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Research Article

Comparative oral and dermal bio accessibility study of Ayurvedic Antidote (*Manjishthadi Agada*) by Franz Diffusion method

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Abstract

Background & objectives: *Manjishtadi Agada* described in Bharat Bhaishajya Ratnakar is indicated in all type of poisoning however the route of administration is not specified. *Agada* formulations are generally administered through various routes both internal (excluding parental routes) and external in cases of poisoning. In emergency condition like poisoning or snake bite, rapid absorption of the drug is essential to achieve peak level and therapeutic effect. The present study was conducted to compare the absorption of *Manjishtadi Agada* through oral and dermal route. Methods: In this study, *Manjishtadi Agada* was prepared and subjected to physicochemical analysis followed by dermal and oral bio accessibility studies using Franz cell diffusion apparatus. Absorbance and corresponding time were recorded and the graphs were plotted for dermal and oral bio accessibility of *Manjishthadi Agada*. Assessment was performed using calibration curve to estimate the absorption of the drugs with different time interval and dilutions. Pearson's Correlation coefficient was calculated to determine the relationship between rate and time of absorption of both the routes. Results: A statistically significant difference was observed in the absorbance of *Manjishthadi Agada* between dermal and oral route. Interpretation and Conclusions: Bio accessibility of *Manjishtadi Agada* was found to be higher through the oral route compared to the dermal route. The formulation may be absorbed more rapidly when administered orally, particularly at higher concentrations. Therefore, in both acute and chronic poisoning conditions, oral administration of *Manjishtadi Agada* is preferable.

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Keywords: Bioaccessibility, Diffusion study, Franz cell diffusion, *Manjishthadi Agada,* Poisoning.

Introduction

Poisoning is a serious public health hazard in the country and it is one of the leading causes of emergency hospitalization. More than 50,000 people die every year due to suicidal and accidental poisoning in India. (1) In rural India, poisoning is the fourth most common cause of mortality. The rate varies from 15-30%. (2,3) Age group 19-40 years has the highest prevalence of poisoning. The overall sex ratio was 1.7, and the male to female ratio was highest in the northern region and lowest in the eastern region. (4) In rural area, people die due to delayed reporting of cases in higher centers. Hence it is necessary to increase the survival period so that the precious time of the patient can be saved.

The Ayurvedic formulations mentioned as *Agada* (Ayurvedic Antidotes) for the treatment of poisoning have not been scientifically studied in detail. Even in the references of most of the *Agada,* dose and duration of administration is not specified. However, the research studies are conducted on some individual drugs included in these *Agada.* Hence, to incorporate *Agada* effectively in the clinical practice, scientific research is necessary to establish standard parameters and to validate their safety and efficacy.

Manjishthadi Agada described in Bharat Bhaishajya Ratnakara is a polyherbal Ayurvedic antidote indicated for use in all types of poisoning. (5) However, its route of administration is not mentioned. *Agada* are generally administered through various routes, both internal (excluding parenteral) and external, in cases of poisoning. In emergency conditions such as poisoning or snake bite, rapid absorption of the drug is required to achieve peak levels and therapeutic effect. Therefore, the present study was undertaken to determine the route through which Manjishthadi Agada is absorbed more rapidly and demonstrates better efficacy. This objective was addressed through an in vitro study comparing oral and trans-dermal diffusion method.

Material and methods

For this study, approval was obtained from the Institutional Ethics Committee (IEC) in a meeting held on 23/05/2022 with Ref. No. MGACHRC/IEC/May-2022/467. The study commenced after

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acceptance from the SPARK team, CCRAS, New Delhi. All experiments were conducted in compliance with Good Laboratory Practices. The study was carried out from January 2023 to March 2023.

