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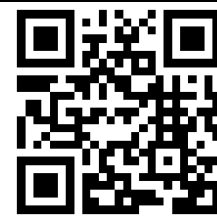
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Pharmaceutical study of Navayas Churna and assessment of its Vardhaman Matra preclinically W.S.R. to RBC and weight gain in Wistar rats.

PAWAR UB¹ AMRUTKAR M²

1. Dept. R.S.B.K. Dr.G.D.Pol Foundation's Y.M.T. Ayurvedic Medical College And Hospital. Kharghar, Navi Mumbai.
2. Associate Professor Of Dept. R.S.B.K.Dr. G.D. Pol Foundation's Y.M.T. Ayurvedic Medical College and Hospital. Kharghar, Navi Mumbai.

Abstract:

Ayurvedic literature emphasizes the use of heavy metals in their formulation due to their particular biological properties for curing disease. Iron deficiency is very common nutritional disorder worldwide & and it affects approximately one third of global population. In Ayurveda Navayas churna is well known iron containing pharmaceutical preparation. The multi-elemental combination of Navayas churna eradicate not only iron deficiency anaemia but also fulfills various demands of the human system. Present study is an attempt to establish standardization and pharmacologically the efficacy of Navayas churna in the alleviation of anaemia in experimental animals. This study conclude that Analytical and TLC study shows presence of inorganic and iron compound in formulation that was also useful in safety and effectiveness of drug and No serious toxicity was observed in acute toxicity study in Increasing dose (Vardhaman matra) for 9 days as well as no Significant histological changes are found in Animal study.

Keywords : Pandu, Iron deficiency anaemia, Vardhaman matra, Navayas churna

Corresponding Author:

VD. USHA B. PAWAR

DEPT. R.S.B.K. DR.G.D.POL FOUNDATION'S

Y.M.T. Ayurvedic Medical College And Hospital Kharghar, Navi Mumbai.

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Introduction

Iron deficiency anaemia is a common problem in the developing countries like India. The modern treatment of iron deficiency anaemia with iron salt preparations like ferrous sulphate results in several adverse effects viz. severe gastrointestinal irritation and necrosis of the mucous membrane, cardiovascular collapse, severe damage to liver or even death may occur. In India the incidence of iron deficiency anaemia is consistently high. Prevalence of anaemia among Indian women 53% and men 23% according to National Family Health Survey (NFHS-4)¹

Iron is being used in Ayurveda since time immemorial in the form of Lauha Bhasma and its preparation Navayas churna for the management of this disease². As per classical Ayurveda point of view, the use of Navayas churna is very much justified. The composition of Navayas churna is excellent formulation. Most of the drug in Navayas churna are Deepan, pachan, strotoshodhan, tridoshaghna, rasaraktavardhan, rasayan, panduhara. Raktavardhak property is also present in lauha bhasma.³ Iron is also present in musta and amalaki. Vidang and Vibhitaki are known krimihara drug that can prevent anaemia because worm infestation is one of the main cause of anaemia in Indian sub continent. Navayas churna is effective, well tolerated and clinically safe to relieve sign and symptom of pandu roga (IDA). The multi-elemental combination of Navayas churna eradicate not only iron deficiency anaemia but also fulfills various demands of the human system.⁴ Present study is an attempt to establish standardization and pharmacologically the efficacy of Navayas churna in the alleviation of anaemia in experimental animals. The study was carried out under the title " Pharmaceutical Study Of Navayas Churna And Assessment Of Its Vardhaman Matra

Preclinically In Pandu W. S. R. To Haemoglobin % (In Vivo) ",

AIM

Preparation and standardization of Navayas Churna & assessment of its vardhaman matra preclinically w.s.r. to haemoglobin % in wistar rats.

OBJECTIVES

Primary objectives

- A) To prepare and standardize Navayas Churna.
 - B) To analyse study drug physico chemically and develop its standards .
 - C) To evaluate haemoglobin percentage of study drug preclinically (in vivo).
- Secondary objectives**
- A) To authenticate each raw drug used in formulation.
 - B) To develop animal models for evaluation of the haemoglobin percentage.

