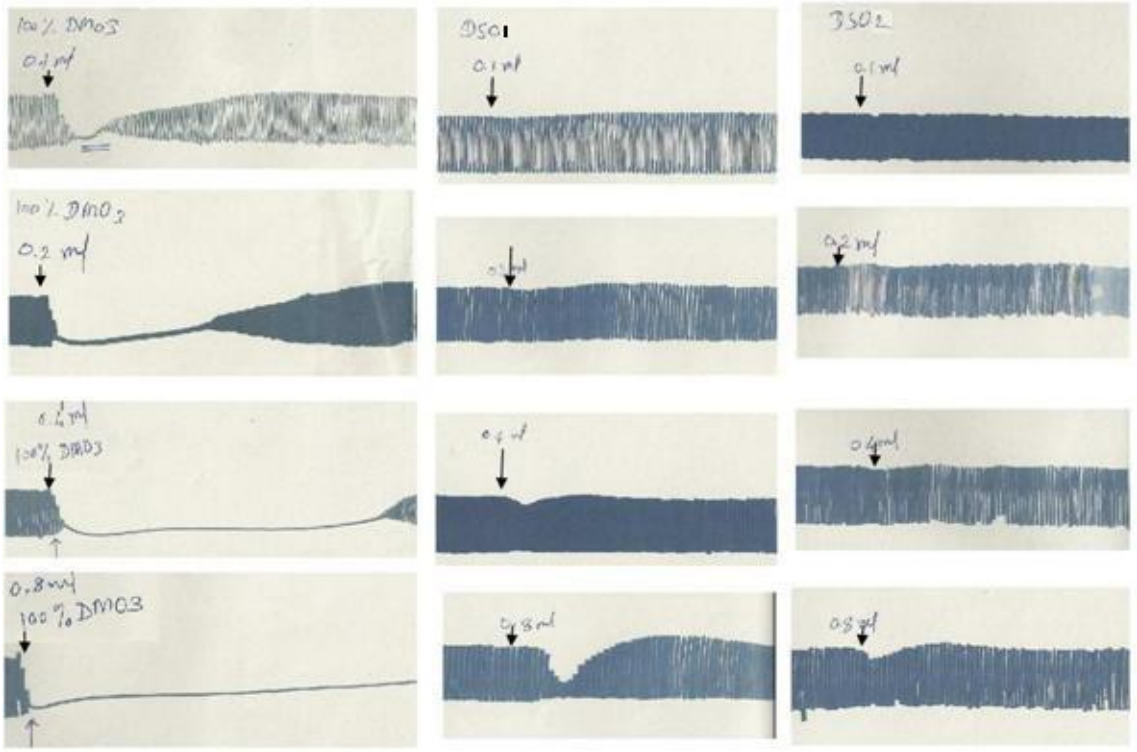

Figure 2: Response of different doses of D. metel on student Kymograph

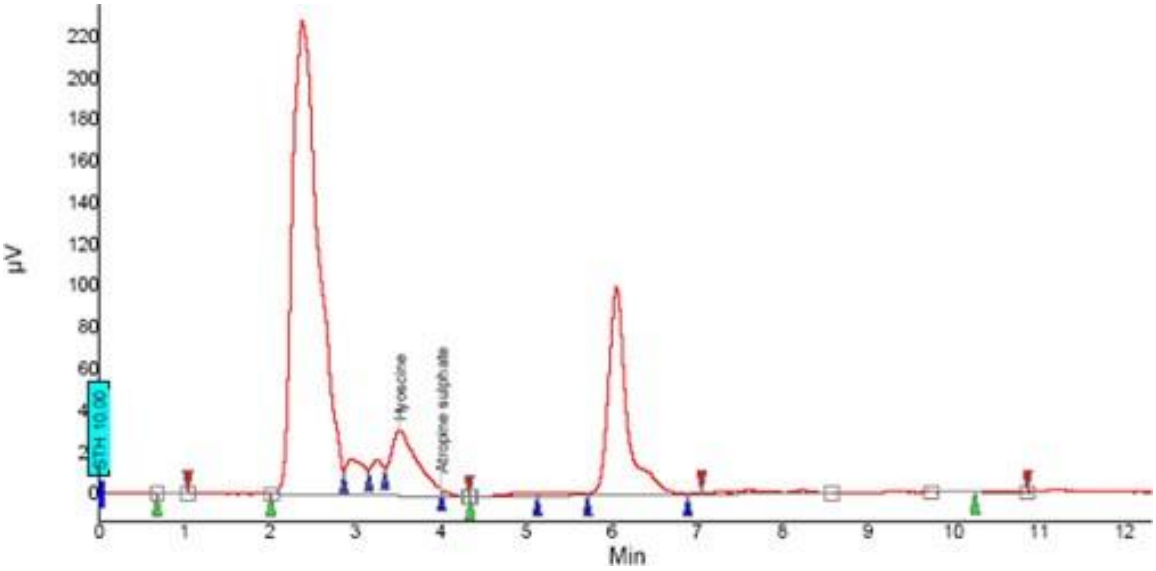

Figure 3: HPLC peak graph for hyoscine and atropine in DM03

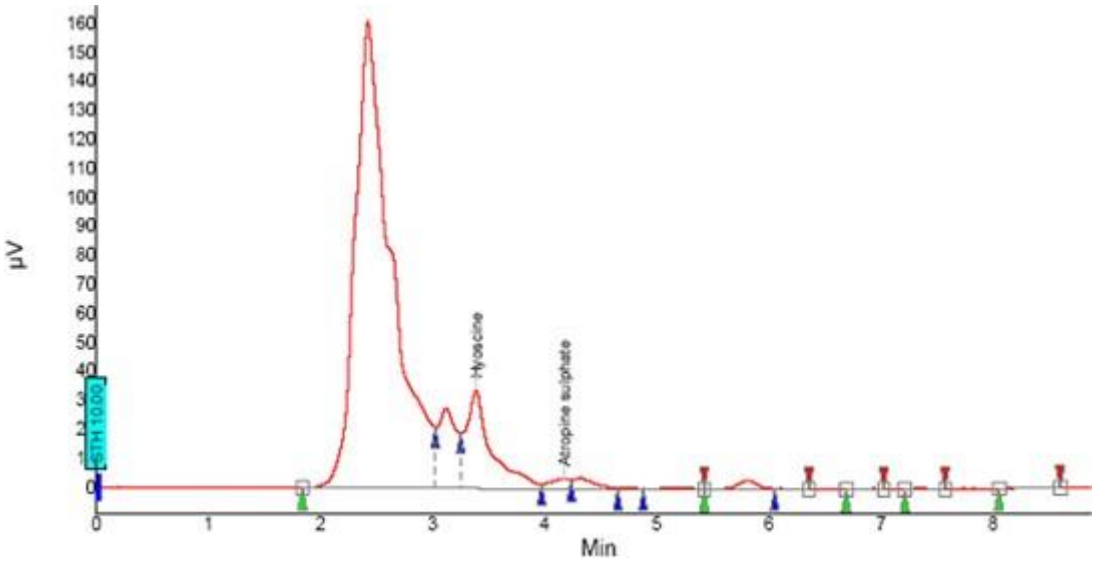

Figure 4: HPLC peak graph for hyoscine and atropine in DS01

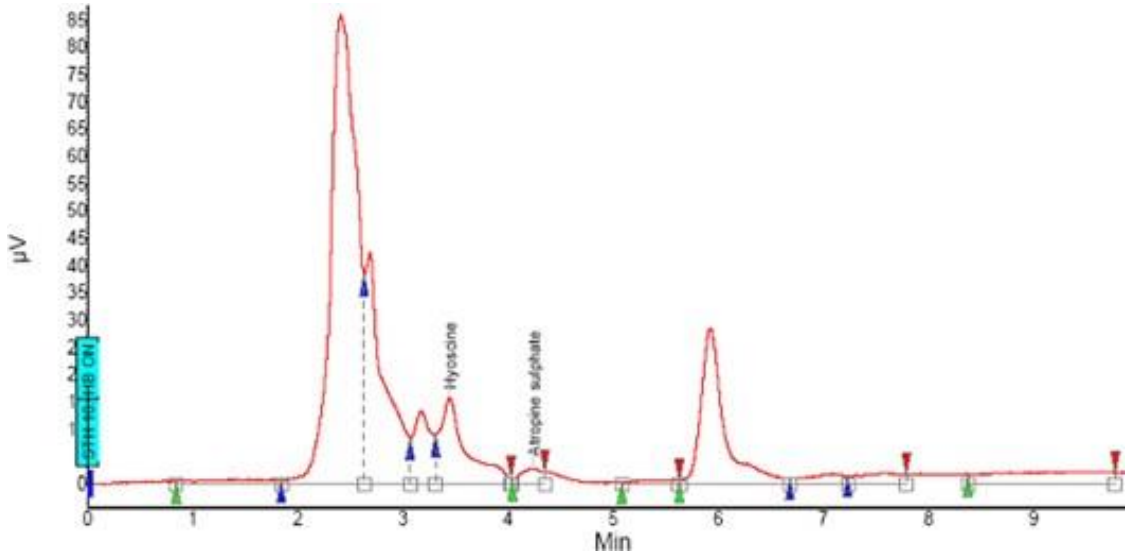

Figure 5: HPLC peak graph for Hyoscine and Atropine in DS02

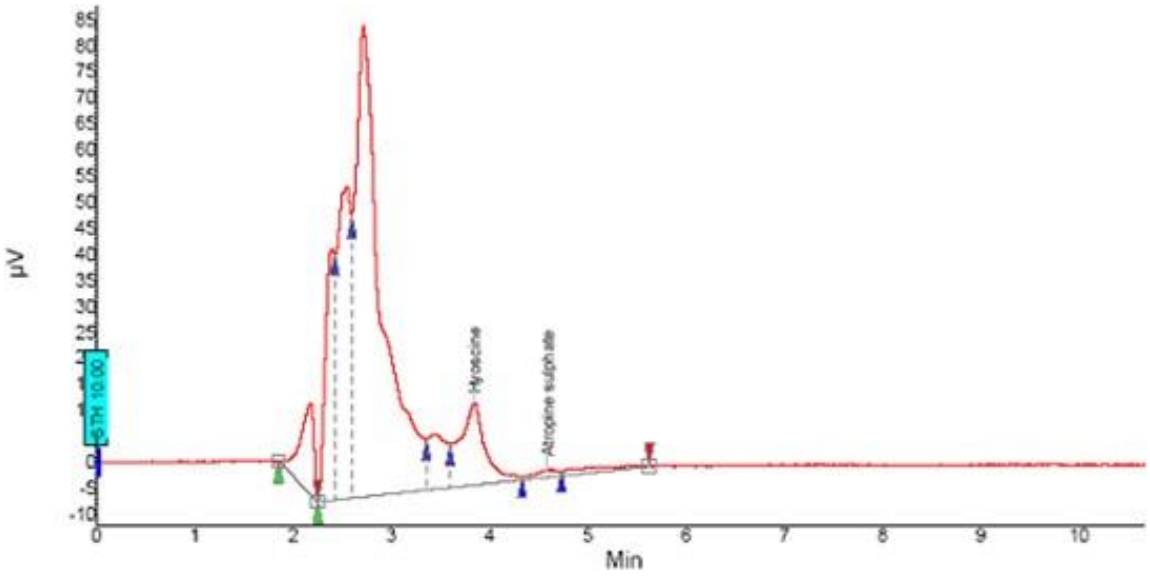

Figure 6: HPLC peak graph for Hyoscine and Atropine in SR01

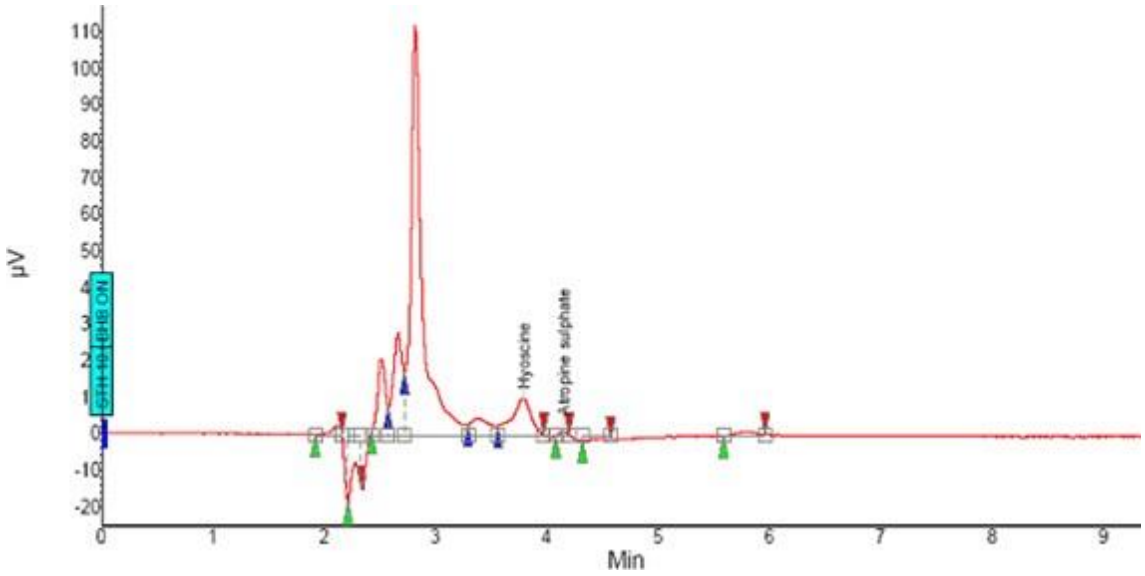

Figure 7: HPLC peak graph for Hyoscine and Atropine in SR02

Cardiotoxicity in the Isolated Heart Preparation of Frog

DM03, DS01 and DS02 samples were evaluated for their cardiotoxicity in the isolated heart preparation of frog. Results obtained are presented in Table 6 and Figure 2.

The DS01 and DS02 did not produce any cardiotoxicity on the perfused heart of frog, whereas DM03 exhibited cardiotoxicity in terms of cardiac arrest for some period for time. This is due to toxic constituents, like hyoscine and atropine in the DM03, which were reduced by 76.74% and 53.84%, respectively, in the DS01; and by 40.7% and 61.54%, respectively, in DS02, thereby reducing the cardiotoxicity. This emphasized the importance of *shodhan* process in reducing the toxicity of toxic and secondary toxic herbal substances. Thus, *shodhan* process is an essential step to reduce the toxicity of DMS before using them in Ayurvedic preparations.

CONCLUSION

The present study justified the mandatory use of *shodhan* process of toxic and secondary toxic drugs, (with special reference to seeds of *D. metel* in the present study) as prescribed in Ayurvedic literature. The present study showed that both the *shodhan* methods of DMS led to the reduction in toxic constituents and that these are necessary to be adopted prior to its use in the therapeutics.

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