Materials

Table 1: Ingredients of *Manjishthadi Agada*

SN	Drug Name	Latin Name	Part used	Quantity
1	*Manjishtha*	*Rubia cardifolia* Linn	Roots	50gm
2	*Ela*	*Elletoria cardamomum* Maton	Seeds	50gm
3	*Draksha*	*Vitis venifera* Linn	Fruit	50gm
4	*Nisha (Haridra)*	*Curucuma longa* Linn	Rhizome	50gm
5	*Jatamansi*	*Nardostachys jatamansi* DC	Rhizome	50gm
6	*Yashtimadhu*	*Glycerrhiza glabra* Linn	Roots	50gm
7	*Harenuka (Renuka)*	*Vitex agnus-castus* Linn	Seeds	50gm

All the seven drugs were procured from the Manakarnika Ayurvedic Aushadhalaya, Pune and were identified and authenticated from the department of Dravyaguna.

Pharmaceutical study

Preparation of *Manjishthadi Agada:*(5)

Manjishthadi Agada was prepared in Dattatraya Rasashala, M.G.A.C. H. & R.C. Salod (Hirapur), Wardha. Fine powders of each ingredient (*Manjishta, Yashtimadhu, Ela, Harenuka, Jatamansi, Haridra*) were prepared separately (Fig. 2). Since *Draksha* has a higher moisture content, it was soaked overnight in water, seeds were removed and pulp was obtained. The pulp was ground into a smooth paste and thoroughly mixed with equal quantities of the powders of the six drugs using a mixer. The mixture was dried in shade. As the *Draksha* has higher moisture content the process of grinding and sieving it with cotton cloth was repeated several times simultaneously until the moisture content was reduced. Using thin cotton cloth, the final mixture was evenly sieved and dried in shade. The powder was filtered through sieve of desired size to get homogenous mixture. (Fig.3) The prepared *Agada* was subjected to physicochemical analysis and diffusion study at Analytical Laboratory, M.G.A.C. H. & R.C. Salod (Hirapur), Wardha.

Figure 1: Raw Ingredients of *Manjishthadi Agada* (Crude drug)

Figure 2: Powder of raw drugs in *Manjishthadi Agada*

Figure 3: *Manjishthadi Agada* (Final product)

Physicochemical Analysis

Loss on drying, Total Ash value, Acid insoluble ash value, Alcohol extractive value, Water extractive value, pH and Microbial load were conducted as per the methods described in Ayurvedic Pharmacopoeia of India (API).

Dermal bio accessibility study

Franz cells diffusion study was used for in vitro experiment. Franz cell diffusion apparatus is made up of two small glass chambers. The upper one is donor chamber and the lower one is recipient chamber.

Diffusion study: (6, 7, 8)

Requirements: 5cm goat intestine, Franz cell, rubber, drug, beaker, Magnetic Stirrer, blade.

Procedure:

The fresh gut of goat was collected. The 5cm goat gut was taken and it was cut open with blade. Fat layer was separated by blade so as to observe the thin skin layer which is to be placed on lower flat flange joint. A buffer solution was prepared in a beaker by adding one buffer tablet of 500mg in water of 100ml and stirred. Solution was poured in Receptor chamber. The donor chamber was placed at top and tied with the rubber to avoid the movement. The drug was loaded in donor chamber. The beaker filled with water was placed on the Magnet stirrer and 37^0 C temperatures was set on the stirrer as that of body temperature. The stirrer was started. The drug in donor chamber was allowed to diffuse from the goat intestinal membrane in the recipient chamber. Sample was collected at 15 min, 30 min, 60 min, 120 min, 180 min and 240 min maintaining pH of 7.0. Each sample collected at different time interval was subjected to dilution. For dilution, a buffer solution was prepared in a beaker by adding one buffer tablet of 500mg in water of 100ml. 2.5ml of buffer solution was added in 2ml sample of *Manjishtadi Agada* to make 25% dilution. 5ml of buffer solution was added in 2ml sample of *Manjishtadi Agada* to make 50% dilution. 7.5ml of buffer solution was added in 2ml sample of *Manjishtadi Agada* to make 75% dilution. 10ml of buffer solution was added in 2ml sample of *Manjishtadi Agada* to make 100% dilution. Total 24 samples were prepared for dermal Trans-diffusion study. All the samples were run under SL244

Double Beam UV-VIS Spectrophotometer of ELICON® on 254nm Wavelength. The reading were taken and was plotted against time and absorbance on x and y axis.