METHODOLOGY

Study design- Experimental and preclinical study.

Study settings-

The study was carried out in two parts.

1. Pharmaceutical and analytical study.
2. Pre-clinical study (in vivo).

1	Ethical	Institutional ethics committee
2	Preparation	Teaching pharmacy of the institute as necessary set up is available
3	Analytical study	Research laboratory of the institute and other scientific research institute as required with subjective
4	In vivo study	Animal study was done in authentic research lab with subjective

Material and Methods- Raw material required in preparation of study drug was collected from

authentic source and was authenticated from certified laboratory as per A.P.I. Guidelines.

Table no. 1 **Ingredients for Navayas churna**^{5,6,7}

Sr.no	Ingredients	Latin name	Part used	Quantity
1	Shunthi	Zingiber officinale	Rhizome	1 Part
2	Marich	Piper nigrum	Fruit	1 Part
3	Pippali	Piper longum	Fruit	1 Part
4	Haritaki	Terminalia chebula	Fruit Pericarp	1 Part
5	Bibhitaki	Terminalia bellirica	Fruit Pericarp	1 Part
6	Amlaki	Emblica officinalis	Fruit Pericarp	1 Part
7	Musta	Cyperus rotundus	Rhizome	1 Part
8	Vidang	Embelia ribes	Fruit	1 Part
9	Chitrak	Plumbago zeylanica	Roots	1 Part
10	Lauha	Iron	Bhasma	9 Part
11	Kadalikandafor samanya shodhan of lauha	Musa paradisiaca	Roots	Q.s.
12	Daradam (hingul) for lauha marana	HgS	-	Q.S.
13	Triphala kashay for vishesh lauha shodhan	Terminalia chebula, Terminalia belerica, Emblica officinalis.	Fruit pericarp	Q.S.
14	Kumari for lauha maran	Aloe barbadensis	Leaf	Q.S.
15	Jambari for hingul shodhan ^l	Citrus lemon	Fruit juice	Q.S

1. Preparation of study drug

Process : the process for the preparation of Navayas churna , a common pharmaceutical proforma was prepared, and was recorded every minute facts and observations regarding these processes. Considering these facts validation master protocol for following processes have been designed.

Pharmaceutical Processing :

1. Validation of process of Samanya Shodhana of Lauha.

2. Validation of process vishesha shodhana of lauha Bhasma
3. Hingul Shodhana
4. Validation of process Marana of lauha Bhasma
5. Validation of process Preparation of Navayas churna

2. Analytical study

Analysis of raw material required for Navayas churna

1. Organoleptic analysis and physicochemical analysis of Raw material

2. T.L.C. of Navayas Churna content
3. XRF,XRD test of loha .
4. XRF analysis of hingul

Analysis of Navayas Churna

1. physicochemical analysis of 3 batches of navayas churna.
2. T.L.C. of navayas churna three batches to compare the Major phytocostituet presence.
3. XRF ,XRD analysis of 3 batches of loha after shodhan Maran .
4. XRF analysis of 3 batches of navayas churna.

3. Pharmacological Study

Study Protocol:

Total 30 wistar rats of either sex, weighing 180 g to 250 g were taken and divided randomly into 5 groups, each containing 6 animals. First groups were treated with Normal control. Group 2 was Disease control, Group 3 was Vehicle control (madhu sarpi)Honey + Ghee (1:1 ratio), Group 4 was Standard (Tab ferdous sulphate 29.25

Observation and results

Table no. 3. Changes In Weigh of Kanta Lohaafter Marana

Loha Maran				
Sr no	PROCEDURE	BATCH A	BATCH B	BATCH C
1	Initial weight of shodhit (purified) kant loha	420 gm	425 gm	450gm
2	Weight after 1 st Gajaput	415 gm	418 gm	422 gm
3	Weight after 2 nd Gajaput	410 gm	412 gm	416 gm

Mg/kg/bodywt) and Group 5 was Study drug Navayas Churna. 9 doses was given to rat. Drugs were administered orally increasing doses. according to the stated dosage schedule. Gross behaviour and exitus (death) were recorded for 9 consecutive days.