Fig.4 Franz Cell diffusion apparatus

Oral bio accessibility study

The same method was used for oral bio accessibility.

Diffusion study: (6, 7, 8)

Requirements: 5cm goat intestine, Franz cell, rubber, drug, beaker, Magnetic Stirrer, blade.

Procedure: The fresh gut of goat was collected. The 5cm goat gut was taken and it was cut open with blade. Fat layer was separated by blade so as to observe the thin skin layer which is to be placed on lower flat flange joint. A buffer solution was prepared in a beaker by adding one buffer tablet of 500mg in water of 100ml and stirred. Solution was poured in receptor chamber. The donor chamber was placed at top and tied with the rubber to avoid the movement. The drug was loaded in donor chamber. The beaker filled with water was placed on the Magnet stirrer and 37^0 C temperatures was set on the stirrer as that of body temperature. The stirrer was started. The drug in donor chamber was allowed to diffuse from the goat intestinal membrane in the recipient chamber. Sample was collected at 15 and 30 min by maintaining pH of 4.0, at 60 and 120 min by maintaining pH of 7.0, at 180 min and at 240 min by maintaining pH of 9.2. Each sample collected at different time interval was subjected to dilution. For dilution, a buffer solution was prepared in a beaker by adding one buffer tablet of 500mg in water of 100ml. 2.5ml of buffer solution was added in 2ml sample of *Manjishthadi Agada* to make 25% dilution. 5ml of buffer solution was added in 2ml sample of *Manjishthadi Agada* to make 50% dilution. 7.5ml of buffer solution was added in 2ml sample of *Manjishthadi Agada* to make 75% dilution. 10ml of buffer solution was added in 2ml sample of *Manjishthadi Agada* to make 100% dilution. Total 24 samples were prepared for oral Trans -diffusion study. All the samples were run under SL244 Double Beam UV-VIS Spectrophotometer (ELICON®) on 240nm Wavelength. The reading were taken and was plotted against time and absorbance on x and y axis.

Results

Figure 5: Absorbance of *Manjishtadi Agada* at dermal diffusion with different time interval

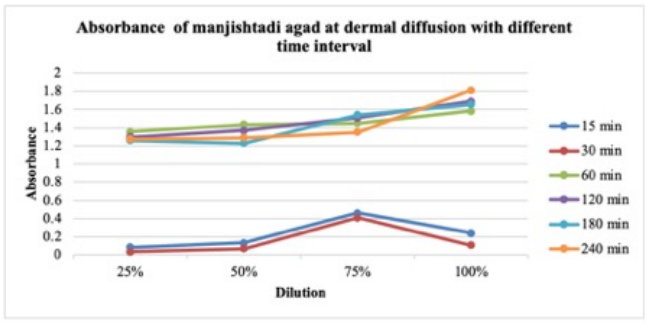


Table 2: Observations of Physicochemical analysis and microbial load

SN.	Analytical Parameter	Observations
1	Loss on drying at 105^0C	0.81%
2	Total Ash Value	1.73%
3	Acid Insoluble Ash	0.31%
4	Water soluble Extractive	2.62%
5	Alcohol soluble extractive	37.15%
6	pH	6.1
	Microbiological load	**Observations**
7	Total Viable Count	Absent
8	Enterobacteriaceae	Absent
9	Total fungus count	Absent
10	E-coli	Absent
11	Salmonella	Absent
12	Staphylococcus aureus	Absent
13	Pseudomonas aueruginosa	Absent

Table 3: Absorbance of *Manjishthadi Agada* at different time interval and different concentration