The animals were divided into 5 groups, each containing 6 animals.

Table no. 2 Grouping for acute toxicity study

Sr. No	Groups	Specification
1	Group1	Normal control
2	Group 2	Disease control
3	Group 3	Vehicle(madhu sarpi)Honey + Ghee (1:1 ratio)
4	Group 4	Standard (Tab ferrous sulphate 29.25 Mg/kg/bodywt)
5	Group 5	Study drug Navayas Churna

4	Weight after 3 rd Gajaput	408 gm	410 gm	414 gm
5	Weight after 4 th Gajaput	402 gm	404 gm	408 gm
6	Weight after 5 th Gajaput	392 gm	400 gm	402 gm
7	Weight after 6 th Gajaput	380 gm	395 gm	397 gm
8	Weight after 7 th Gajaput	372 gm	370 gm	385 gm
	% changes after 7 Gajaputa (loss)	11.4 %	12.9 %	14.4%

TABLE - 4. Properties of Lauha Bhasma

Touch	Colour	Taste	Smell	Magnetic Property
Soft and smooth powder	Purple (Pakwa Jambuphala Varna)	Tasteless	No specific	Magnetic

Table - 5 Observations of classical analytical tests

Sr. No.	Parameter	Lauha Bhasma
01.	Varitara test	+ve
02.	Unam test	+ve
03.	Rekhapurnata test	+ve
04.	Nishchandrata test	+ve

Table no. 6 Analytical Report: Navayas Loha

TEST	RESULTS
APPEARANCE	Dry Powder
COLOUR	Brown
ODOUR	Characteristic
TASTE	Very Bitter & Astringent
MOISTURE CONTENT	3.60 %
DENSITY	1.1183 gm/ml
ASH	52.19 %
A I A	45.31 %

A S E	4.76 %
W S E	15.04 %
pH	4.3

Table 7 XRF of Navayas Churna sample A, B and C XRF test. Analysed results (FP methods)

No.	Component	Result A	Result B	Result C	Unit
1.	SiO ₂	49.1	34.1	41	mass%
2.	Fe ₂ O ₃	43.5	58.4	53.2	mass%
3.	Al ₂ O ₃	1.87	1.26	1.46	mass%
4.	CaO	1.99	1.86	1.47	mass%
5.	K ₂ O	1.93	2.08	1.29	mass%
6.	P ₂ O ₅	0.80	1.22	0.713	mass%
7.	TiO ₂	0.129	0.109	0.121	mass%
8.	SO ₃	0.572	0.782	0.486	mass%
9.	Cr ₂ O ₃	0.0399	0.0343	0.0378	mass%
10.	As ₂ O ₃	0.0078	0.0601	0.0583	mass%
11.	CuO	0.0182	0.0455	0.0392	mass%
12.	ZnO	0.0267	0.077	0.0542	mass%
13.	PbO	0.00015	0.0043	0.0004	mass%
14.	BaO	-	-	0.0074	mass%
15.	MnO	0.0062	<0.0001	0.0076	mass%
16.	HgO	0.0026	0.0063	0.0040	mass%
17.	V ₂ O ₅	-	<0.0001	-	mass%

Table no. 8. Effect of therapy on Individual Body Weight (gm)

Parameter N=6	Individual Body Weight (gm)				
	Mean ± SD (0 day)	Mean ± SD (9 Day)	t	P value	Significance
Normal control	221.9±9.9	227.75± 12;.87	3.610	<0.05	Significant
DC (Phenyl hydrazine)	210±21.6	236.4±15.2	2.414	<0.05	Significant

VC (honey and ghee)	221.5±7	255.1±4.8	9.636	<0.001	Highly significant
STD (Ferrous Sulphate)	218.3±15.6	243.4±13.7	2.958	<0.01	Significant
Test Navayas Churna	223.5±14.2	250±13.7	3.202	<0.01	Significant

Table no. 9. Effect of therapy on RBC count

Groups N=6	RBC count				
	Mean ± SD (0 day)	Mean ± SD (9 Day)	t	P value	Significance
Normal control	6.5±0.3	7.2 ±06	2.874	>0.05	Non significant
DC (Phenyl hydrazine)	3.4±0.1	6.2±0.2	30.431	<0.001	Highly significant
VC (honey and ghee)	4.0±0.3	6.2±0.5	9.659	<0.001	Highly significant
STD (Ferrous Sulphate)	4.3±0.1	6.5±0.4	14.598	<0.001	Highly significant
Test Navayas Churna	3.5±0.1	6.1±0.4	14.623	<0.001	Highly significant

Table no. 10. Effect of therapy on HB%

Parameter N=6	Individual Body Weight (gm)				
	Mean ± SD (0 day)	Mean ± SD (9 Day)	t	P value	Significance
Normal control	12.95±0.4	13.35 ±0.3	1.397	>0.05	Non significant
DC (Phenyl hydrazine)	8.6±0.1	12.5±0.4	25.435	<0.001	Highly significant
VC (honey and ghee)	9.4±0.3	13.9±1.0	9.509	<0.001	Highly significant
STD (Ferrous Sulphate)	8.9±0.7	15.6±0.3	21.958	<0.001	Highly significant

Test Navayas Churna	8.9±0.2	14.0±1.2	10.173	<0.001	Highly significant
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Table no. 11 Comparison between Groups in parameter of Individual Body Weight (gm)

Parameter N=6	Mean ± SD	Individual Body Weight (gm)		
		F	P value	Significance
Normal Control	5.83±3.9	8.398	0.0004	<0.001 Highly Significant
DC (Phenyl hydrazine)	26.01 ±11.23			
VC (honey and ghee)	33.56 ± 6.126			
STD (Ferrous Sulphate)	25.03 ±9.86			
Test Navayas Churna	20.5 ±10.4			

Table no. 12 Comparison between Groups in parameter of RBC

Parameter N=6	Mean ± SD	Individual Body Weight (gm)		
		F	P value	Significance
Normal control	0.7± 0596	9.164	0.0002	<0.001 Highly Significant
DC (Phenyl hydrazine)	2.98 ±0.3			
VC (honey and ghee)	2.13 ± 0.717			
STD (Ferrous Sulphate)	2.26 ±0.372			
Test Navayas Churna	2.18 ±1.137			

Table no. 13 Comparison between Groups in parameter of HB%

Parameter N=6	Mean ± SD	Individual Body Weight (gm)		
		F	P value	Significance
Normal control	0.4± 0.70	21.56	0.0001	<0.001 Highly significant
DC (Phenyl hydrazine)	3.866 ±0.45			
VC (honey and ghee)	4.516 ± 0.45			

STD (Ferrous Sulphate)	6.75 ± 0.84			
Test Navayas Churna (NC)	4.233 ± 2.324			

DISCUSSION

Pharmaceutical Study : To introduce SOP (standard operating procedure) for quality Bhasma preparations, it is mandatory to prepare Loha Bhasma and Navayas Churna preparations as per classical texts and also by mechanical method, and comparison of the finished products should be performed by analytical parameters. By keeping this view in mind both Loha Bhasma was prepared by traditional method in Gajaputa and analysis of the final product was carried out physico-chemically.⁹ In Loha bhasma formed contains Iron in the form of Ferrous or in oxide form which are considered to be the most compatible forms of iron supplementation in the body. The rate of the absorption of the iron depends on the fineness of the powder. Bhasma process makes the metal into very minute particles which are easy to absorb. Colour of Lauha Bhasma was purple (Pakwa Jambu Phala Varna). The average highest temperature in *Gajaputa* was 948.3°C. An average duration of *Putapka* to be 48.24 hr. The weight of *Shuddha Lauha* was loss up to 11% after *Marana* for A Batch. 12.9% for B Batch and 14.4% C Batch.