Time intervals in minutes	Sample concentration	Absorbance	
		During dermal trans -diffusion study	During Oral diffusion study
15	25%	0.0826	2.4879
	50%	0.1330	2.5074
	75%	0.4601	2.4426
	100%	0.2411	2.5657
30	25%	0.0344	2.4358
	50%	0.0671	2.4254
	75%	0.4071	4.0000
	100%	0.1065	4.0000
60	25%	1.3550	3.1220
	50%	1.4331	4.0000
	75%	1.4505	4.0000
	100%	1.5828	2.5871
120	25%	1.2964	4.0000
	50%	1.3708	4.0000
	75%	1.5063	3.9527
	100%	1.6878	4.0000
180	25%	1.2573	3.1139
	50%	1.2294	3.1359
	75%	1.5413	4.0000
	100%	1.6536	4.0000
240	25%	1.2736	4.0000
	50%	1.2901	4.0000
	75%	1.3500	3.0608
	100%	1.8111	4.0000

Figure 6: Absorbance of *Manjishthadi Agada* at oral diffusion with different time interval

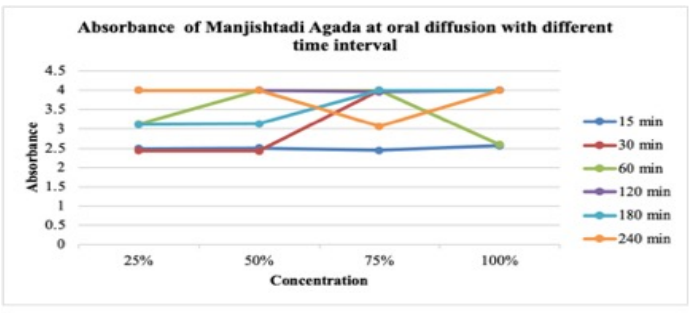


Table 4: Statistical analysis of absorbance with Pearson's correlation

Groups	Count (N)	Sum	Mean	Variance	SD	r	p
Dermal	24	24.621	1.025875	0.390722	0.6119	0.6148	0.0014
Oral	24	81.8372	3.409883	0.467337	0.6692		

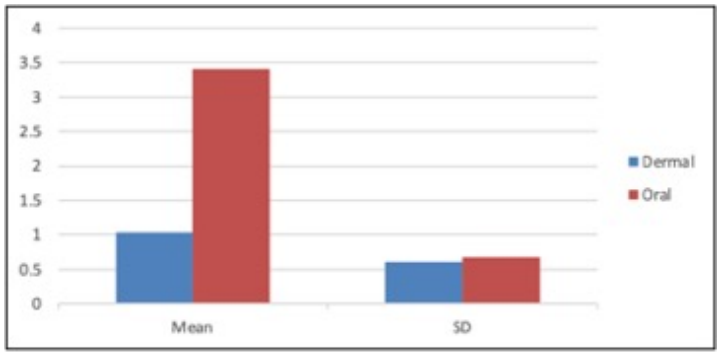
There was a significant relation (a = 0.05).

Table 5: Statistical analysis of absorbance in dermal and oral diffusion study of *Manjishtadi Agada*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	68.20195	1	68.20195	158.968	<0.025	4.051749
Within Groups	19.73535	46	0.429029			
Total	87.9373	47				

There was a significant difference found (a = 0.05).

Figure 7: Mean and SD for both the group of dermal and oral diffusion study of *Manjishtadi Agada*



Discussion

In the present study, *Manjishthadi Agada* was prepared using seven ingredients in equal proportion. The finished product obtained was yellowish brown in colour with characteristic smell of *Ela, Draksha* and *Manjishtha*. Since *Draksha* contains more moisture, a little modification was conducted which is a routine practice where *Draksha* is an ingredient. This modification was done to mix *Draksha* homogeneously with other ingredients. In physicochemical analysis, it was onserved that the moisture content was relatively high, possibly due to sticky and water retaining nature of *Draksha*. Hence, it is recommended to prepare tablets of *Manjishthadi Agada* and compare the shelf life of powder and tablet form for use in human beings.