Analytical study: The observations indicate that Lauha Bhasma samples pass the classical tests Varitara test, Unam test, Rekhapurnata test and Nishchandrata test mentioned for evaluation of

the quality of Bhasma preparations.⁸ Analytical study of raw drugs and final product show normal values. Also TLC study of raw drug and Navayas churna shows all the essential phytoconstituent. In the TLC, the major bands of the test sample of Navayas Churna A, B and C comply with the bands of Suntha (*Zingiber officinale*) at Rf 0.06, 0.24, 0.30, 0.35 & 0.50. The test samples of Navayas Churna A, B and C show the presence of Gingerol-6, the major phytoconstituent of Sunthi (*Zingiber officinale*) whereas Gingerol 10 was not detected in all three batches of Navayas Churna. In X-ray Fluorescence (XRF) and X-Ray Diffraction (XRD) of Raw Kantaloha, Hingula, Loha Bhasma and Final Product Navayas Churna shows many oxide and minerals.^{10,11}

Pharmacological Study: wistar rats of either sex weighing between 180 g to 250 g were used for experiments. The dose of Navayas churna 18 ratti. Considering adult human dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio. Test dose was given in Vardhaman matra for 9 days in rats. The animals were divided into 5 groups, each containing 6 animals. Normal control, Disease control, Vehicle (madhu sarpi) Honey + Ghee (1:1 ratio), Standard (Tab ferrous sulphate 29.25 Mg/kg/bodywt) and Study drug Navayas Churna. In RBC and Hb%, after

applying paired t test, Statistically highly significant result <0.001 were found in DC (Phenyl hydrazine), VC (honey and ghee) STD (Ferrous Sulphate) and Test Navayas Churna group on 9th day as compared to starting day. Histopathological study shows that among 4 organs studied, in 1st batch non-significant pathological changes were observed in only one organ i.e. spleen, in Liver DC (Phenyl hydrazine) shows Minimal changes where as other groups shows no abnormality. In kidney NAD to Minimal (+1) changes found. In heart DC (Phenyl hydrazine) shows minimal changes, while STD (Ferrous Sulphate) and Test Navayas Churna shows NAD to Minimal (+1) changes, also VC (honey and ghee) shows no abnormality. 2nd batch non-significant pathological changes were observed in only one organ i.e. spleen, in Liver and kidney NAD to Minimal (+1) changes found. In heart DC (Phenyl hydrazine) and STD (Ferrous Sulphate) shows minimal changes, while VC (honey and ghee) and Test Navayas Churna shows no abnormality. This can be conclude that non any abnormality or mild changes were found in organs in Test Navayas Churna groups which indicate the safety profile of drug in Vardhaman Matra for 9 days in rats. The observed changes may be attributable to the enhanced availability of free iron.

all herbs used in preparation of Navayasa lauha are stomachic and carminative and helps to improve indigestion which occurs due to

consumption of iron. However, the herbs increase the bioavailability of lauha bhasma and favor its absorption which increases the haemoglobin content in blood. Loha Bhasma was prepared by traditional method in Gajaputa. Bhasma passed all the classical physico-chemical parameters so the Analytical study provides the objective parameters to set up the standards for quality finished products. Analysis of the findings of phase identification of different samples of Lauha, Hingula and Navayas Churna by X-ray diffraction (XRD) method. and X-ray fluorescence (XRF) methods reveals metallic iron in raw Lauha was converted to iron oxide (Fe_2O_3). TLC of raw herbal drugs shows all herbal drugs present in Navayas churna is authentic and contain essential phytoconstituent. In this study Navayas Churna was given in increasing dose for consecutive 9 day. Analysis of the data of the toxicity study reveals Navayas Churna do not show any serious toxic effect on hematological and histopathological parameters and vital organs. Navayas churna contain Loha Bhasma. It contains Iron in the form of Ferrous or in oxide form which are considered to be the most compatible forms of iron supplementation in the body. The rate of the absorption of the iron depends on the fineness of the powder. Bhasma process makes the metal into very minute particles which are easy to absorb. Anemia occurs especially in iron deficiency condition. However since the drug

itself provides iron, hence it shows safe theopoetic value.