The ash value was found to be 1.73, which may be attributed to the inorganic content of the herbal drugs. The formulation was soluble in both water and alcohol and exhibited a weakly acidic nature with a pH of 6.1. No bacterial contamination was detected in the prepared Agada. The physicochemical parameters established in this study may serve as reference standards for further research. Following physicochemical analysis, bio accessibility study by diffusion method. Bio accessibility is essential for bioavailability as the bioactive compounds must be released from food matrix and modified in the GI tract before they are bio available.

Franz diffusion method is a gold standard technique to study the penetration of the drug in to the skin and GIT. (8) Franz diffusion test is proposed to analyse the drug diffused from skin membrane by using Franz diffusion cell apparatus. Dermal diffusion study indicates that the absorption of *Manjishthadi Agada* starts after 15 minutes and attains maximum absorption at 240 minutes. The absorbance of *Manjishthadi Agada* is maximum with 75% dilution at 15 and 30 minutes interval. The absorbance of *Manjishthadi Agada* is maximum with 100% dilution at 60, 120, 180 and 240 minutes interval. At 25%, 50% and 75% dilution, absorbance is maximum at 60 minutes. At 100% dilution, absorbance is maximum at 240 minutes. It indicates that the formulation requires 60 minutes to 240 minutes to attain its peak absorption at different dilutions (table 3). Hence this *Agada* should be applied in the form of *lepa* or ointment so that it can be retained up to 4 hours. The pH of all the samples were maintained at 7.0 as that the pH of the skin.

In oral bio accessibility study, it is observed that the absorption of *Manjishthadi Agada* starts after 15 minutes and attains maximum absorption at 240 minutes. The absorbance of *Manjishthadi Agada* is maximum with 100% dilution at all time intervals. At 25 % dilution, *Manjishthadi Agada* is absorbed maximum at 120 minutes. At 50 % dilution, *Manjishthadi Agada* is absorbed maximum at 60 minutes and retains up to 240 minutes. At 75 % and 100% dilution, *Manjishthadi Agada* is absorbed maximum at 30 minutes and retains up to 240 minutes. The absorbance is faster with 75% and 100% dilution. Hence, this concentration may be considered for acute conditions.

The samples were collected at different intervals maintaining the pH present in stomach, duodenum, small and large intestine. It is observed that the absorbance is more at 60 min interval at 50% and 75% dilution. At 120 min and 240 min interval, the absorbance is more at all dilutions (table 3). It indicates that *Manjishthadi Agada* is absorbed more from duodenum, small and large intestines. When both the routes are compared, it is observed that the absorbance of *Manjishthadi Agada* is more with oral administration than the dermal route. The same is verified statistically.

As the data is quantitative in nature, ANOVA test was applied. As the p value is less than 0.025, a significant difference was found in both groups. So it can be said that there is significant difference in absorbance of *Manjishthadi Agada* with oral and dermal through routes of administration. *Manjishthadi Agada* is absorbed more through oral route than the dermal route. For external use, *Manjishthadi Agada* should be applied in the form of *lepa* or ointment to ensure its retention up to 4 hours.

Conclusion

The bioaccessibility of *Manjishthadi Agada* was found to be greater through the oral route compared to the dermal route. This indicates that the formulation may be absorbed earlier when administered orally, particularly at higher concentrations. Therefore, in both acute and chronic poisoning conditions, oral administration of *Manjishthadi Agada* is preferable. In the future, its safety and efficacy in increasing survival time can be evaluated first in animal models and subsequently in human beings. Dose-escalation studies followed by randomized controlled trials may further establish *Manjishthadi Agada* as a primary or adjuvant therapy in the management of various types of poisoning.

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Conflict of Interest: There is no conflict of interest.

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