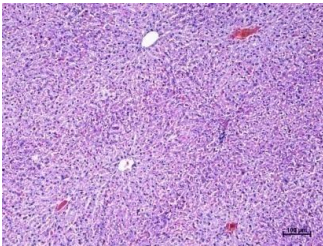
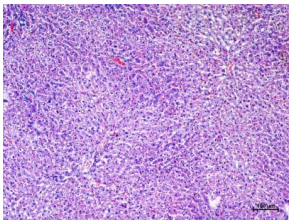
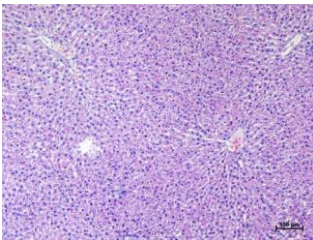
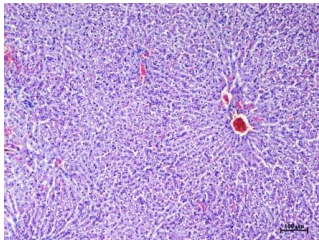
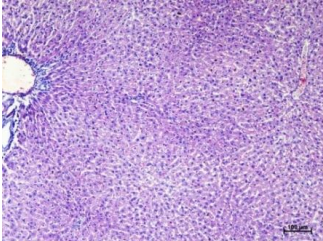
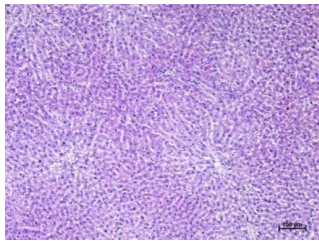
Conclusion: Navayas churna is a herbomineral preparation which is used in pandu to increase hemoglobin.

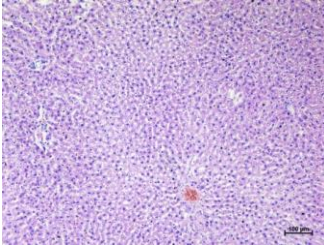
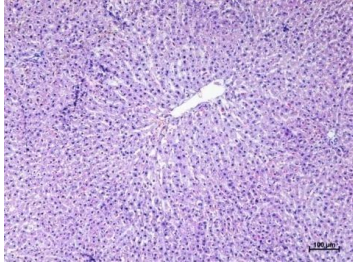


1. Analytical and TLC study shows presence of inorganic and iron compound in formulation that was also useful in safety and effectiveness of drug
2. No serious toxicity was observed in acute toxicity study in Increasing dose (Vardhaman matra) for 9 days as well as no Significant

histological changes are found in Animal study.

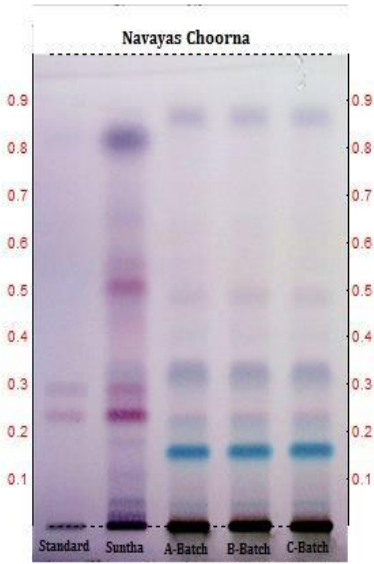
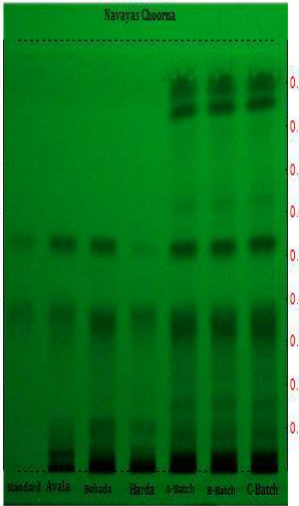
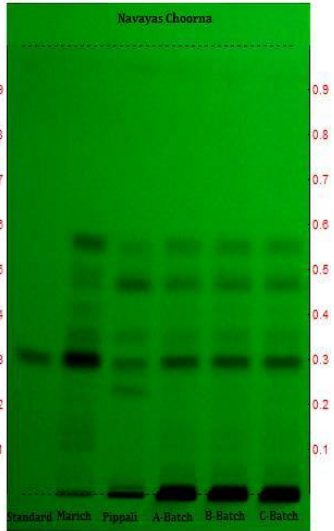
3. Significant increase in HB and RBC count was pharmacological study. Which shows Navayas churna possess hematinic effect and hemoglobin regeneration efficiency.
4. On results of this research study it may be concluded that navayas churn In vardhman matra for 9 days was therapeutically safe and highly Significant in increasing hb% and rbc in experimental animals.

Histopathological study

	
DC (Phenyl hydrazine)- 1	DC (Phenyl hydrazine)- 2
	
VC (honey and water)-1	VC (honey and water)-2
	
STD (Ferrous Sulphate) -1	STD (Ferrous Sulphate) -2

	
TEST (NAVAYAS Churna)- 1	TEST (NAVAYAS Churna)- 2
	
Oral Dosing	Necropsy

TLC profile of Navayas choorna

		
Fig 1: TLC plate of test sample of Navayas Choorna for Gingerol	Fig 2: TLC plate of test sample of Navayas Choorna for Ellagic acid and Gallic acid	TLC of Navayas Choorna for Piperine

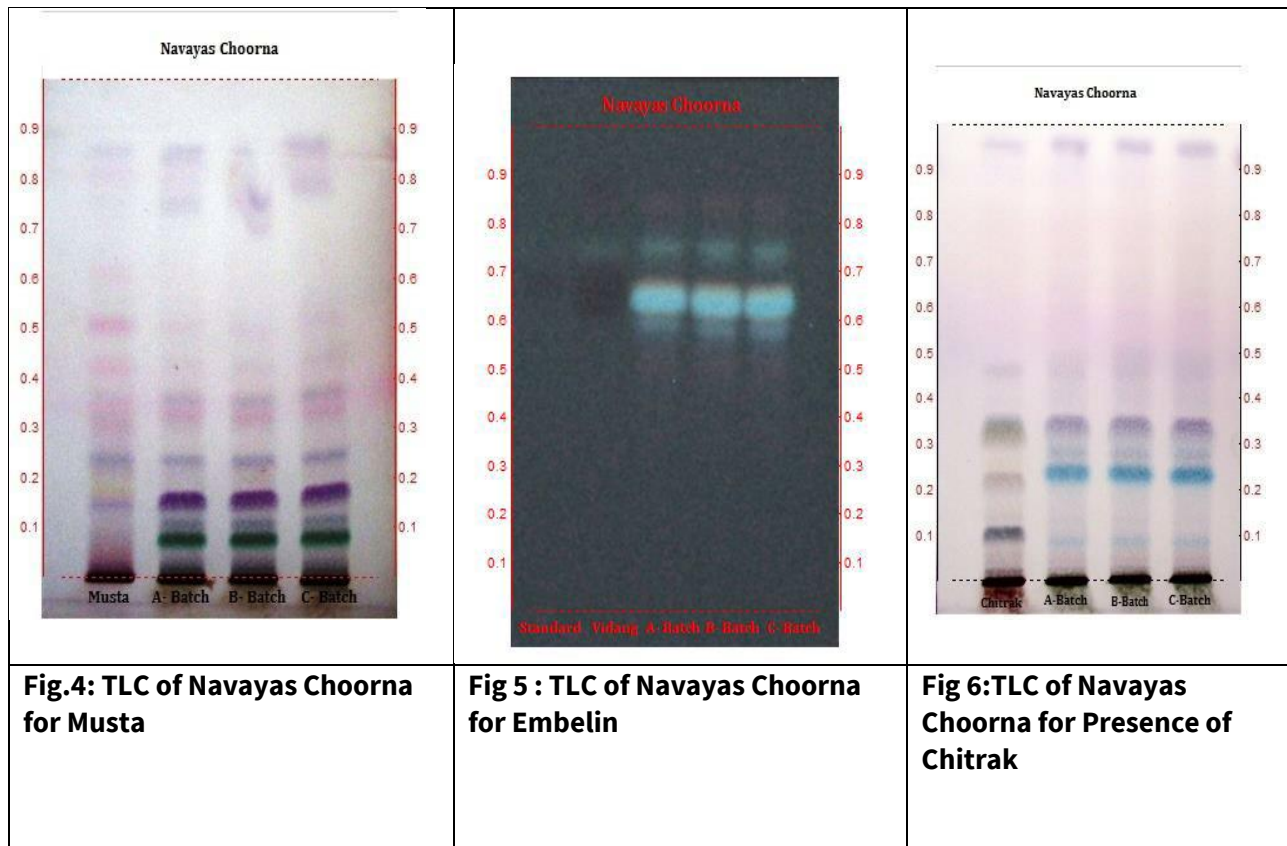


Fig no. 1 XRD of Lauha Bhasma sample A

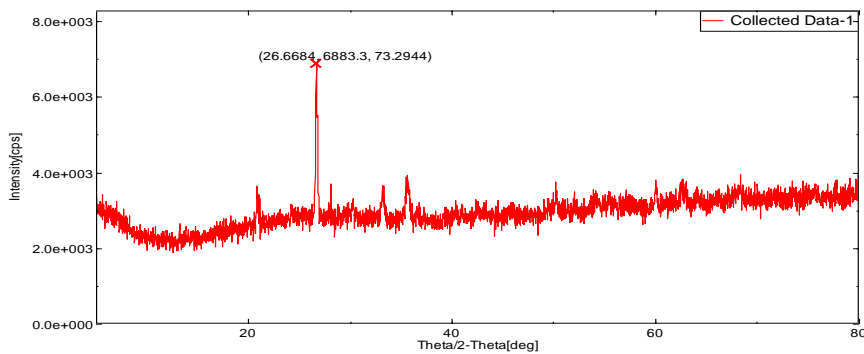


Fig 2. XRD of Lauha Bhasma sample B

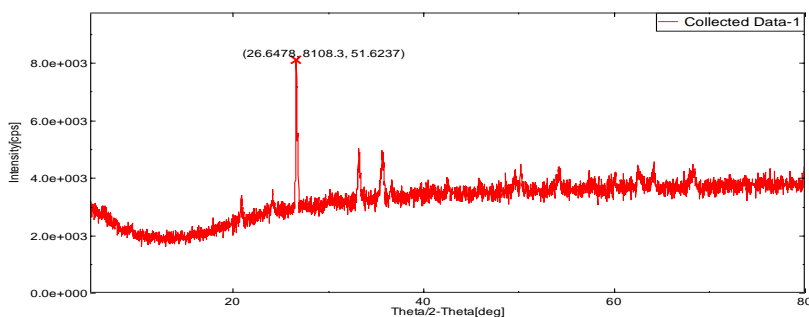
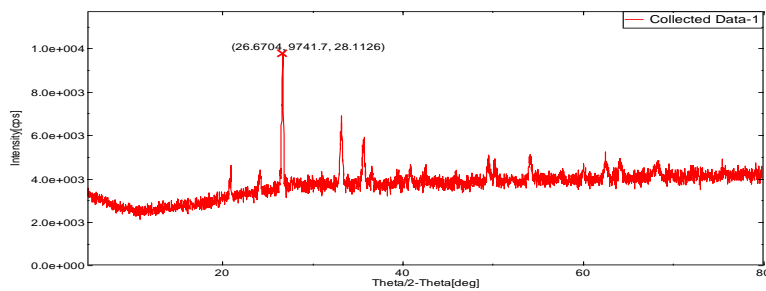


Fig 3. XRD of Lauha Bhasma sample C

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